CHAPTER III

OBSERVATIONS AND DISCUSSION

Observations

- There are 19 experimental groups.
- There are five regions of brain.
- Array of 33-histochemical techniques are used to analyze proteoglycans and sialic acid.
- It is not possible to present all the data in plates of all the staining reactions though data is with the author.
- Therefore basic and technique significant data is presented through the plates.
- Total data is presented in tables to avoid the repetition and similar data.

Abbreviations used-

1. GAGs- Glycosaminoglycans
2. NGAG- Neutral glycosaminoglycans
3. SGAG- Sulfated glycosaminoglycans
4. SA- Sialic acid
5. HA-Hyaluronic acid

Abbreviations used in Tables-

1. Mesen- Mesencephalon
2. Mete- Metencephalon
3. Myelen- Myelencephalon
4. Dien- Diencephalon
5. Telen- Telencephalon
6. Cyt.-Cytoplasm
7. Cs- Cell surface
8. Ics- intercellular space, ML- Marginal layer
9. - : Absence of reaction.
10. ± - Trace intensity of coloration
11. + - Weak intensity of coloration
12. ++ - Moderate intensity of coloration
13. +++ - Intense intensity of coloration
14. ++++ - Highly intensity of coloration.
15. +++++- Significant intensity of coloration.

Staining techniques-

1. PAS- periodic acid Schiff's technique
2. Amy-dig-PAS- amylase digestion periodic acid Schiff's reagent techniques
3. Dia-dig-PAS- diastase digetion periodic acid Schiff's reagent technique
4. Phy-hyd-PAS- phenyl hydrazin periodic acid Schiff's reagent technique
5. Sap-PAS- saponification periodic acid Schiff's reagent technique
6. Ace-PAS- Acetylation periodic acid Schiff's reagent technique
7. Deace-PAS- deacetylation periodic acid Schiff 's reagent technique

8. Phe-deace-sap-PAS- Phenylhydrazin deacetylation saponification periodic acid Schiff's technique
9. Sulfur–indu-metachro- sulfur induced metachromacia
10. AB pH 2.5- Alcian blue at pH 2.5
11. PA*- Bh-Sap-PAS- Selective periodic acid borohydrate saponification periodic acid Schiff's reagent technique.
12. AZ-metach –pH 3.5- Azure metachromacia at pH 3.5
13. Sap-AB pH 2.5- Saponification Alcian blue at pH 2.5
14. Mild-met-AB pH 2.5- Mild Methylation alcian blue at pH 2.5
15. Mild-met-Sap-AB pH 2.5- Mild Methylation saponification alcian blue at pH 2.5
16. Act.-met-AB pH 2.5- Active Methylation alcian blue at pH 2.5
17. Act-met-Sap-AB pH 2.5- Active Methylation saponification Alcian blue at pH 2.5
18. AB 2.5-PAS- Alcian blue at pH 2.5 +periodic acid Schiff's reagent technique.
19. AF- AB pH 2.5- Aldehyde fuschin-alcian blue at pH 2.5.
20. Acid-hyd-AB pH 2.5- acid hydrolysis Alcian blue at pH 2.5
21. AB pH 1- Alcian blue at pH 1
22. AF- Aldehyde fuschin
23. 0.0M- Alcian blue pH 5.6 with 0.0M $MgCl_2$
24. 0.1M - Alcian blue pH 5.6 with 0.1M $MgCl_2$
25. 0.2M - Alcian blue pH 5.6 with 0.2M $MgCl_2$
26. 0.4M - Alcian blue pH 5.6 with 0.4M $MgCl_2$
27. 0.5M - Alcian blue pH 5.6 with 0.5M $MgCl_2$
28. 0.6M - Alcian blue pH 5.6 with 0.6M $MgCl_2$
29. 0.8M - Alcian blue pH 5.6 with 0.8M $MgCl_2$
30. 1.0M - Alcian blue pH 5.6 with 1.0M $MgCl_2$
31. AZ pH 0.5- Azure at pH 0.5
32. AZ pH 0.5- Azure at pH 1.0
33. AZ pH 0.5- Azure at pH 1.5
34. AZ pH 4.5- Azure at pH 4.5.

OBSERVATIONS AND DISCUSSION

Section I: Mortality

Alterations in mortality percentage are depicted in Table no. 1

Single dose exposure initiated at 24 hrs of incubation further continued the incubation for 24, 48, 72 and 96 hrs. At these intervals, the developmental stages of embryos were 48, 72, 96 and 120 hrs respectively. At 48, 72, 96 and 120 hrs of development normal embryos showed 10% mortality at each hrs. The embryos only in HBSS as control embryos showed 5% mortality at 48 hrs of development while it was decreased by 5% as compared with the mortality noted at 48 hrs of development in normal condition indicating improvement in survival of the embryos at all the intervals studied.

Treatment of 0.05 mM H_2O_2 given at 24 hrs to the embryos showed 20, 20, 20 and 25% mortality at 48, 72, 96 and 120 hrs of the final incubation intervals respectively. The increase was 10-15% as compared with mortality observed in normal embryos. Initiation of treatment at 24 hrs and 24, 48, 72 hrs of exposure showed same percentage of mortality but 96 hrs of exposure showed 25% increase in mortality. While at 34 hrs of initiation of exposure and duration of exposure further at 24, 48, 72 hrs showed 25% decreased mortality but 96 hrs of continued exposure showed 30% mortality which indicated that for 0.05 mM dose 96 hrs of exposure mortality was highest .Initiation of exposure of 0.05 mM H_2O_2 dose at 40 hrs decreased mortality by 25% after 24 hrs of exposure but for remaining exposure hrs it remained as 20%. Thus except one exposure the mortality percentage remained between 15-25 at different initiation exposure time and total exposure hrs. Initiation of exposure at 48 hrs showed mortality similar to normal at exposure of 24 hrs while it was 20% for 48 and 72 hrs exposures. Initiation of exposure of 0.05 mM H_2O_2 at 72, 96 and 120 hrs for 24 hrs exposure no change in mortality observed in normal embryos.

Treatment of 0.5 mM H_2O_2 to the embryos of 24 hrs showed 50% mortality at 48 and 72 hrs of incubation while at 96 hrs of incubation 52% mortality was observed while at 120 hrs of incubation the mortality observed was 55%. 0.5 mM dose at all the initiation of treatment hrs and treatment exposure hrs remained in the range of 45-55%, thus indicating that irrespective of their development status, initiation of

exposure developmental stages and total exposure period/duration the mortality is in the range of 45-55% and thus all the developmental stages are equally vulnerable to death induced by 0.5 mM H_2O_2.

Treatment of 1.0 mM H_2O_2 at 24 hrs showed 80, 80, 85 and 90% mortality at 48, 72, 96 and 120 hrs of final developmental stages. Initiation of treatment at 34 and 120 hrs with 24 hrs exposure and initiation of treatment at 40 hrs with 24 and 48 hrs exposure showed 75% mortality. With this dose the mortality varied in the range of 75-90%. Except these three exposures remaining treatments showed 80-90% mortality showing increase in mortality by 30-35% at all experimental conditions.

Treatment of 1.5 mM H_2O_2 to the embryos of all hrs of incubation showed 100% mortality. Thus, the dose dependant increase in mortality was observed with H_2O_2 treatment.

Among the four doses of H_2O_2 (0.05 mM, 0.5 mM, 1 mM and 1.5 mm) 1.5 mM dose mortality was not altered by any of the vitamin C doses, 0.5 mM dose showed average 50% mortality for all the hrs of development. This dose was used to study influence of vitamin C on mortality of embryos as it may give the subtle developmental alterations that may lead to abnormalities in addition to survival. Three mg of vitamin C dose is known to improve hatchability (Ipek *et al.*, 2004) and hence same and other higher doses (4 mg and 5 mg) were used to test their protective efficacy against H_2O_2 induced damage.

Treatment of 0.5 mM H_2O_2 + 3 mg vitamin C given as a single dose at all the developmental hours showed 10 % mortality which was similar to the mortality noted in normal embryos. Simultaneous treatment of 3 mg vitamin C to the embryos with 0.5 mM H_2O_2 treatment showed 100% depletion in mortality for all the hours of exposures.

Remaining experimental schedule showed percent mortality range 25-30% i.e. prevention of mortality percentage in the range of 25-20 without any abnormalities on hatching (data observed not given). The results indicate that 24, 40, 72, 96,120 hrs initiations are equally preventable while 34 hrs stage exposure damage is totally prevented by 3 mg vitamin C dose.

Treatment of 0.5 mM H_2O_2 + 4 mg vitamin C at 24 hrs showed 40, 40, 50 and 50% mortality at 48, 72, 96 and 120 hrs of development respectively. Initiation of treatment at 34 for 24, 48 and 72 exposure and at 40 hrs initiation for 24 hrs exposure showed 35% mortality. Initiation at 40 hrs for 48 hrs exposure showed 40% mortality

while 34 hrs initiation and 96 hrs exposure showed 55% mortality. At 40 hrs of initiation for 72 and 96 hrs exposure 70% mortality was noted. While initiation at 48 hrs for 24, 48 and 72 hrs mortality varies between 20-30. Mortality observed in treatment initiated at 72 hrs for 24 and 48 hrs exposure, initiation at 96 and 120 for 24 hrs exposure the mortality varies between 10-12% which was similar to mortality noted in normal. Therefore with this dose mortality varies between 10-70%.

In 0.5 mM H_2O_2 +5 mg vitamin C treated embryos at 24 hrs 40% mortality was observed at 48 and 72 hrs of development. At 96 and 120 hrs of development 45 and 55% mortality was observed respectively. Initiation of treatment at 34 and 40 hrs for 24 and 48 hrs showed 35 and 40%, while initiation 35 for 72 and 96 hrs 35 and 60%. Initiation at 40 hrs for 72 and 96 hrs showed 75% mortality. Initiation at 48 hrs for 24 , 48 and 72 hrs exposure showed 20, 70 and 100% mortality. Treatment initiated at 72 hr for 24 and 48 hrs showed 15% mortality while initiation at 96 and 120 hrs foe 24 hrs exposure showed 25 and 20% mortality respectively.

In vitamin C (3 mg) treated control embryos 5% mortality was noted for all the hrs of development for all the exposures. Dose of 4 and 5 mg vitamin C Treatment of 4 mg vitamin C initiated at 24 and 34 hrs showed mortality within the range of 24-28%. Initiation at 40 hrs for 24 hrs exposure showed 35% and for 48, 72 and 96 hrs mortality noted was 75 and 80% respectively. Initiation of treatment of 4 and 5 mg vitamin C at 48 hrs for 24 hrs exposure showed 35 and 30% mortality. Initiation of treatment at 72 hrs for 24 and 48 hrs exposure and 120 hrs initiation for 24 hrs showed 15% mortality. While 4 and 5 mg vitamin C initiated at 96 hrs for 24 hrs exposure showed 10% mortality.

Vitamin C 3 mg dose given at 24 and 34 hrs and continued at latter hrs had improver the mortality rate while same dose of vitamin C given at 40 hr had not affected the mortality rate in normal embryos indicating its hatching improvement potency (Ipek *et al.*, 2004) which is confirmed in present results but 4 and 5 mg doses deflected from these results as they had increased mortality of embryos treated at 40 hrs and continued at 48, 72 and 96 hrs. These intervals seem to be more sensitive to vitamin C for survival. Since vitamin C is known to inhibit cell death induced by oxidative stress in glutathione depleted HL- 60 cells (Guaiquil *et al.*, 2001). Similarly microvascular endothelial cells in lung have shown vitamin c induced loss of redox dependant viability (Varadharaj *et al.*, 2005). It is possible that the higher doses may

be causing decreased cell death which may be obligatory for the survival of embryos leading the increase in mortality.

Besides number of ascorbic acid isomer derivatives were synthesized and 2/6 or both positions in ascorbic acid have marked cytoxicity on malignant and non malignant cell lines in vitro and in vivo (Park and Kimler, 1991; Roomi *et al.*, 1998; Roomi *et al.*, 1998). Thus vitamin C is responsible for cell survival and cell death in different conditions , increase and decrease in mortality percentage of embryos may consequences of such effects in vivo leading to differential mortality. Similarly it seems that 3 mg dose of vitamin C seem to optimize the mortality percentage in induced free radical generation by 0.5 mM H_2O_2 in embryos at different hrs of development.

Dose of 3 mg vitamin C prevents mortality induced by 0.5 mM H_2O_2 leading to survival of embryos as observed in normal embryos at all the hrs of development for all the exposure. But 4 mg vitamin C with 0.5 mM H_2O_2 did not improve mortality over 3 mg dose in case of 48 hrs of treatment initiation continued for 24, 48 and 72 hrs. But mortality similar to that was observed in normal was shown in embryos where initiation of dose was at 72 hrs continued for 24 and 48 hrs, dose initiation at 96 and 120 hrs and continued for 24 hrs. thus treatment of 4 mg vitamin C dose simultaneous with 0.5 mM H_2O_2 has protected the embryos as they survived in normal condition. 3 mg dose showed similar influence as 4 mg dose at 48 hrs of initiation with all the intervals but dose dependant improvement was noted with these doses at 72, 96 and 120 hrs initiation and continuation hrs studied. But 5 mg dose increased mortality at 48 hrs initiation and initiation at remaining hrs showed marginal increase in percent mortality i.e. 4 mg vitamin C dose alone is preventing 50 % mortality to maintain normal mortality in embryos. But 4 and 5 mg vitamin C doses (alone) increased the mortality when the dose was initiated at 48 hrs with continuation 24, 48 and 72 hrs , dose and duration dependant increase in mortality was observed the results indicated that 3 mg dose of vitamin C had improved the mortality but not the remaining doses.

But results of 3 mg vitamin C dose given alone indicate improver survival at 72, 96 120 hrs of treatment initiation and continued hrs studied. While 4 and 5 mg vitamin C doses initiated at 72, 96 and 120 hrs marginally increased the mortality

Table 3 : Effect of H_2O_2 and Vitamin C on mortality (in %) of chick embryos at different stages of development.

(Result expressed as mean of six embryos).

Treatments	Groups (Treatment given at in hrs)																		
	I 24				II 34				III 40				IV 48			V 72		VI 96	VII 120
	Exposure time in hrs																		
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	A	B	A	A
	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	24	48	24	24
i) Normal	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
ii) HBSS control	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
iii) 0.05 mM H_2O_2	20	20	20	25	15	15	15	30	15	20	20	20	10	20	20	10	20	10	10
iv) 0.5 mM H_2O_2	50	50	52	55	48	49	50	50	45	45	48	50	45	50	50	50	51	50	45
v)1.0 mM H_2O_2	80	80	85	90	80	75	85	90	75	75	90	90	80	80	90	85	85	80	75
vi)1.5 mM H_2O_2	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
vii) 0.5 mM H_2O_2+ 3 mg Vitamin C	9	10	10	11	9	9	9	12	9	9	10	11	9	10	10	10	10	10	10
viii) 0.5 mM H_2O_2+ 4 mg Vitamin C	40	40	50	50	35	35	35	55	35	40	70	70	20	30	30	12	12	10	10
ix) 0.5 mM H_2O_2+ 5 mg Vitamin C	40	40	45	55	35	40	35	60	35	40	75	75	20	70	100	15	15	25	20
x) 3 mg Vit. C	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
xi) 4 mg Vit. C	25	25-	24	28	25	25	25	25	25	75	75	75	35	75	75	15	15	10	15
xii) 5 mg Vit. C	24	25	28	28	25	25	25	25	25	80	80	80	30	80	75	15	15	10	15

indicating its negligible influence on embryos at all continuation hrs studies indicating sensitivity of embryos to vitamin C treated at 48 hrs of incubation .

The result showed 10% mortality in normal embryos and 5% mortality in HBSS control 1.5 mM H_2O_2 exposure at any hrs of development lead to 100% mortality. Dose dependant increase in mortality was shown by 0.05, 0.5, 1.0 and 1.5 mM H_2O_2 treatment at different hrs. 0. 5 mM showed nearly 50% mortality at all hrs of treatment and hence was used for vitamin C induced prevention studies. Tree mg dose of vitamin C showed improved mortality which was similar to HBSS control but remaining doses showed (4 and 5 mg) showed increased mortality (75-85%) at 40 and 48 hrs treatment and with remaining intervals 25% mortality was observed. But since 3 mg dose of vitamin C had shown 10-30% mortality thus is capable of reducing 85 to 97.5% mortality. No abnormalities were observed in these chicks on hatching.

Initiation of treatment at 34, 40, 48 and 72 hrs of development showed abnormalities like retarded growth in embryos with 0.5 and 1.0 mM H_2O_2 While increased weight in 0.5 mM H_2O_2 + vitamin C and control 5 mg vitamin C treated embryos on hatching but the percentage of abnormalities was less than the treatment initiated at 24 hrs. In late hrs treatment i.e. 96 and 120 hrs no abnormalities were observed on hatching in any of the experimental group. At 24 hrs of initiation 0.5 mM H_2O_2 + 3 mg vitamin C, only 3 mg vitamin C, normal and HBSS control showed no abnormalities on hatching but remaining schedules showed abnormalities viz. extended neck, increased weight/growth retardation associated with serious difficulties in movement in many of the hatched ones. The results showed that concentrations of both Vitamin C and free radical generated (naturally and by H_2O_2 cumulatively) both modulate the mortality at different levels of chick development.

Section II Histology

Observations:

Alterations in histology are depicted in Plate no I-VII.

Normal:

48 hrs development:

Double staining and triple staining methods were used to analyze histology. Histologically the brain showed five brain vesicles as Mesencephalon, metencephalon, myelencephalon, diencephalons and telencephalon which could be

distinguished. The histological architecture of normal embryo brain vesicles showed multilayered neuroepithalial cells. The nuclei of neuroepithelial cells are arranged at all possible planes indicating multilayered histological arrangement of neuroepithelium. The multilayered epithelium divided as per the developmental stages of the cells as follows-

The inner ventricular/ intermediate/mantle/dense layer was distinct. Cell free marginal outer layer with numerous cell processes but few cell bodies were distinct. Ventricular (ependyma) innermost layer was of columnar epithelium and in mitosis. Oblong cells were present in the ependymal and mantle layer. Nuclei were intensely basophilic, cytoplasm was eosinophilic. Mitotic stages noted at ependymal layer. Axonol processes were not observed. The marginal layer did not appear. Ependymal, mantle and marginal layers was not observed.

58 hrs development-

There was increased thickness of the neuroepithelial cells with large nuclei. Ependymal layer was with the dividing cells. Migratory cells were observed in the mantle layer. Marginal layer was not defined.

64 hrs development-

The features observed earlier were in more advanced stage.

72 hrs development

At 72 hrs thickening of the neuroepithelial cells was observed. Neuroepithelium was three layered. Ependymal layer was in mitosis. Mantle layer was with radially and laterally moving cells to outwards. The migratory cells in mantle layer were unipolar or bipolar with intensely stained nuclei. The marginal layer was not prominent. The axonal outgrowths were aggregated.

82 hrs development-

The growth features of 72hrs proceeded.

88 hrs development-

Growth features of 72 and 82 hrs continued to advance further at this stage of development.

96 hrs development-

Neuroepithelial layer continued to increase in thickness to undergo migration of neuroepithelial cells were prominent. Ependymal layer migrated towards the mantle layer reducing the thickening of ependymal layer and increasining the thickening of mantle layer. In the mantle layer cells with unipolar and bipolar

PLATE I

PLATE I- Initial incubation (24 hrs) + Dose exposure incubation (24 hrs) = Final development (48 hrs).

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen .- Neuroepithelial layer present. Compactly arranged cells without cell surfaces, intensely stained basophilic nuclei.

Fig. 2 - Meten. - Cell compactly arranged without intercellular spaces, basophilic nuclei.

Fig. 3 - Myelen - Cells compactly arranged without intercellular spaces intensely stained basophilic nuclei.

Fig. 4 - Dien - Compactly arranged cells without intercellular spaces.

Fig. 5 - Telen. - Compactly arranged cell without intercellular spaces.

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000.

Fig. 6 - Mesen. - Cells compactly arranged with little intercellular spaces. Foggy cells towards the luminal side.

Fig. 7 - Meten. - Cells compactly arranged, foggy cell towards the luminal side.

Fig. 8 - Myelen - Cells compactly arranged, foggy cells towards the luminal side

Fig. 9 - Dien - Cells compactly arranged without intercellular spaces, foggy cell towards the luminal side.

Fig. 10 - Telen- Cells compactly arranged with foggy appearance.

Fig. 11-15- 0.5 mM H2O2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen - Cells compactly arranged without intensely stained nuclei.

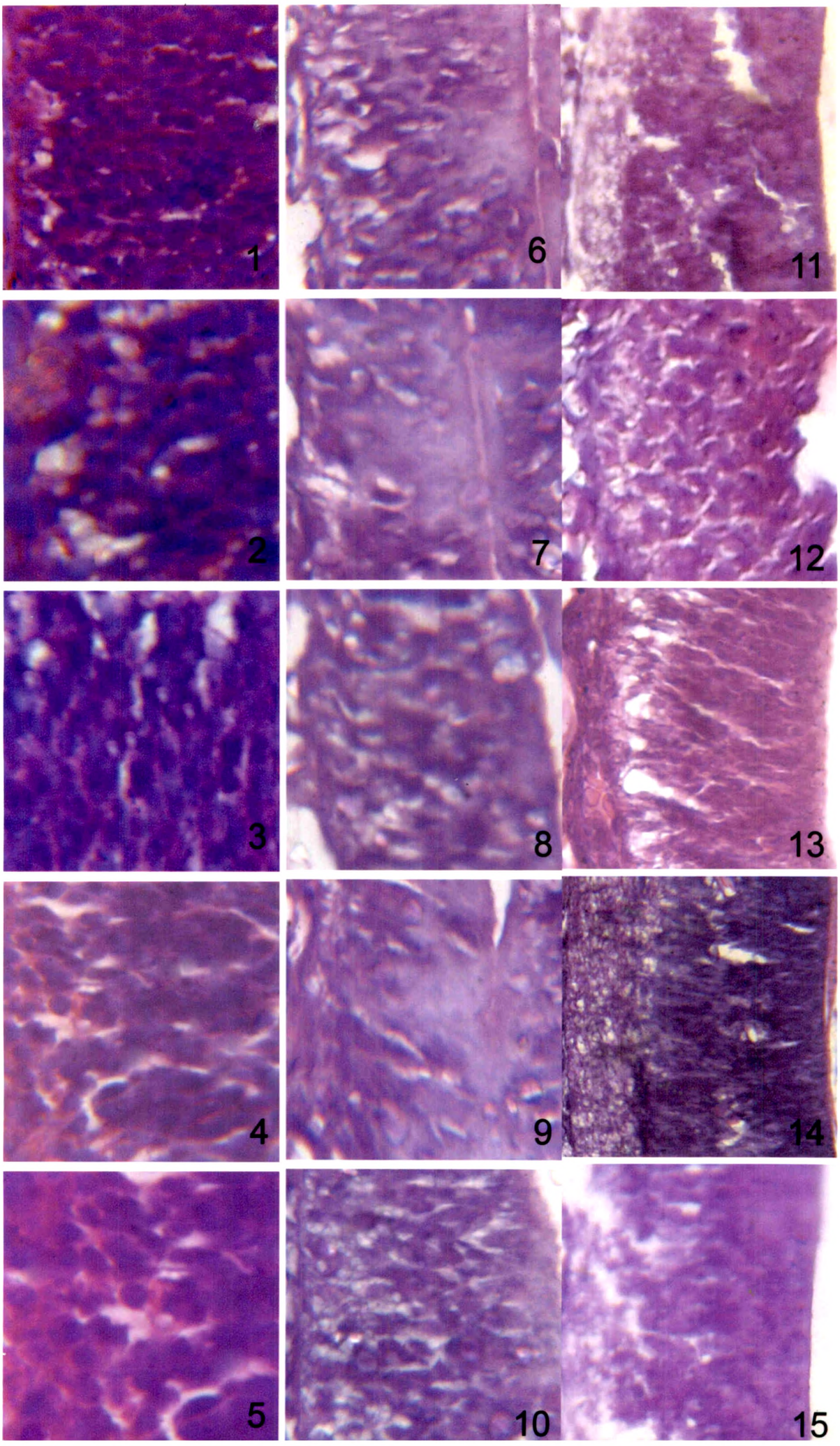
Fig. 12 - Meten. - Cells compactly arranged not well marked cell boundaries.

Fig. 13 - Myelen - Cells compactly arranged without cell boundaries.

Fig. 14 - Dien - Cells compactly arranged without well stained cells.

Fig. 15 - Telen. - Cells compactly arranged without intercellular spaces. No well marked cell boundaries.

PLATE I



processes were clearly visible. Cells were compactly arranged in the mantle layer. The marginal layer showed complex network of outgrowths, which were eosinophilic.

106 hrs development-

Enlargement of all the brain regions continued 96 hrs onward.

112 hrs development-

Histological architecture similar to that was observed at 96 hrs and continued to grow through 106 hrs.

120 hrs development-

Neuroepithelial layer was more conspicuous. Mesencephalon showed more thickening to lead enlargement of tissue. At this hour of development three layers of neuroepithelial cells noted. Mesencephalon was with prominent cell bodies and enlarged cell outgrowths. Ependymal layer reduced in thickness due to migration of cells outwards to thicken the mantle layer. Cells with basophilic nuclei and unipolar, bipolar and multipolar outgrowths were moving outward from the ependymal layer. The radial migration was prominent. Outgrowth of metencephalon, myelencephlon, diencephalons and telencephalon cells was distinct. The marginal layer was with more complex network of outgrowths and gave thick layer; cell bodies were not present in this layer.

130 hrs development-

Histological picture was observed at 120 hrs showed further growth in all the features.

136 hrs development-

Histological elements showed advanced growth features of the development.

144 hrs development-

The growth of various brain ventricle regions showed advancement in growth and cell migration in different layers.

Control HBSS

Histological architecture observed at 24, 64, 82, 88, 106, 112, 120, 130, 136, 144 hrs of development did not show any difference from the histological features observed in normal embryos of respective hours.

Control 3mg vitamin C

All the histological features studied at different developmental hrs mentioned under control HBSS were similar in features observed in normal developing embryos at respective hours and HBSS control embryo. Therefore results are not presented.

0.5 mM H_2O_2 treated

Initiation at 24hrs of development

i) Initial incubation (24hrs) + Dose exposure incubation (24hrs) = Final development (48hrs) -

All the regions of brain viz. mesencephalon, metencephalon, myelencephalon, diencephalons and telencephalon showed similar type of alterations. The cells at this hour were compactly arranged, with eosinophilic oblong nuclei. Some of the cells of ependymal and mantle layer showed foggy appearance. These cells did not show distinct nuclear or cytoplasmic staining. Number of cells with foggy appearance were more in myelencephalon, diencephalons and telencephalon as compared to mesencephalon and metencephalon.

ii) Initial incubation (24 hrs) + Dose exposure incubation (48hrs) = Final development (72 hrs) -

Embryos showed advanced stages of foggy cells. The cells were compactly arranged with increased thickness. Three layers of neuroepithelial cells were distinct. The neuroepithelial cells were foggy in large number at luminal and mantle region. Intensely stained eosinophilic nuclei were observed.

iii) Initial incubation (24hrs) + Dose exposure incubation (72hrs) = Final developmental (96 hrs) -

All the five brain regions viz. mesencephalon, metencephalon, myelencephalon, diencephalon and telencephalon showed similar histological alterations. Increased numbers of necrotic cells were observed than the earlier treatment interval. Reduction in thickness of neuroepithelial cells was noted. Foggy cells stained with haematoxylin and eosin were without the well demarked boundaries in all the regions. There number was high.

Initial incubation (24 hrs) + Dose exposure incubation (96hrs) = Final development (120 hrs) -

Mesencephalon: The neuroepithelial cells appeared swollen with foggy eosinophilic cytoplasm and intensely stained nuclei. Their entity as a cell was not marked. They were observed as clumps of the cells, the intracellular spaces were with foggy opaque appearance. Patches of foggy cells with intensely stained nuclei were intermittently evident. In the few cells axonal outgrowths were observed but they were scanty.

PLATE II

PLATE II- Initial incubation (24 hrs) + Dose exposure incubation (96 hrs) = Final development (120 hrs).

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen. - Neuroepithelial layer showed three zones ependymal, mantle and marginal. Cells migrating, intense nuclei, well formed axonal network.

Fig. 2 - Meten. – Intensely stained cells with well formed axonal network.

Fig. 3 - Myelen - Intensely stained cells with well formed axonal network.

Fig. 4 - Dien - Intensely stained cells with well formed axonal network.

Fig. 5 - Telen. - Intensely stained cells with well formed axonal network.

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000.

Fig. 6 - Mesen. - Three zones, foggy, aggregated cells. Migration not proper.

Fig. 7 - Meten, – Foggy cells with aggregations increased intercellular spaces

Fig. 8 - Myelen – Foggy cells with eosinophilic nuclei, cells aggregated.

Fig. 9 - Dien – Foggy patches of cells, nuclei basophilic with empty spaces.

Fig. 10 - Telen- Cells aggregated, foggy with patchy nuclei.

Fig. 11-15- 0.5 mM H2O2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen - Three zones, intensely stained nuclei, axonal network, migration normal.

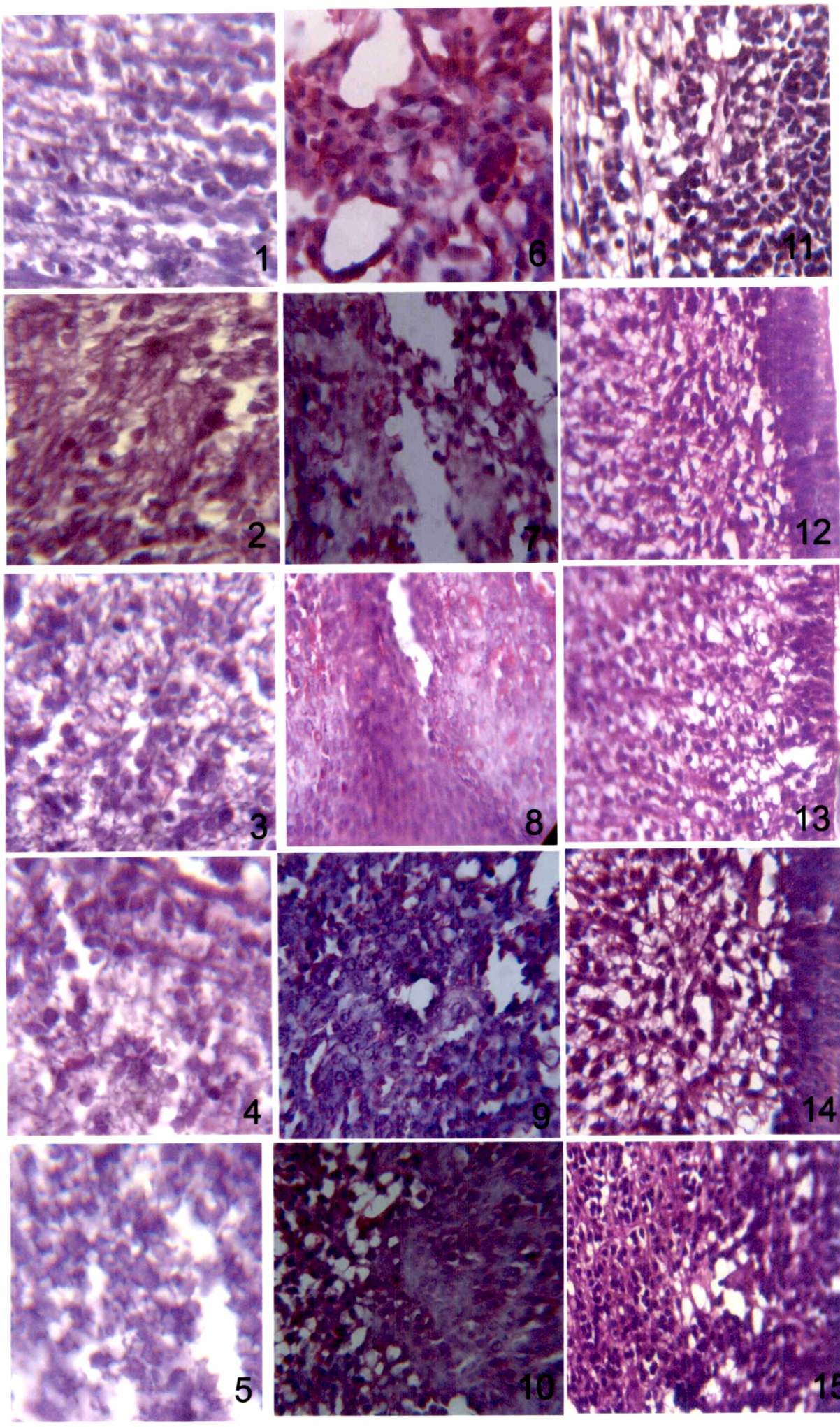
Fig. 12 - Meten. - Ependymal zone with decreased cells, nuclei intensely stained migration normal.

Fig. 13 - Myelen - Cells with intense staining, axonal networking, migration normal.

Fig. 14 - Dien – Cells migrating with well stained nuclei. Axonal network well formed.

Fig. 15 - Telen. - Ependymal zone with decreased cells, nuclei intensely stained migration normal

PLATE II



Metencephalon: The cells of metencephalon showed similar histopathology. The clumped cells were abundant in this region. Foggy appearance of cells shadowed the nuclear staining and appeared faint. The large empty areas were predominant possibly through swelling or clumping of the dead cells making the spaces. Many cells showed neuronal outgrowth but they were not organized as in normal or control (HBSS and 3mg vitamin C treated) brain.

Myelencephalon: This region showed crowding of the cells as compared to mesencephalon and metencephalon. Intercellular spaces were present but were restricted to luminal side of the ventricle. Cloudy opaque appearance was a common feature of the region. Cloudy, foggy appearance had shadowed the appearance of karyolytic empty nuclei were common, and they were eosinophilic. Eosinophilic patches observed surrounding or in the vicisinity of cells, may be clumping of neuronal network. There were regions where cells appeared devoid of nuclei.

Diencephalon: Cells were with intensely stained nuclei and foggy, cloudy basophilic cytoplasm. The intracellular spaces appeared evenly distributed. Eosinophilic clumped areas with marginized appearance in certain regions, appear to represent neuroaxonal outgrowths in pathological condition of clumping.

Telencephalon: In mantle layer cells were clumped and showed intensely basophilic nuclei with shadowed foggy, opaque faintly basophilic cytoplasm. Very few cells were distinctly identified. The region was with less number of empty spaces.

In contrast the marginal zone was crowded with the spaces; which were transversed by the eosinophilic neuroaxonal extensions. The cells were identified with intensely stained nuclei and basophilic cytoplasm. These cells were distributed in aggregates. Majority of these cells showed normal histological appearance of neuronal cells but in same regions foggy areas were identified.

Initiation at 34hrs of development

v) Initial incubation (34hrs) + dose exposure incubation (24 hrs) = Final development (58 hrs) –

The histopathological alterations in brain were similar to 48 hrs (subgroup i) of exposure given embryos. No significant difference was noted.

vi) Initial incubation (34hrs) + Dose exposure incubation (48hrs) = Final development (82hrs) –

The results were similar to the histopatholgical observations noted in brains of embryos subjected to exposure of 48 hrs for 24 hrs initiation subgroup (subgroup ii).

vii) Initial incubation (34hrs) + Dose exposure incubation (72hrs) = Final development (106 hrs) –

The histopathological results were similar to the alterations noted in 96hrs of exposure (subgroup iii) given embryos..

viii) Initial incubation (34 hrs) + Dose exposure incubation (96 hrs) = Final development (130 hrs) –

The histopathology was similar to that was observed in (24+96=120hrs-subgroup iv) embryos. No significant difference in observations was noted.

Initiation at 40hrs of development

ix) Initial incubation (40hrs) + Dose exposure incubation (24hrs) = Final development (64 hrs) –

Thickened neuroepithelial layer was noted. Marginal layer was distinct with axonal network appearance of which was stunted. Necrotic cells were observed in mantle and ependymal regions. Large number of cells were not distinguished by their boundaries but by their nuclei. Foggy patchy eosinophilic cytoplasm was observed.

x) Initial incubation (40hrs) + Dose exposure incubation (48hrs) = Final development (88 hrs) –

Histopathological alterations were similar to those were noted in embryos (subgroup iii) at 98 hrs on treatment.

xi) Initial incubation (40hrs) + Dose exposure incubation (72hrs) = Final development (112 hrs) –

Mesencephalon: The histological changes showed clumping and aggregation of mesencephalon cells. Increased intracellular spaces were noted probably due to removal of cells by cell death or due to aggregation of cells. Balloon like cells with intensely stained nuclei were noted. The nuclei of neuronal cells showed more intensely stained nuclei than the cytoplasm. Reduced network of the axonal outgrowths.

Metencephalon : Migratory path observed of neuronal cells in normal/controls (HBSS and 3 mg vitamin C treated) was obliterated. Metencephalic region of the brain also showed the necrotic cells. Cell aggregations were increased, the intracellular spaces were also increased. Intensely stained nuclei were with unstained cytoplasm. Neuronal cells were aggregated with reduced axonal outgrowths.

PLATE III

PLATE III- Initial incubation (48 hrs) + Dose exposure incubation (24 hrs) = Final development (72 hrs).

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen. – Appearance of marginal zone with axonal network. Compactly arranged cells without cell surfaces, intensely stained basophilic nuclei.

Fig. 2 - Meten. - Cell compactly arranged without intercellular spaces, basophilic nuclei.

Fig. 3 - Myelen - Cells compactly arranged without intercellular spaces intensely stained basophilic nuclei.

Fig. 4 - Dien - Compactly arranged cells without intercellular spaces.

Fig. 5 - Telen. - Compactly arranged cell without intercellular spaces.

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000.

Fig. 6 - Mesen. - Cells compactly arranged with little intercellular spaces. Foggy cells towards the luminal side.

Fig. 7 - Meten. - Cells compactly arranged, foggy cell towards the luminal side.

Fig. 8 - Myelen - Cells compactly arranged, foggy cells towards the luminal side

Fig. 9 - Dien - Cells compactly arranged without intercellular spaces, foggy cell towards the luminal side.

Fig. 10 - Telen- Cells compactly arranged with foggy appearance.

Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen - Cells compactly arranged without intensely stained nuclei.

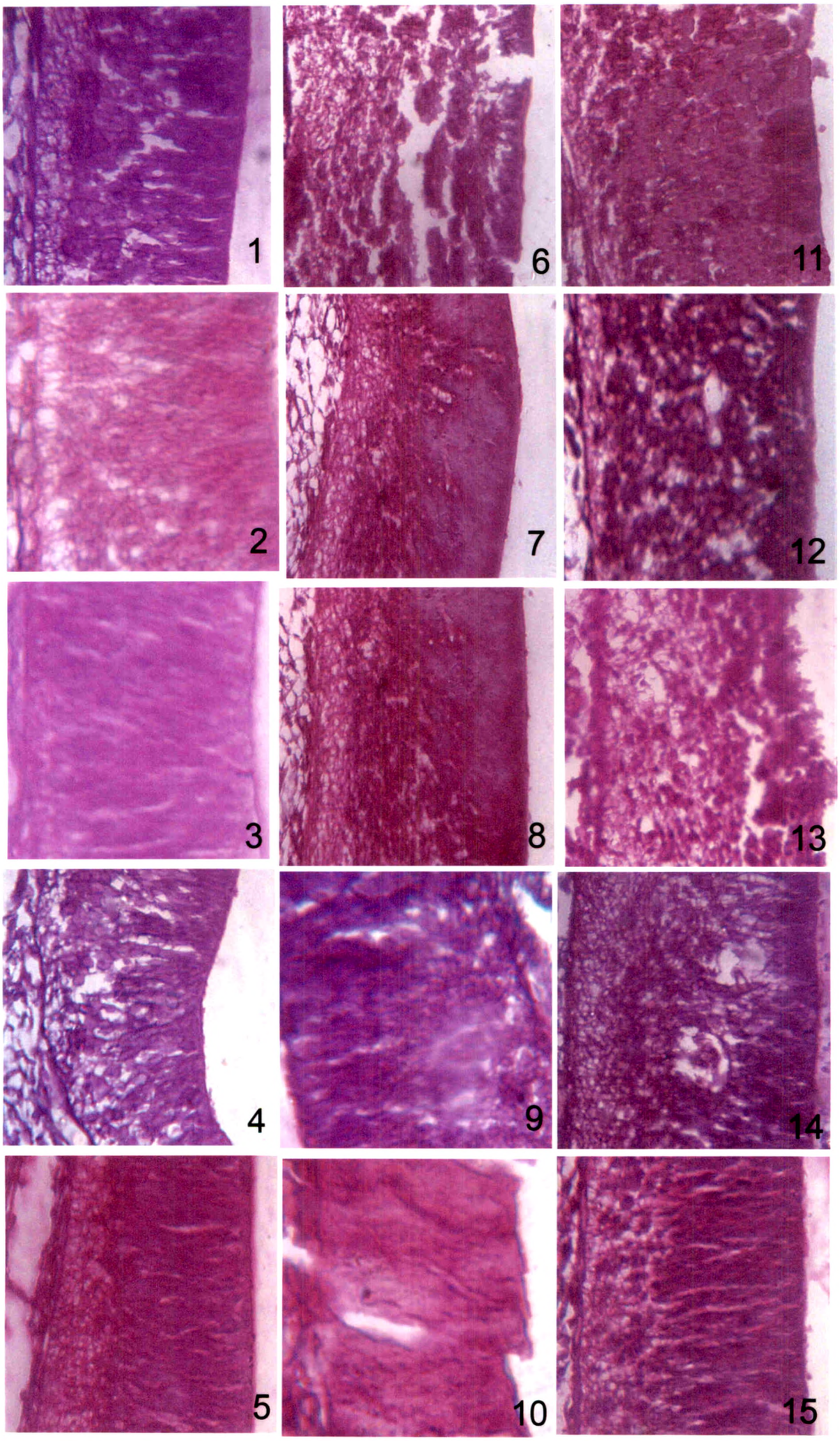
Fig. 12 - Meten. - Cells compactly arranged not well marked cell boundaries.

Fig. 13 - Myelen - Cells compactly arranged without cell boundaries.

Fig. 14 - Dien - Cells compactly arranged without well stained cells.

Fig. 15 - Telen. - Cells compactly arranged without intercellular spaces. No well marked cell boundaries.

PLATE III



Myelencephalon: Cells were with intensely stained nuclei and foggy cytoplasm. Reduced axonal outgrowths.

Diencephalon: Necrotic cells were observed with intensely stained nuclei and foggy cytoplasm. The axonal outgrowths showed reduced length and heavy aggregations. The telencephalon showed increased number of dead cells resulting into increased intracellular spaces. The nuclei of cells were intensely basophilic. Network of the axonal outgrowths was reduced and appeared clumped.

xii) Initial incubation (40 hrs) + Dose exposure incubation (96hrs) = Final development (136hrs) –

Reduced ependymal zone was observed. Mantle and marginal layer was thickened. The neuronal cells with reduced axonal outgrowths were noted. Cells of mantle and ependymal layer were intensely stained (basophilic nuclei, cytoplasm with foggy eosinophilic appearance).

Initiation at 48hrs of development

xiii) Initial incubation (48hrs) + Dose exposure incubation (24hrs) = Final development (72 hrs)-

Histopathological changes showed three layered neuroepithelial layer. Cells were faint basophilic intensely stained foggy cytoplasm shadowed, basophilic nuclei. Increased intracellular space was noted.

xiv) Initial incubation (48hrs) + Dose exposure incubation (48hrs) = Final developmental (96hrs) -

Damaged necrotic cells in the mantle and in ependymal layer were observed. Cells with disturbed cell boundaries were increased. Foggy patchy basophilic cytoplasm was observed, that shadowed the appearance of intensely stained nuclei.

xv) Initial incubation (48hrs) + Dose exposure incubation (72hrs) = Final developmental (120 hrs) –

Histopathological alterations observed in subgroup –ix were similar and are presented in plates.

Initiation at 72hrs of development

xvi) Initial incubation (72 hrs) + Dose exposure incubation (24hrs) = Final development (96 hrs) -

All the brain regions viz: mesencephalon, metencephalon, myelencephalon, diencephalon, and telencephalon showed following alterations. Ependymal cells were foggy with demarking cell boundaries. Intracellular spaces were less. Migratory path can be identified and were observed as in normal brain region. The marginal layer was similar to corresponding zone in normal embryo with normal networking of the axonal outgrowths. Cellular staining was as observed in normal /control embryos. Axonal outgrowth networking was observed as in normal.

xvii) Initial incubation (72 hrs) + Dose exposure incubation (48hrs) = Final development (120 hrs) -

Mesencephalon: Necrotic cells were noted in the mesencephalon. They were aggregated masses. The boundaries were not marked and reduced axonal outgrowth's were observed. Intensely stained nuclei were with foggy basophilic cytoplasm. Shadowing nuclear intensity of staining.

Metencephalon: Necrotic cells were distributed in clumped zones with increased intracellular space. Reduction in axonal outgrowths was conspicuous. Aggregated cells with intensely stained nuclei. Eosinophilic axonal outgrowths were identified. Migratory path could not be marked.

Myelencephalon: Increased necrotic cells were observed. Axonal networking reduced and appeared as aggregated knobs. The nuclei of neuronal cells were intensely stained.

Diencephalons: Foggy cells were observed. They were with intensely stained nuclei and faint basophilic cytoplasmic appearance. Cells were observed with many small outgrowths indicating limited effect on axonal out outgrowths.

Telencephalon: Cells were with foggy nuclei and foggy opaque cytoplasm. Axonal outgrowths appeared shrunken.

PLATE IV

PLATE IV- Initial incubation (48 hrs) + Dose exposure incubation (96 hrs) = Final development (120 hrs).

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen .- Cells intensely stained, axonal network well formed, migrating cells.

Fig. 2 - Meten. – Cells with intensely stained nuclei, axonal outgrowths well formed.

Fig. 3 - Myelen – Cells intensely stained nuclei, axonal outgrowths are well formed

Fig. 4 - Dien – Normal histological cells with migrating cells.

Fig. 5 - Telen. – Basophilic nuclei with well formed axonal outgrowths.

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000.

Fig. 6 - Mesen. – Foggy cells with increased intercellular spaces, migration not normal.

Fig. 7 - Meten. – Cells with foggy appearance, necrotic, stunted axonal outgrowths.

Fig. 8 - Myelen – Foggy cells with patchy nuclei, axonal outgrowths stunted.

Fig. 9 - Dien – necrotic cells with, with foggy appearance, axonal outgrowths not normal.

Fig. 10 - Telen- foggy cells with reduced axonal outgrowths.

Fig. 11-15- 0.5 mM H2O2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen – Intensely stained nuclei with proper migrating path.

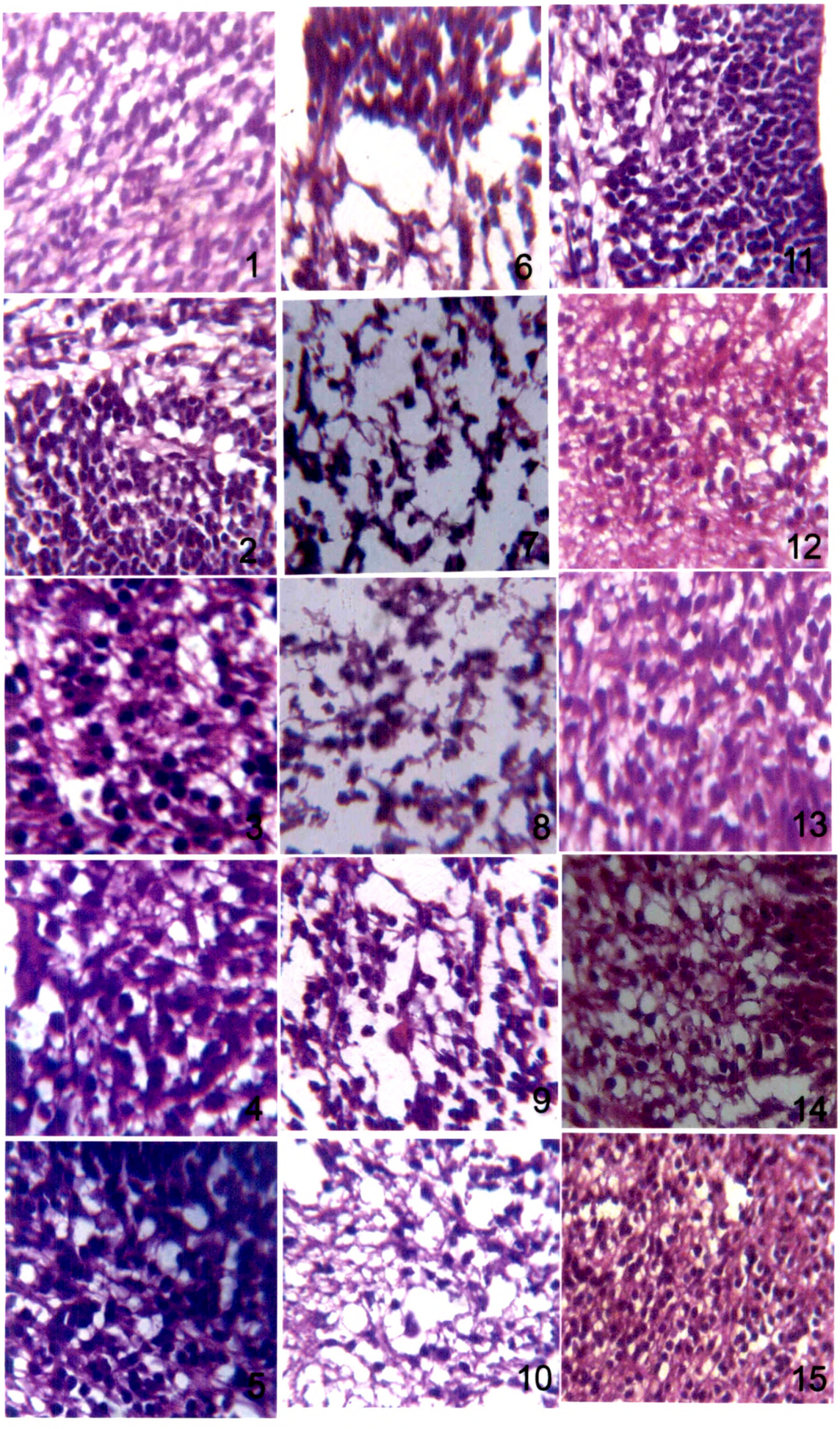
Fig. 12 - Meten. – Cells normal in shape with moving outsidely.

Fig. 13 - Myelen - Cells with intensely stained nuclei, axonal network well formed.

Fig. 14 - Dien – Moving cells with well formed axonal outgrowths.

Fig. 15 - Telen. – Cell normal, with axonal networking.

PLATE IV



Initiation at 96hrs of development

xviii) Initial incubation (96hrs) + Dose exposure incubation (24hrs) = Final development (120 hrs) -

Mesencephalon: Cells with not properly stained nucleus. Some of the cells were with bust nuclei, diffused into the cytoplasm. Basophilic axonal outgrowths. Mantle layer did not show proper migratory cells.

Metencephalon: Less aggregated cells with few intracellular spaces. Many small axonal outgrowths. Disturbed migratory path of the cells. Blood cells in some of the site.

Myelencephalon: Aggregation of cells in the ependymal layer. Necrotic cells in clumps with disturbed migratory path. Intracellular space in-between the clumped cells. Nucleus of the cells were intensely stained.

Diencephalon: Cell cytoplasm foggy and cloudy in appearance. Axonal outgrowths aggregated and reduced in number.

Telencephalon: Ependymal cells with intensely stained nuclei, with shadowed cytoplasm. Cell clumped. Axonal outgrowths reduced. Retarded migration could be sensed. Mantle was nearly normal but cells and nuclei appeared swollen. Intensely basophilic nuclei and thin acidophilic cytoplasm identified the cells. Neuroaxonal networking was evident. But in some regions foggy cells similar to those observed in ependymal regions were noted.

Initiation at 120hrs of development

xix) Initial incubation (120hrs) + Dose exposure incubation (24hrs) = Final development (144 hrs) –

144 hrs of development showed less alterations. Few cells with the foggy appearance. Most of the cellular structures observed with the normal histological structures. Migratory path of the neuroepithelial cells was also observed as in normal, indicating the less free radical damage to the cells induced by the H_2O_2.

0.5mM H_2O_2 + 3mg vitamin C treated

Initiation at 24hrs of development

i) Initial incubation (24hrs) + Dose exposure incubation (24hrs) = Final development (48 hrs) –

All five embryonic regions viz. mesencephalon, metencephalon, myelencephalon, diencephalon and telencephalon, showed similar histological

features. Compactly arranged neuroepithelial cells were loosened, but in some regions they continued to remain clumped. Nuclei improved in their morphology and remained basophilic but that was not completely normal. Few foggy cells still persisted indicated partial protection. They showed intensely stained nucleus with foggy cytoplasm. Few dead cells were observed at this interval brain was not totally protected from free radical damage.

ii) Initial incubation (24hrs) + Dose exposure incubation (48hrs) = Final development (72 hrs) –

Number of necrotic cells were decreased significantly as compared to 0.5mM H_2O_2 treatment. All the brain regions showed normal histological features as observed at the normal developmental hour and similar to that was repeated in control HBSS and vitamin C.

iii) Initial incubation (24hrs) + Dose exposure incubation (72hrs) = Final development (96hrs)-

Normal histological structures with compactly arranged neuroepithelial cells at the ependymal layer of each brain regions were noted. The intracellular spaces were as in normal. Mantle layer showed the basophilic nuclei and weakly stained eosinophilic cytoplasm. Unipolar, bipolar axonal outgrowth was noted in this region. Marginal zones showed the well-formed networking of the axonal outgrowths stained with eosin.

iv) Initial incubation (24hrs) + Dose exposure incubation (96hrs) = Final development (120 hrs) –

All embryonic brain regions viz. mesencephalon, metencephalon, myelencephalon, diencephalon and telencephalon showed compactly arranged neuroepithelial layer. Nuclei intensely stained with strong basophilia. Network of axonal outgrowths was stained with eosin along with cell body cytoplasm the intercellular space between the neuronal cells and axonal outgrowths was as in normal. Migratory path of neuronal cells i.e. radial and lateral was also observed as normal cells, moving outward from the ependymal layer. At the ependymal layer cells were continuously migrating resulting in the thinning of the cell layers. Cells at this layer, were oblong in shape with heavily stained nucleus and less eosinophilic cytoplasm. The marginal layer showed complex network of axonal outgrowths. The thickness of marginal layer was observed as in normal.

PLATE V

PLATE V- Initial incubation (72 hrs) + Dose exposure incubation (24 hrs) = Final development (96 hrs). stained with TB.

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen. – Ependyma, mantle and marginal zones visible. With well stained nuclei, migrating cells in mantle layer.

Fig. 2 - Meten. – Cells moving in the mantle zone with well stained nuclei. .

Fig. 3 - Myelen – Cells with axonal outgrowths.

Fig. 4 - Dien – Cells moving outward from the ependymal zone, intensely stained nuclei.

Fig. 5 - Telen. – Cells well formed axonal outgrowths, migration normal.

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000.

Fig. 6 - Mesen. - Foggy cells towards the ependymal layer and in mantle layer.

Fig. 7 - Meten. – Cells with foggy appearance.

Fig. 8 - Myelen – Necrotic cells with foggy appearance.

Fig. 9 - Dien – Cells without normal axonal outgrowths.

Fig. 10 - Telen- Cell with foggy appearance.

Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen – Few cells with foggy appearance.

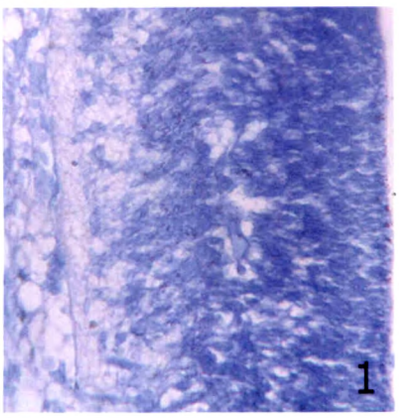
Fig. 12 - Meten. - Foggy less few in number.

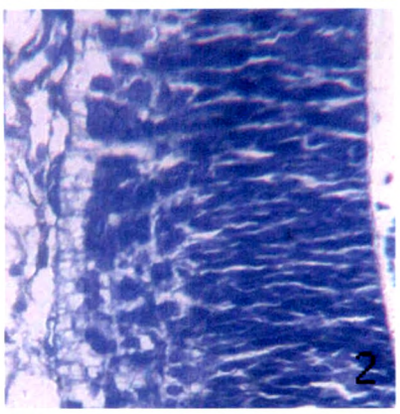
Fig. 13 - Myelen – Cells without well formed axonal outgrowths.

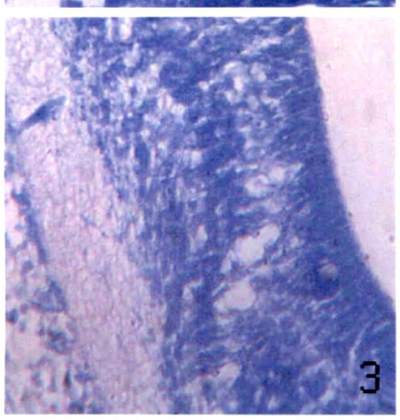
Fig. 14 - Dien - Cells not normal in structures. Few cells with foggy appearrence.

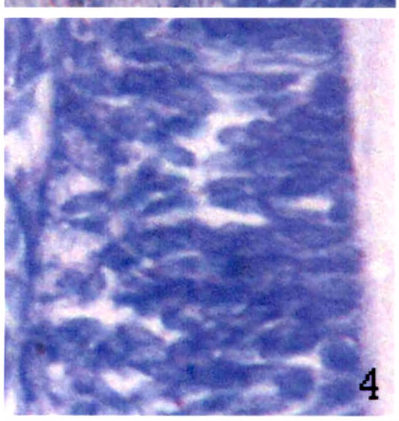
Fig. 15 - Telen. – Few cells with foggy appearance.

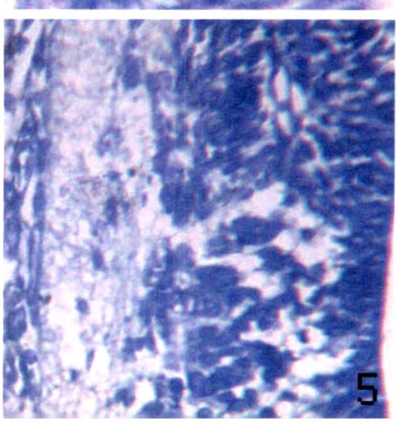
PLATE V

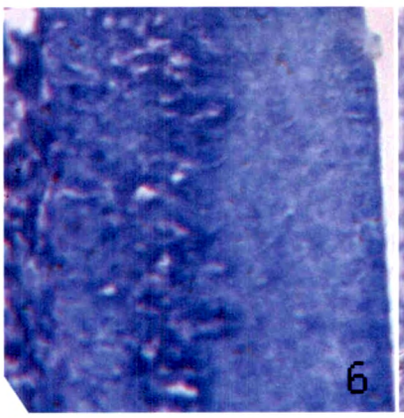


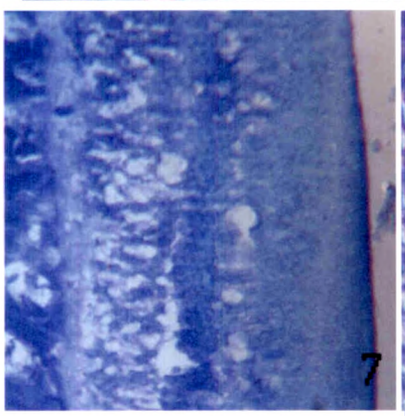


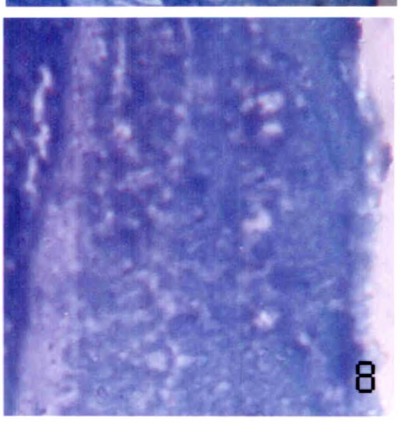


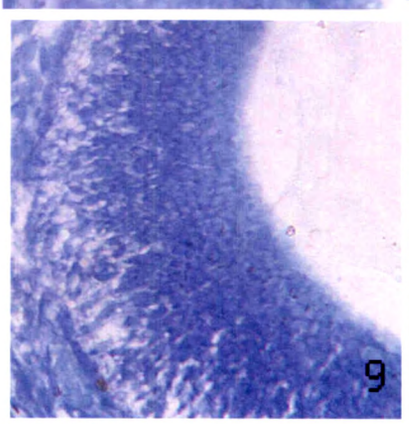


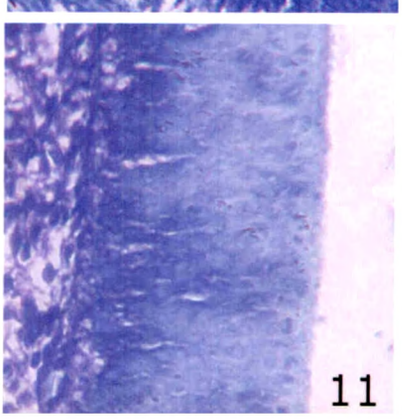


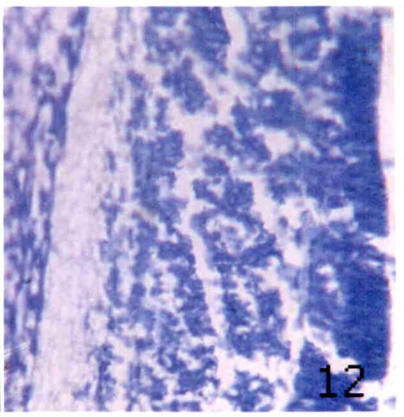


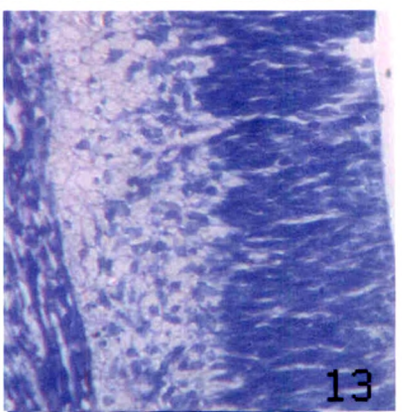


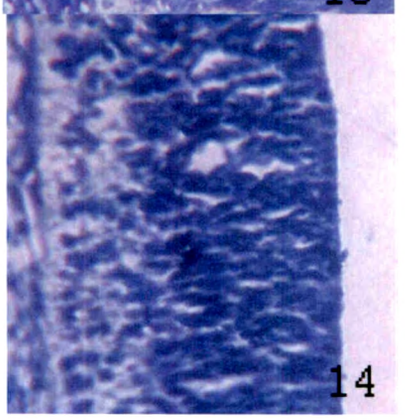


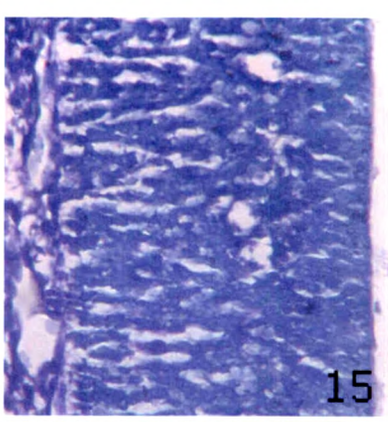


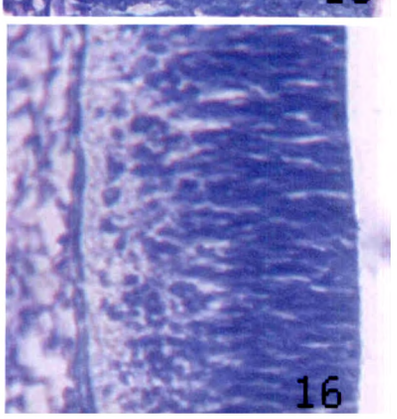












Initiation at 34 hrs of development

v) Initial incubation (34hrs) + Dose exposure incubation (24hrs) = Final development (58 hrs) –

Few of the cells of each brain region viz. mesencephalon, metencephalon, myelencephalon, diencephalon and telencephalon showed foggy cells. Eosinophilic cytoplasm. Few cells were with reduced axonal outgrowths.

vi) Initial incubation (34hrs) + Dose exposure incubation (48hrs) = Final development (82 hrs) –

Histological features observed were similar to the 82hrs normal or HBSS treated control/vitamin C (3 mg) treated control embryos.

viii) Initial incubation (34 hrs) + Dose exposure incubation (72hrs) = Final development (106 hrs) –

All the normal features of the histology were observed in the brain regions studied.

vii) Initial incubation (34hrs) + Dose exposure incubation (96hrs) = Final development (130 hrs) –

Histological alterations were completely protected by vitamin C at this developmental hour of treatment and hence all the features were normal.

Initiation at 40hrs of development

ix) Initial incubation (40hrs) + Dose exposure incubation (24hrs) = Final developmental (64 hrs) –

All brain regions of the treated group of embryos remained normal with scanty foggy cells.

x) Initial incubation (40hrs) + Dose exposure incubation (48hrs) = Final developmental (88 hrs) –

No alterations were not noted at this developmental hour of the treatment. Embryo brain was completely normal.

xi) Initial incubation (40 hrs) + Dose exposure incubation (72hrs) = Final development (112 hrs) –

The histology was similar to the results obtained in the brain of normal embryo of same developmental group.

xii) Initial incubation (40 hrs) + Dose exposure incubation (96hrs) = Final development (136 hrs) –

Histology of brain regions was normal and similar to corresponding normal, HBSS and vitamin C control.

Initiation at 48hrs of development

xiii) Initial incubation (48 hrs) + Dose exposure incubation (24hrs) = Final development (72 hrs) –

At all the brain regions were with normal zonal features and matched their HBSS control and Vitamin C (3 mg) control brain regions and with normal brain of corresponding developmental stage.

xiv) Initial incubation (48 hrs) + Dose exposure incubation (48 hrs) = Final development (96 hrs) –

Neuronal cells of all five brain regions were with well-marked cell boundaries, normally stained nucleus and cytoplasm. Compactly arranged cells at ependymal and mantle layers were noted. Marginal layer was observed with the axonal outgrowths i.e. showing all the normal features.

xv) Initial incubation (48 hrs) + Dose exposure incubation (96hrs) = Final development (120 hrs) –

The histological features were observed similar to that was observed in the normal or untreated group of control embryos at the same developmental stage. Basophilic intensely stained nuclei. Long, eosinophilic axonal outgrowths. No dead, necrotic or foggy cells were noted. Ependymal layer of all five brain regions decreased in thickness because of migrating cells outwardly. Migrating cells of mantle layer was with well stained nuclei and axonal outgrowths. Complex network of axonal outgrowths was observed.

Initiation at 72hrs of development

xvi) Initial incubation (72 hrs) + Dose exposure incubation (24hrs) = Final development (96 hrs) –

At all five brain regions, damaged cellular features with foggy cells at the lumenal side persisted partially. Intracellular spaces were evident in different regions. Cellular boundaries were not distinct in some area i.e. the histology showed persistence of features of histopathology that was observed in 0.5 mM H2O2 treated animals in brain was observed in some zones indicating no protection of brain completely.

PLATE VI

PLATE VI- Initial incubation (72 hrs) + Dose exposure incubation (48 hrs) = Final development (120 hrs).

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen. - Normal cells with well formed axonal outgrowths.

Fig. 2 - Meten. - Normal cells with well formed axonal outgrowths.

Fig. 3 - Myelen - Normal cells with well formed axonal outgrowths.

Fig. 4 - Dien - Normal cells with well formed axonal outgrowths.

Fig. 5 - Telen. - Normal cells with well formed axonal outgrowths.

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000.

Fig. 6 - Mesen. – Cells with necrotic appearance, foggy cells with reduced axonal outgrowths.

Fig. 7 - Meten. – Foggy cells with increased intercellular spaces.

Fig. 8 - Myelen – Foggy necrotic cells with reduced axonal growth, cell without normal

Fig. 9 - Dien – Foggy necrotic cells with stunted axonal outgrowths

Fig. 10 - Telen- Basophilic nuclei, with foggy cytoplasm.

Fig. 11-15- 0.5 mM H2O2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen - Cells compactly arranged without intensely stained nuclei.

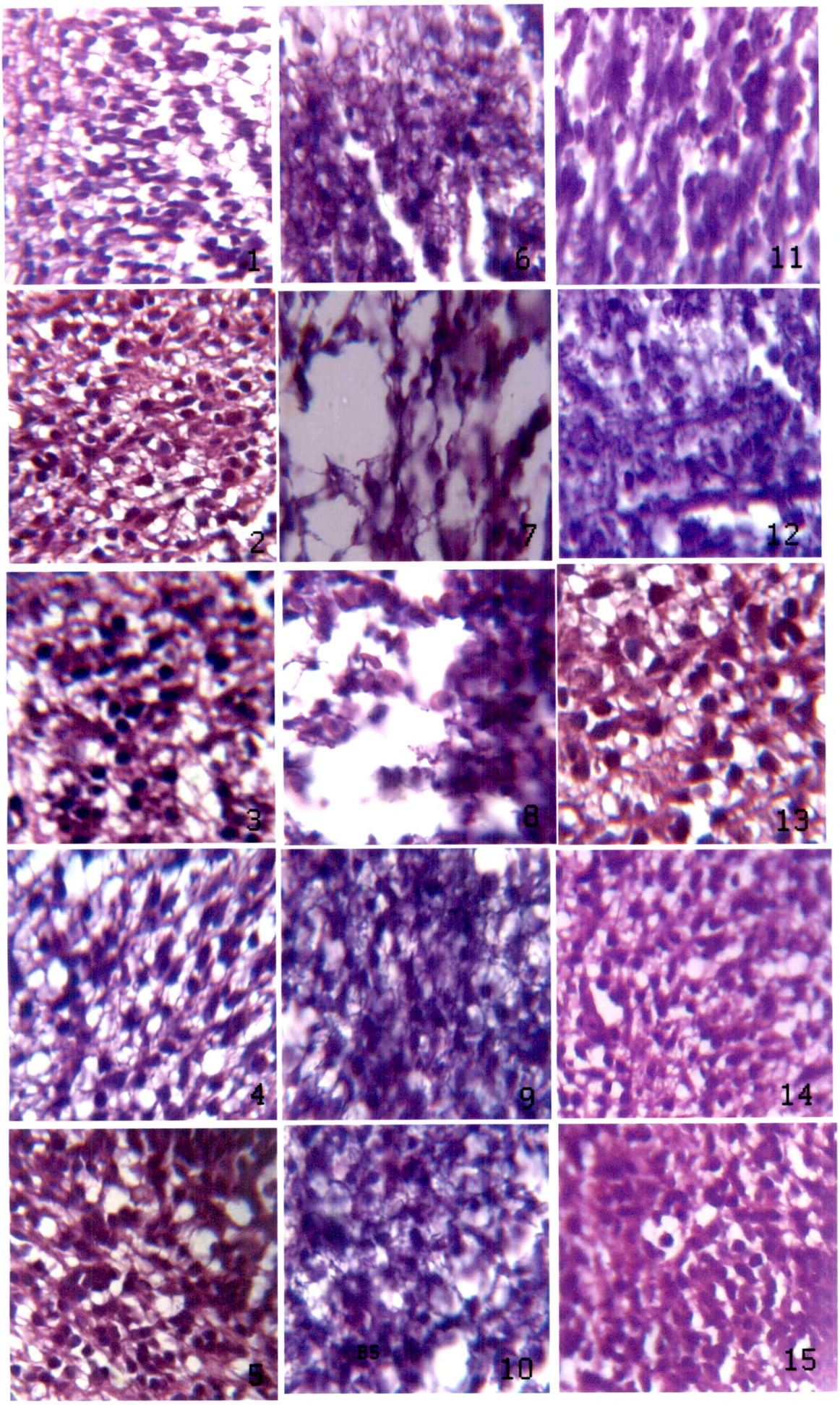
Fig. 12 - Meten. - Cells compactly arranged not well marked cell boundaries.

Fig. 13 - Myelen - Cells compactly arranged without cell boundaries.

Fig. 14 - Dien - Cells compactly arranged without well stained cells.

Fig. 15 - Telen. - Cells compactly arranged without intercellular spaces. No well marked cell boundaries.

PLATE VI



xvii) Initial incubation (72 hrs) + Dose exposure incubation (48hrs) = Final development (120 hrs) –

Cells were with basophilic nuclei, eosinophilic cytoplasm, and axonal outgrowths. Cells were on normal migratory path as in normal embryo brain at corresponding developmental status.

Initiation at 96 hrs of development

xviii) Initial incubation (96 hrs) + Dose exposure incubation (24 hrs) = Final development (120 hrs) –

Histological observations noted were similar to that were noted at the normal developmental 120 hrs. Cells were evident with intensely stained nuclei with eosinophilic cytoplasm and long extended axonal outgrowths.

Initiation at 120 hrs of development

xix) Initial incubation (120 hrs) + Dose exposure incubation (24 hrs) = Final development (144 hrs) –

Embryo brain at 144 hrs of development showed the advanced stage of development as compared to the 120hrs of development. Histological features remained similar to that were observed with normal group of embryos.

The HBSS control and vitamin C (3 mg) control embryos did not alter any of the features or shifted the developmental hours and therefore data in not presented here.

As stated in the experimental protocol in specified developmental hours that were studied overlapping and were used to aim to distinguish the most sensitive developmental stage for H_2O_2 damage and also of recovery by vitamin C (3mg). The histopathological observations in those of the overlapping intervals remained similar because of the similarity of histological elements and it made of development pattern of brain i. e. dividing and proliferating growth in ependymal layer and cellular migration in mantle layer and extensions of neuroaxonal growth networking pattern. To avoid the repetitive similar data histological observations of significance are presented in Plates

But the data is described in observations in detail.

Discussion:

The features of the early development of brain seemed with differentiation of five brain regions and associated growth and development. During development of 24 hrs ---------120 hrs and further growth from 120hrs of development onward.

As mentioned in experimental protocol the observations of overlapping hrs seem common and have the impact on cell migration neuroaxonal network and associated histopathological interactions in different regions of brain described in normal development.

The pathological alterations can be summarized in various regions of brain in following categories.

The ependymal region on 0.5 mM H_2O_2 exposure showed in all the regions as a intact slab of cells which were foggy with opaque faint basophilic material where cellular integrity was not identified.

These observations indicted that the cells tend to remain in clump as they were observed in normal 24 hrs of development and also in control HBSS and vitamin C (3 mg) but the cells were not foggy in normal conditions which appeared on 0.5 mM H_2O_2 exposure (with any of the treatment hrs of 19 groups). These observations indicate that, cellular divisions may have suspended but their migration is inhibited at all hrs of initiation of treatment and at all hrs of treatment intervals and their cell-cell interactive forces seem to be more cohesive in presence of H_2O_2 and hence migration of cells may have inhibited. It is also possible that the molecules involved in cellular migration i.e. membrane surface molecules and extracellular matrix molecules may have been inhibited in playing their role in molecules since. Cellular entities can't be identified and the cellular foggy appearance are indicators of inhibitory actions involving both the molecules in inhibition of migration i.e. receptors cell adhesion molecules and extracellular matrix molecules which participate in radial and non radial migration (Rio *et al.,* 1957; Rakie, 1990; O'Rourke *et al.,* 1995; Golen *et al.,* 1997; Hatten, 1999; Heffron and Golden, 2000). Thus free radicals generated on H_2O_2 exposure from H_2O_2 itself in early hrs of treatment and metabolic products of H_2O_2 generated free radicals of various varieties may be contributing to the stabilization of cells, specifically cell membranes and other above stated participant molecules involved in migration.

Mantle layer seems to be the hub of the migratory activities coupled with cytoplasmic extensions to produce neuroaxonal networking. In normal embryo brain these areas in all the 5 parts of brain and developing hours in range 32 hrs -144 hrs of which the various overlapping time intervals were studied indicated the distinct radial filaments along which migration specifically radial migrations are guided in which after 96hrs, with the cooperation of glial of cells. The axonal and dendritic extensions

PLATE VII

PLATE VIII- Initial incubation (96 hrs) + Dose exposure incubation (24 hrs) = Final development (120 hrs).

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen .- Neuroepithelial layer present. Compactly arranged cells without cell surfaces, intensely stained basophilic nuclei.

Fig. 2 - Meten. - Cell compactly arranged without intercellular spaces, basophilic nuclei.

Fig. 3 - Myelen - Cells compactly arranged without intercellular spaces intensely stained basophilic nuclei.

Fig. 4 - Dien - Compactly arranged cells without intercellular spaces.

Fig. 5 - Telen. - Compactly arranged cell without intercellular spaces.

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000.

Fig. 6 - Mesen. - Cells compactly arranged with little intercellular spaces. Foggy cells towards the luminal side.

Fig. 7 - Meten. - Cells compactly arranged, foggy cell towards the luminal side.

Fig. 8 - Myelen - Cells compactly arranged, foggy cells towards the luminal side

Fig. 9 - Dien - Cells compactly arranged without intercellular spaces, foggy cell towards the luminal side.

Fig. 10 - Telen- Cells compactly arranged with foggy appearance.

Fig. 11-15- 0.5 mM H2O2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen - Cells compactly arranged without intensely stained nuclei.

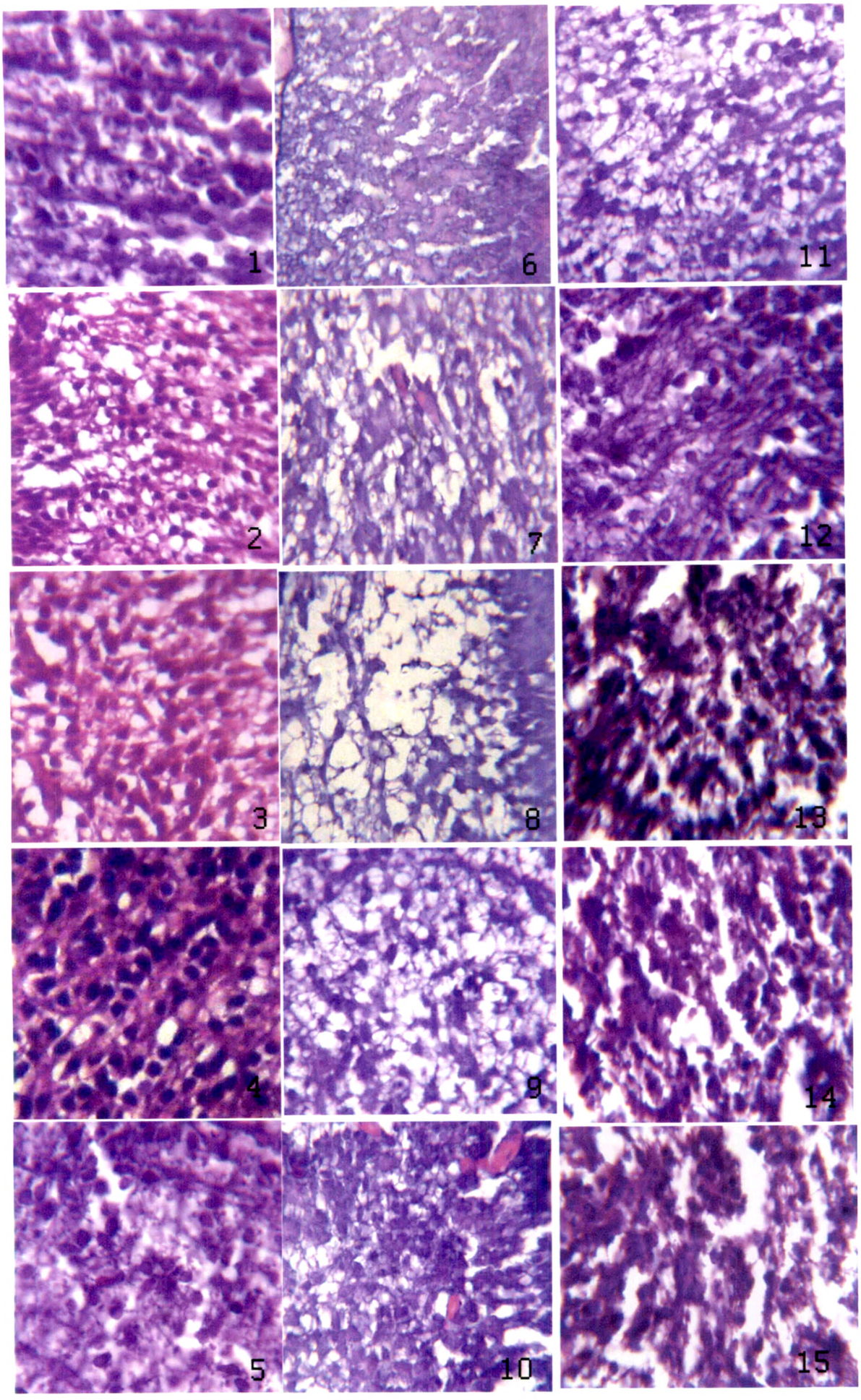
Fig. 12 - Meten. - Cells compactly arranged not well marked cell boundaries.

Fig. 13 - Myelen - Cells compactly arranged without cell boundaries.

Fig. 14 - Dien - Cells compactly arranged without well stained cells.

Fig. 15 - Telen. - Cells compactly arranged without intercellular spaces. No well marked cell boundaries.

PLATE VII



from cell bodies traversed the distances between the cell bodies and their anathematization filled the paces between the same non radial migratory routes were also observed in the diencephalon region specifically.

Exposure of 0.5 mM H_2O_2 initiating at different hours and concluding at different hrs indicated a pattern of histopathological alterations in which the mantle zone was filled with clumped cell bodies where the neuroaxonal extensions could not be identified. The clumps showed two types of regions in one large sheets of cells where cellular identity was lost and slab was identified as faint basophilic opaque material in which sometimes this material showed nuclei (with faint basophilia) could be observed with difficulties. But in other zone the cell clumps were not slab like but aggregates like where intensely stained nuclei could be made out but membranes could not be made out. In some of these parts partially extended neuroaxonal, dendridal outreaching trunks of cytoplasm could be identified.

The spaces in which cell bodies and neuroaxonal outstretches were crowed in normal and both the controls' embryo brain regions remained empty in 0.5 mM H_2O_2 exposed embryos because of aggregation of cell bodies and stunting of axons and other cytoplasmic extensions. Thus large empty spaces could be visible in the mantle area depending on the interval times and interval initiations.

Accumulation of foggy material in early and prolong intervals may be immediate effects of free radicals generated by 0.5mM H_2O_2 introduced and/or these free radicals associated products of free radical metabolisms. The material accumulated/altered was opaque and basophilic. In some cases the nuclei seemed trapped but were stained through faintly i. e. the stain entered the material and the material was also stained faint basophilic.

As the consequence of H_2O_2 the membranes of the cells seemed to be stabilized in which the cytoplasmic extensions which are guided by heterogeneous neurofascin in chick (Vokmer *et al.,* 1992) in normal conditions seems to have failed in their extending activities. This may be true in case of other molecules which keep cell bodies isolated or in guided migration e.g. Neuregulin and erbB-receptors which are known to be involved in neuronal migration (Rio *et al.,* 1997).

Marginal zone being dominated by neuroaxonal networks. Only broken fibers seems to suspend in empty large spaces. These alterations confirm the earlier effect of H_2O_2 exposure i.e. to inhibit migration of cells, stabilization of cell membranes leading to clumping of cells, loss of intercellular spaces in ependymal regions. But

exposed large spaces in mantle and marginal zone due to broken axons, failure of axon extension due to inhibition of migration on either of the routes, radial/non radial and either of migrations i.e. guided independently or guided by glial cells.

Simultaneous treatment of vitamin C–3 mg protected all the above alterations in all the three zones of five regions of brain when simultaneously introduced with H_2O_2. But this concentration (3 mg) of vitamin C is very critical since the higher doses lead to mortality and also the abnormalities.

Thus free radicals like O* and OH* which are naturally generated also needed to be removed immediately and shouldn't be accumulated at any stage of the brain development which may lead to brain abnormalities.

Vitamin C seems to play very limited role and that also concentration specific and therefore there is a need of free radical scavenger which will be useful in broad range for protection of brain in early stage of development which may have chance of exposure to free radicals because the development of brain is in itself is critical for development of healthy organism.

The trend of alterations described above increased with the prolong exposure. More the exposure acute the pathological alterations. Dose initiated at 24 hrs and observed after 24 hrs. showed 50% protection at 24 hrs of exposure. In other treatment interval histopathological alterations were protected by 3 mg vitamin C. The protection potency was directly proportional to exposure time. Treatment of 0.5 mM H_2O_2 treated at 120 hrs and observed after 24hrs minimum alterations, may be advanced stage embryo resisted the free radicals and H_2O_2 mediated pathological alterations.

Section III Glycosaminoglycans (GAGs):

A) Neutral glycosaminoglycans (NGAGs):

Observations:

Alterations in NGAGs are depicted in Plate no I- VII and Table no I a-Id

The observations narrated under neutral glycosaminoglycans are based on the inferences studied under different staining methods given in Chapter II (Table 3) and observations as per inferences semiqualitated on visual observations in Table I in present chapter. Similarly, the regions of brain were five and the histological zones were ependymal, mantle and marginal layers. The cell bodies were more in

Neutral GAGs.

Table no. I.a Initial incubation (24 hrs) + exposure incubation (24 hrs) =Final incubation (48 hrs).

Staining technique	Brain Region	Normal				0.5 mM H_2O_2 treated			
		Neuroepithelial layer				Neuroepithelial layer			
		Cyt	Cs	Ics	Inference to observations	Cyt	Cs	Ics	Inference to observations
1)PAS	Mesen	±	+	+	Trace reaction at Cs, Cyt and Ics	+++	+++	+++	Intense reaction at all 4 regions
	Meten	±	+	+		+++	+++	+++	
	Myelan	±	+	+		+++	+++	+++	
	Dien	±	+	+		+++	+++	+++	
	Telen	±	+	+		+++	+++	+++	
2)Amy-PAS	Mesen	±	+	+	No histochemically detectable glycogen at any of the sites	+++	+++	+++	No histochemically detectable glycogen at any of the sites
	Meten	±	+	+		+++	+++	+++	
	Myelan	±	+	+		+++	+++	+++	
	Dien	±	+	+		+++	+++	+++	
	Telen	±	+	+		+++	+++	+++	
3)Dia-PAS	Mesen	±	+	+	No histochemically detectable glycogen at any of the sites	+++	+++	+++	No histochemically detectable glycogen at any of the sites
	Meten	±	+	+		+++	+++	+++	
	Myelan	±	+	+		+++	+++	+++	
	Dien	±	+	+		+++	+++	+++	
	Telen	±	+	+		+++	+++	+++	
4)Phe-hyd-PAS	Mesen	++	++	++	Blocked regained with equal intensity at all sites	+++	+++	+++	Blocked regained with equal intensity at all sites
	Meten	++	++	++		+++	+++	+++	
	Myelan	++	++	++		+++	+++	+++	
	Dien	++	++	++		+++	+++	+++	
	Telen	++	++	++		+++	+++	+++	

Table no. I. a continued (Initial incubation (24 hrs) + Dose exposure incubation (24 hrs) =Final incubation (48 hrs).

E	Brain zones	Normal				0.5 mM H2O2			
		Neuroepithelial layer				Neuroepithelial layer			
		Cyt	Cs	Ics	Inference to observations	Cyt	Cs	Ics	Inference to observations
5) Sap- PAS	Mesen	++	++	++	Regained with equal intensity in all sites	+++	+++	+++	Regained with equal intensity in all sites
	Meten	++	++	++		+++	+++	+++	
	Myelan	++	++	++		+++	+++	+++	
	Dien	++	++	++		+++	+++	+++	
	Telen	++	++	++		+++	+++	+++	
6) Ace-PAS	Mesen	++	++	++	Blocked periodate reactive groups at all sites and all regions	+++	+++	+++	Blocked periodate reactive groups at all sites and all regions
	Meten	++	++	++		+++	+++	+++	
	Myelan	++	++	++		+++	+++	+++	
	Dien	++	++	++		+++	+++	+++	
	Telen	++	++	++		+++	+++	+++	
7)Acety- Dace-PAS	Mesen	±	±	±	PAS +tivity regained all sites in all regions	+	+	+	PAS +tivity regained all sites in all regions
	Meten	±	±	±		+	+	+	
	Myelan	±	±	±		+	+	+	
	Dien	±	±	±		+	+	+	
	Telen	±	±	±		+	+	+	
8)Phe-Deacy Sap-PAS	Mesen	++	++	++	PAS positivity increases	+++	+++	+++	PAS positivity increases
	Meten	++	++	++		+++	+++	+++	
	Myelan	++	++	++		+++	+++	+++	
	Dien	++	++	++		+++	+++	+++	
	Telen	++	++	++		+++	+++	+++	
9) Sulfer induced metachromacia	Mesen	++	++	++	PAS positivity increases	+++	+++	+++	PAS positivity increases
	Meten	++	++	++		+++	+++	+++	
	Myelan	++	++	++		+++	+++	+++	
	Dien	++	++	++		+++	+++	+++	
	Telen	++	++	++		+++	+++	+++	

Table no. I.a continued (Initial incubation (24 hrs) + Dose exposure incubation (24 hrs) =Final incubation (48 hrs)

Staining technique	Brain zones	0.5 mM H_2O_2+ 3 mg vitamin C treated				Control 3 mg vitamin C treated			
		Neuroepithelial layer				Neuroepithelial layer			
		Cyt	Cs	Ics	Inference to observations	Cyt	Cs	Ics	Inference to oservations
1) PAS	Mesen	++	+++	++	As Compared to normal reaction was intense at PM and no change at all other sites	±	±	±	Reaction intensity and distribution was as in normal
	Meten	++	+++	++		±	±	±	
	Myelan	++	+++	++		±	±	±	
	Dien	++	+++	++		±	±	±	
	Telen	++	+++	++		±	±	±	
2)Amy-PAS	Mesen	++	+++	++	As compared to normal reaction was intense at PM and no change at all other sites	±	±	±	Reaction intensity and distribution was as in normal
	Meten	++	+++	++		±	±	±	
	Myelan	++	+++	++		±	±	±	
	Dien	++	+++	++		±	±	±	
	Telen	++	+++	++		±	±	±	
3)Dia -PAS	Mesen	++	+++	++	As compared to normal reaction was intense at PM and no change at all other sites	±	±	±	Reaction intensity and distribution was as in normal
	Meten	++	+++	++		±	±	±	
	Myelan	++	+++	++		±	±	±	
	Dien	++	+++	++		±	±	±	
	Telen	++	+++	++		±	±	±	
4) Phe hyd PAS	Mesen	++++	++++	++++	As compared to normal reaction was intense at PM and no change at all other sites	++	++	++	Reaction intensity and distribution was as in normal
	Meten	++++	++++	++++		++	++	++	
	Myelan	++++	++++	++++		++	++	++	
	Dien	++++	++++	++++		++	++	++	
	Telen	++++	++++	++++		++	++	++	

Table no. I.a continued (Initial incubation (24 hrs) + Dose exposure incubation (24 hrs) =Final incubation (48 hrs)

Staining technique	Brain zones	0.5 mM H_2O_2+ 3 mg vitamin C treated				Control 3 mg vitamin C treated			
		Neuroepithelial layer				Neuroepithelial layer			
		Cyt	Cs	Ics	Inference to observations	Cyt	Cs	Ics	Inference to observations
5)Sap-PAS	Mesen	++++	++++	++++	As compared to normal reaction was intense at PM and no change at all other sites	++	++	++	Reaction intensity and distribution was as in normal
	Meten	++++	++++	++++		++	++	++	
	Myelan	++++	++++	++++		++	++	++	
	Dien	++++	++++	++++		++	++	++	
	Telen	++++	++++	++++		++	++	++	
6)Ace-PAS	Mesen	+++	+++	+++	Blocked regained with equal intensity at all sites	++	++	++	Reaction intensity and distribution was as in normal
	Meten	+++	+++	+++		++	++	++	
	Myelan	+++	+++	+++		++	++	++	
	Dien	+++	+++	+++		++	++	++	
	Telen	+++	+++	+++		++	++	++	
7) Acety-Dace-PAS	Mesen	+++	+++	+++	Regained with equal intensity in all sites	±	±	±	Reaction intensity and distribution was as in normal
	Meten	+++	+++	+++		±	±	±	
	Myelan	+++	+++	+++		±	±	±	
	Dien	+++	+++	+++		±	±	±	
	Telen	+++	+++	+++		±	±	±	
8)Phe Daceat	Mesen	+++	+++	+++	PAS positivity increases	++	++	++	PAS positivity increases
	Meten	+++	+++	+++		++	++	++	
	Myelan	+++	+++	+++		++	++	++	
	Dien	+++	+++	+++		++	++	++	
	Telen	+++	+++	+++		++	++	++	
9) Induced sulfation Metachro macia	Mesen	+++	+++	+++	PAS positivity increases	++	++	++	PAS positivity increases
	Meten	+++	+++	+++		++	++	++	
	Myelan	+++	+++	+++		++	++	++	
	Dien	+++	+++	+++		++	++	++	
	Telen	+++	+++	+++		++	++	++	

Table no. I. b Initial incubation (24 hrs) + exposure incubation (48 hrs) =Final incubation (72 hrs).

Staining Tech.	Brain region	Normal								0.5 mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)PAS	Mes	±	±	±	±	±	±	±	Trace reaction at all brain regions.	+	+++	++	+	+++	++	+++	Weak reac. at cyto, Moderat Reac.at Ics,Intesnse rea at PM, ML
	Met	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
	Mye	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
	Die	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
	Tel	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
2)Amy Dig PAS	Mes	±	±	±	±	±	±	±	No histo-Chemically detectable glycogen at any of the sites	+	+++	++	+	+++	++	+++	No histo-Chemically detectable glycogn at any of the sites
	Met	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
	Mye	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
	Die	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
	Tel	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
3)Dias digPAS	Mes	±	±	±	±	±	±	±	No histo-Chemically detectable glycogen at any of the sites	+	+++	++	+	+++	++	+++	No histo-chemically detectable glycogn at any of the sites
	Met	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
	Mye	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
	Die	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
	Tel	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
4) Amy dig.-Sap-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity in all sites	+++	+++	+++	+++	+++	+++	+++	Regained with equal intensity in all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no. I. b continued Initial incubation (24 hrs) + exposure incubation (48 hrs) =Final incubation (72 hrs).

5)Phe hyd-PAS	Mes	++	++	++	++	++	++	++	Increased color intensity	+++	+++	+++	+++	+++	+++	+++	Increased color intensity
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
6) Phe-Deace PAS	Mes	++	++	++	++	++	++	++	Regained color reaction	+++	+++	+++	+++	+++	+++	+++	Regained color reaction
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
7) Ace-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity at all sites	+++	+++	+++	+++	+++	+++	+++	Blocked regained with equal intensity at all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
8) Dace-PAS	Mes	±	±	±	±	±	±	±	Blocked Regained with equal intensity in all sites	±	±	±	±	±	±	±	Blocked regained with equal intensity at all sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9) Sulfer induced metachromacia	Mes	++	++	++	++	++	++	++	Increased color intensity	+++	+++	+++	+++	+++	+++	+++	Increased color intensity
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no. I. b continued Initial incubation (24 hrs) + exposure incubation (48 hrs) =Final incubation (72 hrs).

Staining Tech.	Brain zone	0.5 mM H_2O_2 + 3mg vitamin C treated								Control 3mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1) PAS	Mes	++	++	++	++	++	++	++	Trace reac.at all brain regions	±	±	±	±	±	±	±	Weak reac.at cyto, Ics, PM, ML
	Met	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Mye	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Die	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Tel	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
2) Amy Dig PAS	Mes	++	++	++	++	++	++	++	No histo Chemically detectableglycogen at any of the sites	±	±	±	±	±	±	±	No histo chemically detectableglycogen at any of the sites
	Met	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Mye	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Die	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Tel	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
3)Dias dig -PAS	Mes	++	++	++	++	++	++	++	No histo Chemically detectable glycogen at any of thesites	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of hesites
	Met	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Mye	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Die	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Tel	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
4)Amy dig. Sap-PAS	Mes	+++	+++	+++	+++	+++	+++	+++	Regained with equal intensity in all sites	++	++	++	++	++	++	++	Regained with equal intensity in all sites
	Met	+++	+++	+++	+++	+++	+++	+++		++	++	++	++	++	++	++	
	Mye	+++	+++	+++	+++	+++	+++	+++		++	++	++	++	++	++	++	
	Die	+++	+++	+++	+++	+++	+++	+++		++	++	++	++	++	++	++	
	Tel	+++	+++	+++	+++	+++	+++	+++		++	++	++	++	++	++	++	

Table no. I. b continued Initial incubation (24 hrs) + exposure incubation (48 hrs) =Final incubation (72 hrs).

5)Phe. Hya PAS	Mes	++	++	++	++	++	++	++	Increased color intensity	++	++	++	++	++	++	++	Increased color intensity
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
6) Phe-Deace PAS	Mes	++	++	++	++	++	++	++	Regained color reaction	++	++	++	++	++	++	++	Regained color reaction
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tcl	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
7) Ace-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity at all sites	++	++	++	++	++	++	++	Blocked regained with equal intensity at all sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
8) Dace-PAS	Mes	±	±	±	±	±	±	±	Blocked Regained with equal intensity in all sites	±	±	±	±	±	±	±	Blocked regained with equal intensity at all sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9) Sulfer induced metachro macia	Mes	++	++	++	++	++	++	++	Increased color intensity	++	++	++	++	++	++	++	Increased color intensity
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	

Table no. I. c Initial incubation (24 hrs) + exposure incubation (72 hrs) =Final incubation (96 hrs).

Staning techn	Brain zone	Normal								0.5 mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)PAS	Mes	±	±	±	±	±	±	±	Trace reaction at all brain regions	+++	+++	++	++	+++	++	+++	Moderat Reac. at cyto Ics, Intesnse rea at PM, ML
	Met	±	±	±	±	±	±	±		+++	+++	++	++	+++	++	+++	
	Mye	±	±	±	±	±	±	±		+++	+++	++	++	+++	++	+++	
	Die	±	±	±	±	±	±	±		+++	+++	++	++	+++	++	+++	
	Tel	±	±	±	±	±	±	±		+++	+++	++	++	+++	++	+++	
2)Amy Dig PAS	Mes	±	±	±	±	±	±	±	No histo-Chemically detcctable glycogen at any of the sites	+++	+++	++	++	+++	++	+++	No histo-Chemically detectable glycogn at any of the sites
	Met	±	±	±	±	±	±	±		+++	+++	++	++	+++	++	+++	
	Mye	±	±	±	±	±	±	±		+++	+++	++	++	+++	++	+++	
	Die	±	±	±	±	±	±	±		+++	+++	++	++	+++	++	+++	
	Tel	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
3)Dias dig - PAS	Mes	±	±	±	±	±	±	±	No histo-Chemically detectable glycogen at any of the sites	++	+++	++	++	+++	++	+++	No histo-chemically detectable glycogn at any of the sites
	Met	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Mye	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Die	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Tel	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
4)Amy digSap-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity in all sites	+++	+++	+++	+++	+++	+++	+++	Regained with equal intensity in all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no. I .c Initial incubation (24 hrs) + exposure incubation (72 hrs) =Final incubation (96 hrs). continued.....

5)Phe hyd-PAS	Mes	++	++	++	++	++	++	++	Increased color intensity	+++	+++	+++	+++	+++	+++	+++	Increased color intensity
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
6) Phe-Deace PAS	Mes	++	++	++	++	++	++	++	Regained color reaction	+++	+++	+++	+++	+++	+++	+++	Regained color reaction
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
7) Ace-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity at all sites	+++	+++	+++	+++	+++	+++	+++	Blocked regained with equal intensity at all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
8) Dace-PAS	Mes	±	±	±	±	±	±	±	Blocked Regained with equal intensity in all sites	±	±	±	±	±	±	±	Blocked regained with equal intensity at all sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9) sulfer induced metachromacia	Mes	++	++	++	++	++	++	++	Increased intensity of coloration at all sites.	+++	+++	+++	+++	+++	+++	+++	Increased intensity of coloration at all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no. I.c continued Initial incubation (24 hrs) + exposure incubation (72 hrs) =Final incubation (96 hrs).

Staining Tech.	Brain zones	0.5 mM H_2O_2 + 3 mg vitamin C treated								Control 3 mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1) PAS	Mes	±	±	±	±	±	±	±	Trace reac.at all brain regions	±	±	±	±	±	±	±	Weak reac.at cyto, Moderat Reac.at Ics,Intesnse rea at PM, ML
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
2)Amy Dig PAS	Mes	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
3)Dias dig -PAS	Mes	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of thesites	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
4) Amy dig-Sap-PAS	Mes	++	++	++	++	++	++	++	Rcgainccl with equal intensity in all sites	++	++	++	++	++	++	++	Regained with equal intensity in all sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	

Table no. I.c continued Initial incubation (24 hrs) + exposure incubation (72 hrs) =Final incubation (96 hrs).

5)Phe. Hya PAS	Mes	++	++	++	++	++	++	++	Increased color intensity	++	++	++	++	++	++	++	Increased color intensity
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
6) Phe-Deace PAS	Mes	++	++	++	++	++	++	++	Regained color reaction	++	++	++	++	++	++	++	Regained color reaction
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
7) Ace-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity at all sites	++	++	++	++	++	++	++	Blocked regained with equal intensity at all sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
8) Dace-PAS	Mes	±	±	±	±	±	±	±	Blocked Regained with equal intensity in all sites	±	±	±	±	±	±	±	Blocked regained with equal intensity at all sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9) Sulfur induced metachromacia	Mes	++	++	++	++	++	++	++	Increased intensity of coloration at all the sites	++	++	++	++	++	++	++	Increased intensity of coloration at all the sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	

Table no. I. d Initial incubation (24 hrs) + exposure incubation (96 hrs) =Final incubation (120 hrs).

Staning techn	Brain zone	Normal								0.5 mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)PAS	Mes	±	±	±	±	±	±	±	Trace reaction at all brain regions	+++	+++	++	++	+++	++	+++	Moderat Reac. at cyto Ics, Intesnse rea at PM, ML
	Met	±	±	±	±	±	±	±		+++	+++	++	++	+++	++	+++	
	Mye	±	±	±	±	±	±	±		+++	+++	++	++	+++	++	+++	
	Die	±	±	±	±	±	±	±		+++	+++	++	++	+++	++	+++	
	Tel	±	±	±	±	±	±	±		+++	+++	++	++	+++	++	+++	
2)Amy Dig PAS	Mes	±	±	±	±	±	±	±	No histo-Chemically detectable glycogen at any of the sites	+++	+++	++	++	+++	++	+++	No histo-Chemically detectable glycogn at any of the sites
	Met	±	±	±	±	±	±	±		+++	+++	++	++	+++	++	+++	
	Mye	±	±	±	±	±	±	±		+++	+++	++	++	+++	++	+++	
	Die	±	±	±	±	±	±	±		+++	+++	++	++	+++	++	+++	
	Tel	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
3)Dias dig - PAS	Mes	±	±	±	±	±	±	±	No histo-Chemically detectable glycogen at any of the sites	++	+++	++	++	+++	++	+++	No histo-chemically detectable glycogn at any of the sites
	Met	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Mye	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Die	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Tel	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
4)Amy digSap-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity in all sites	+++	+++	+++	+++	+++	+++	+++	Regained with equal intensity in all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no. I .d continued Initial incubation (24 hrs) + exposure incubation (96 hrs) =Final incubation (120 hrs).

5)Phe hyd-PAS	Mes	++	++	++	++	++	++	++	Increased color intensity	+++	+++	+++	+++	+++	+++	+++	Increased color intensity
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
6) Phe-Deace PAS	Mes	++	++	++	++	++	++	++	Regained color reaction	+++	+++	+++	+++	+++	+++	+++	Regained color reaction
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
7) Ace-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity at all sites	+++	+++	+++	+++	+++	+++	+++	Blocked regained with equal intensity at all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
8) Dace-PAS	Mes	±	±	±	±	±	±	±	Blocked Regained with equal intensity in all sites	±	±	±	±	±	±	±	Blocked regained with equal intensity at all sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9) sulfer induced metachromacia	Mes	++	++	++	++	++	++	++	Increased intensity of coloration at all sites.	+++	+++	+++	+++	+++	+++	+++	Increased intensity of coloration at all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no. I..d Initial incubation (24 hrs) + exposure incubation (96 hrs) =Final incubation (120hrs).continued

Staining Tech.	Brain zones	0.5 mM H_2O_2 + 3 mg vitamin C treated								Control 3 mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1) PAS	Mes	±	±	±	±	±	±	±	Trace reac.at all brain regions	±	±	±	±	±	±	±	Weak reac.at cyto, Moderat Reac.at Ics,Intesnse rea at PM, ML
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
2)Amy Dig PAS	Mes	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
3)Dias dig -PAS	Mes	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of thesites	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
4) Amy dig-Sap-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity in all sites	++	++	++	++	++	++	++	Regained with equal intensity in all sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	

Table no. I..d Initial incubation (24 hrs) + exposure incubation (96 hrs) =Final incubation(120 hrs).continued

5)Phe. Hya PAS	Mes	++	++	++	++	++	++	++	Increased color intensity	++	++	++	++	++	++	++	Increased color intensity
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
6) Phe-Deace PAS	Mes	++	++	++	++	++	++	++	Regained color reaction	++	++	++	++	++	++	++	Regained color reaction
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
7) Ace-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity at all sites	++	++	++	++	++	++	++	Blocked regained with equal intensity at all sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
8) Dace-PAS	Mes	±	±	±	±	±	±	±	Blocked Regained with equal intensity in all sites	±	±	±	±	±	±	±	Blocked regained with equal intensity at all sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9) Sulfur induced metachromacia	Mes	++	++	++	++	++	++	++	Increased intensity of coloration at all the sites	++	++	++	++	++	++	++	Increased intensity of coloration at all the sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	

Neutral GAGs.

Table no. IV .a Initial incubation (48 hrs) + exposure incubation (24 hrs) =Final incubation (72 hrs).

Staining technique	Brain Region	Normal				0.5 mM H_2O_2 treated			
		Neuroepithelial layer				Neuroepithelial layer			
		Cyt	Cs	Ics	Inference to observations	Cyt	Cs	Ics	Inference to observations
1)PAS	Mesen	±	+	+	Trace reaction at Cs, Cyt and Ics	+++	+++	+++	Intense reaction at all 4 regions
	Meten	±	+	+		+++	+++	+++	
	Myelan	±	+	+		+++	+++	+++	
	Dicn	±	+	+		+++	+++	+++	
	Telen	±	+	+		+++	+++	+++	
2)Amy-PAS	Mesen	±	+	+	No histochemically detectable glycogen at any of the sites	+++	+++	+++	No histochemically detectable glycogen at any of the sites
	Meten	±	+	+		+++	+++	+++	
	Myelan	±	+	+		+++	+++	+++	
	Dien	±	+	+		+++	+++	+++	
	Telen	±	+	+		+++	+++	+++	
3)Dia-PAS	Mesen	±	+	+	No histochemically detectable glycogen at any of the sites	+++	+++	+++	No histochemically detectable glycogen at any of the sites
	Meten	±	+	+		+++	+++	+++	
	Myelan	±	+	+		+++	+++	+++	
	Dien	±	+	+		+++	+++	+++	
	Telen	±	+	+		+++	+++	+++	
4)Phe-hyd-PAS	Mesen	++	++	++	Blocked regained with equal intensity at all sites	+++	+++	+++	Blocked regained with equal intensity at all sites
	Meten	++	++	++		+++	+++	+++	
	Myelan	++	++	++		+++	+++	+++	
	Dien	++	++	++		+++	+++	+++	
	Telen	++	++	++		+++	+++	+++	

Table no. IV .a Initial incubation (48 hrs) + exposure incubation (24 hrs) =Final incubation (72 hrs).

Staining technique	Brain zones	Normal				0.5 mM H2O2			
		Neuroepithelial layer				Neuroepithelial layer			
		Cyt	Cs	Ics	Inference to observations	Cyt	Cs	Ics	Inference to observations
5) Sap- PAS	Mesen	++	++	++	Regained with equal intensity in all sites	+++	+++	+++	Regained with equal intensity in all sites
	Meten	++	++	++		+++	+++	+++	
	Myelan	++	++	++		+++	+++	+++	
	Dien	++	++	++		+++	+++	+++	
	Telen	++	++	++		+++	+++	+++	
6) Ace-PAS	Mesen	++	++	++	Blocked periodate rcactive groups at all sites and all regions	+++	+++	+++	Blocked periodate reactive groups at all sites and all regions
	Meten	++	++	++		+++	+++	+++	
	Myelan	++	++	++		+++	+++	+++	
	Dien	++	++	++		+++	+++	+++	
	Telen	++	++	++		+++	+++	+++	
7) Dace-PAS	Mesen	±	±	±	PAS +tivity regained all sites in all regions	+	+	+	PAS +tivity regained all sites in all regions
	Meten	±	±	±		+	+	+	
	Myelan	±	±	±		+	+	+	
	Dien	±	±	±		+	+	+	
	Telen	±	±	±		+	+	+	
8)Phe-Deacy Sap-PAS	Mesen	++	++	++	PAS positivity increases	+++	+++	+++	PAS positivity increases
	Meten	++	++	++		+++	+++	+++	
	Myelan	++	++	++		+++	+++	+++	
	Dien	++	++	++		+++	+++	+++	
	Telen	++	++	++		+++	+++	+++	
9) Sulfer induced metachromacia	Mesen	++	++	++	PAS positivity increases	+++	+++	+++	PAS positivity increases
	Meten	++	++	++		+++	+++	+++	
	Myelan	++	++	++		+++	+++	+++	
	Dien	++	++	++		+++	+++	+++	
	Telen	++	++	++		+++	+++	+++	

Table no. IV .a Initial incubation (48 hrs) + exposure incubation (24 hrs) =Final incubation (72 hrs).

Staining technique	Brain zones	0.5 mM H_2O_2+ 3 mg vitamin C treated				Control 3 mg vitamin C treated			
		Neuroepithelial layer				Neuroepithelial layer			
		Cyt	Cs	Ics	Inferencc to observations	Cyt	Cs	Ics	Inference to oservations
1) PAS	Mesen	++	+++	++	As Compared to normal reaction was intense at PM and no change at all other sites	±	±	±	Reaction intensity and distribution was as in normal
	Meten	++	+++	++		±	±	±	
	Myelan	++	+++	++		±	±	±	
	Dien	++	+++	++		±	±	±	
	Telen	++	+++	++		±	±	±	
2)Amy-PAS	Mesen	++	+++	++	As compared to normal reaction was intense at PM and no change at all other sites	±	±	±	Reaction intensity and distribution was as in normal
	Meten	++	+++	++		±	±	±	
	Myelan	++	+++	++		±	±	±	
	Dien	++	+++	++		±	±	±	
	Telen	++	+++	++		±	±	±	
3)Dia -PAS	Mesen	++	+++	++	As compared to normal reaction was intense at PM and no change at all other sites	±	±	±	Reaction intensity and distribution was as in normal
	Meten	++	+++	++		±	±	±	
	Myelan	++	+++	++		±	±	±	
	Dien	++	+++	++		±	±	±	
	Telen	++	+++	++		±	±	±	
4) Phe hyd-PAS	Mesen	++++	++++	++++	As compared to normal reaction was intense at PM and no change at all other sites	++	++	++	Reaction intensity and distribution was as in normal
	Meten	++++	++++	++++		++	++	++	
	Myelan	++++	++++	++++		++	++	++	
	Dien	++++	++++	++++		++	++	++	
	Telen	++++	++++	++++		++	++	++	

Table no. IV .a Initial incubation (48 hrs) + exposure incubation (24 hrs) =Final incubation (72 hrs).

Staining technique	Brain zones	0.5 mM H_2O_2+ 3 mg vitamin C treated				Control 3 mg vitamin C treated			
		Neuroepithelial layer				Neuroepithelial layer			
		Cyt	Cs	Ics	Inference to observations	Cyt	Cs	Ics	Inference to observations
5)Sap-PAS	Mesen	++++	++++	++++	As compared to normal reaction was intense at PM and no change at all other sites	++	++	++	Reaction intensity and distribution was as in normal
	Meten	++++	++++	++++		++	++	++	
	Myelan	++++	++++	++++		++	++	++	
	Dien	++++	++++	++++		++	++	++	
	Telen	++++	++++	++++		++	++	++	
6)Ace-PAS	Mesen	+++	+++	+++	Blocked regained with equal intensity at all sites	++	++	++	Reaction intensity and distribution was as in normal
	Meten	+++	+++	+++		++	++	++	
	Myelan	+++	+++	+++		++	++	++	
	Dien	+++	+++	+++		++	++	++	
	Telen	+++	+++	+++		++	++	++	
7)Dace-PAS	Mesen	+++	+++	+++	Regained with equal intensity in all sites	±	±	±	Reaction intensity and distribution was as in normal
	Meten	+++	+++	+++		±	±	±	
	Myelan	+++	+++	+++		±	±	±	
	Dien	+++	+++	+++		±	±	±	
	Telen	+++	+++	+++		±	±	±	
8)Phe Daceat	Mesen	+++	+++	+++	PAS positivity increases	++	++	++	PAS positivity increases
	Meten	+++	+++	+++		++	++	++	
	Myelan	+++	+++	+++		++	++	++	
	Dien	+++	+++	+++		++	++	++	
	Telen	+++	+++	+++		++	++	++	
9) Induced sulfation Metachromacia	Mesen	+++	+++	+++	PAS positivity increases	++	++	++	PAS positivity increases
	Meten	+++	+++	+++		++	++	++	
	Myelan	+++	+++	+++		++	++	++	
	Dien	+++	+++	+++		++	++	++	
	Telen	+++	+++	+++		++	++	++	

Table no. IV. C Initial incubation (48 hrs) + Dose exposure incubation (48 hrs) = Final incubation (96 hrs).

Staning techn	Brain zone	Normal								0.5 mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)PAS	Mes	±	±	±	±	±	±	±	Trace reaction at all brain regions	++	+++	++	++	+++	++	+++	Moderat Reac. at cyto Ics, Intesnse rea at PM, ML
	Met	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Mye	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Die	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Tel	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
2)Amy Dig PAS	Mes	±	±	±	±	±	±	±	No histo-Chemically detectable glycogen at any of the sites	++	+++	++	++	+++	++	+++	No histo-Chemically detcctable glycogn at any of the sites
	Met	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Mye	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Die	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Tel	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
3)Dias dig - PAS	Mes	±	±	±	±	±	±	±	No histo-Chemically detectable glycogen at any of the sites	++	+++	++	++	+++	++	+++	No histo-chemically detectable glycogn at any of the sites
	Met	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Mye	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Die	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Tel	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
4)Amy digSap-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity in all sites	+++	+++	+++	+++	+++	+++	+++	Regained with equal intensity in all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no. IV. C Initial incubation (48 hrs) + Dose exposure incubation (48 hrs) = Final incubation (96 hrs).

4)Phe hyd-PAS	Mes	++	++	++	++	++	++	++	Increased color intensity	+++	+++	+++	+++	+++	+++	+++	Increased color intensity
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
6) Phe-Deace PAS	Mes	++	++	++	++	++	++	++	Regained color reaction	+++	+++	+++	+++	+++	+++	+++	Regained color reaction
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
7) Ace-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity at all sites	+++	+++	+++	+++	+++	+++	+++	Blocked regained with equal intensity at all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
8) Dace-PAS	Mes	±	±	±	±	±	±	±	Blocked Regained with equal intensity in all sites	±	±	±	±	±	±	±	Blocked regained with equal intensity at all sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9) sulfer induced metachro macia	Mes	++	++	++	++	++	++	++	Increased intensity of coloration at all sites.	+++	+++	+++	+++	+++	+++	+++	Increased intensity of coloration at all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no. IV. C Initial incubation (48 hrs) + Dose exposure incubation (48 hrs) = Final incubation (96 hrs).

Staining Tech.	Brain zones	0.5 mM H_2O_2 + 3 mg vitamin C treated								Control 3 mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1) PAS	Mes	±	±	±	±	±	±	±	Trace reac.at all brain regions	±	±	±	±	±	±	±	Weak reac.at cyto, Moderat Reac.at Ics,Intesnse rea at PM, ML
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tcl	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
2)Amy Dig PAS	Mes	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites
	Met	±	±	±	±	+	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
3)Dias dig -PAS	Mes	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of thesites	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
4) Amy dig-Sap-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity in all sites	++	++	++	++	++	++	++	Regained with equal intensity in all sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	

Table no. IV. C Initial incubation (48 hrs) + Dose exposure incubation (48 hrs) = Final incubation (96 hrs).

5)Phe. Hya PAS	Mes	++	++	++	++	++	++	++	Increased color intensity	++	++	++	++	++	++	++	Increased color intensity
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
6) Phe-Deace PAS	Mes	++	++	++	++	++	++	++	Regained color reaction	++	++	++	++	++	++	++	Regained color reaction
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
7) Ace-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity at all sites	++	++	++	++	++	++	++	Blocked regained with equal intensity at all sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
8) Dace-PAS	Mes	±	±	±	±	±	±	±	Blocked Regained with equal intensity in all sites	±	±	±	±	±	±	±	Blocked regained with equal intensity at all sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9) Sulfur induced metachromacia	Mes	++	++	++	++	++	++	++	Increased intensity of coloration at all the sites	++	++	++	++	++	++	++	Increased intensity of coloration at all the sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	

Table no. IV .d Initial incubation (48 hrs) + exposure incubation (96 hrs) =Final incubation (120 hrs).

Staning techn	Brain zone	Normal								0.5 mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)PAS	Mes	±	±	±	±	±	±	±	Trace reaction at all brain regions	+++	+++	+++	+++	+++	+++	+++	Moderat Reac. at cyto Ics, Intesnse rea at PM, ML
	Met	±	±	±	±	±	±	±		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	±	±	±	±	±	±		+++	+++	+++	+++	+++	+++	+++	
	Die	±	±	±	±	±	±	±		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	±	±	±	±	±	±		+++	+++	+++	+++	+++	+++	+++	
2)Amy Dig PAS	Mes	±	±	±	±	±	±	±	No histo-Chemically detectable glycogen at any of the sites	+++	+++	+++	+++	+++	+++	+++	No histo-Chemically detectable glycogn at any of thc sites
	Met	±	±	±	±	±	±	±		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	±	±	±	±	±	±		+++	+++	+++	+++	+++	+++	+++	
	Die	±	±	±	±	±	±	±		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	±	±	±	±	±	±		+++	+++	+++	+++	+++	+++	+++	
3)Dias dig -PAS	Mes	±	±	±	±	±	±	±	No histo-Chemically detectable glycogen at any of the sites	+++	+++	+++	+++	+++	+++	+++	No histo-chemically detectable glycogn at any of the sites
	Met	±	±	±	±	±	±	±		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	±	±	±	±	±	±		+++	+++	+++	+++	+++	+++	+++	
	Die	±	±	±	±	±	±	±		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	±	±	±	±	±	±		+++	+++	+++	+++	+++	+++	+++	
4)Amydi gSap-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity in all sites	+++	+++	+++	+++	+++	+++	+++	Regained with equal intensity in all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no. IV .d Initial incubation (48 hrs) + exposure incubation (96 hrs) =Final incubation (120 hrs).

4)Phe hyd-PAS	Mes	++	++	++	++	++	++	++	Increased color intensity	+++	+++	+++	+++	+++	+++	+++	Increased color intensity
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
6) Phe-Deace PAS	Mes	++	++	++	++	++	++	++	Regained color reaction	+++	+++	+++	+++	+++	+++	+++	Regained color reaction
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
7) Ace-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity at all sites	+++	+++	+++	+++	+++	+++	+++	Blocked regained with equal intensity at all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
8) Dace-PAS	Mes	±	±	±	±	±	±	±	Blocked Regained with equal intensity in all sites	±	±	±	±	±	±	±	Blocked regained with equal intensity at all sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9) sulfer induced metachro macia	Mes	++	++	++	++	++	++	++	Increased intensity of coloration at all sites.	+++	+++	+++	+++	+++	+++	+++	Increased intensity of coloration at all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no. IV .d Initial incubation (48 hrs) + exposure incubation (96 hrs) =Final incubation (120 hrs).

Staining Tech.	Brain zones	0.5 mM H_2O_2 + 3 mg vitamin C treated								Control 3 mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1) PAS	Mes	±	±	±	±	±	±	±	Trace reac.at all brain regions	±	±	±	±	±	±	±	Weak reac.at cyto, Moderat Reac.at Ics,Intesnse rea at PM, ML
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
2)Amy Dig PAS	Mes	±	±	±	±	±	±	±	No histo chemicaldetectabl glycogn at any of the sites	±	±	±	±	±	±	±	No histo chemicaldetectabl glycogn at any of the sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
3)Dias dig -PAS	Mes	±	±	±	±	±	±	±	No histo chemicaldetectabl glycogn at any of the sites	±	±	±	±	±	±	±	No histo chemicaldetectabl glycogn at any of the sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
4) Amy dig-Sap- PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity in all sites	++	++	++	++	++	++	++	Regained with equal intensity in all sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	

Table no. IV .d Initial incubation (48 hrs) + exposure incubation (96 hrs) =Final incubation (120 hrs).

5)Phe. Hya PAS	Mes	++	++	++	++	++	++	++	Increased color intensity	++	++	++	++	++	++	++	Increased color intensity
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
6) Phe-Deace PAS	Mes	++	++	++	++	++	++	++	Regained color reaction	++	++	++	++	++	++	++	Regained color reaction
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
7) Ace-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity at all sites	++	++	++	++	++	++	++	Blocked regained with equal intensity at all sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
8) Dace-PAS	Mes	±	±	±	±	±	±	±	Blocked Regained with equal intensity in all sites	±	±	±	±	±	±	±	Blocked regained with equal intensity at all sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9) Sulfur induced metachromacia	Mes	++	++	++	++	++	++	++	Increased intensity of coloration at all the sites	++	++	++	++	++	++	++	Increased intensity of coloration at all the sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	

Table no. V. a Initial incubation (72 hrs) + dose exposure incubation (24 hrs) =Final incubation (96 hrs).

Staning techn	Brain zone	Normal								0.5 mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)PAS	Mes	±	±	±	±	±	±	±	Trace reaction at all brain regions	++	+++	++	++	+++	++	+++	Moderat Reac. at cyto Ics, Intesnse rea at PM, ML
	Met	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Mye	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Die	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Tel	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
2)Amy Dig PAS	Mes	±	±	±	±	±	±	±	No histo-Chemically detectable glycogen at any of the sites	++	+++	++	++	+++	++	+++	No histo-Chemically detectable glycogn at any of the sites
	Met	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Mye	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Die	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Tel	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
3)Dias dig -PAS	Mes	±	±	±	±	±	±	±	No histo-Chemically detectable glycogen at any of the sites	++	+++	++	++	+++	++	+++	No histo-chemically detectable glycogn at any of the sites
	Met	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Mye	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Die	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Tel	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
4)Amydi gSap-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity in all sites	+++	+++	+++	+++	+++	+++	+++	Regained with equal intensity in all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no. V. a Initial incubation (72 hrs) + dose exposure incubation (24 hrs) =Final incubation (96 hrs).

4)Phe hyd-PAS	Mes	++	++	++	++	++	++	++	Increased color intensity	+++	+++	+++	+++	+++	+++	+++	Increased color intensity
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
6) Phe-Deace PAS	Mes	++	++	++	++	++	++	++	Regained color reaction	+++	+++	+++	+++	+++	+++	+++	Regained color reaction
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
7) Ace-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity at all sites	+++	+++	+++	+++	+++	+++	+++	Blocked regained with equal intensity at all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
8) Dace-PAS	Mes	±	±	±	±	±	±	±	Blocked Regained with equal intensity in all sites	±	±	±	±	±	±	±	Blocked regained with equal intensity at all sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9) sulfer induced metachromacia	Mes	++	++	++	++	++	++	++	Increased intensity of coloration at all sites.	+++	+++	+++	+++	+++	+++	+++	Increased intensity of coloration at all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no. V. a Initial incubation (72 hrs) + dose exposure incubation (24 hrs) =Final incubation (96 hrs).

Staining Tech.	Brain zones	0.5 mM H_2O_2 + 3 mg vitamin C treated								Control 3 mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1) PAS	Mes	±	±	±	±	±	±	±	Trace reac.at all brain regions	±	±	±	±	±	±	±	Weak reac.at cyto, Moderat Reac.at Ics,Intesnse rea at PM, ML
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
2)Amy Dig PAS	Mes	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
3)Dias dig -PAS	Mes	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of thesites	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
4) Amy dig-Sap-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity in all sites	++	++	++	++	++	++	++	Regained with equal intensity in all sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	

Table no. V. a Initial incubation (72 hrs) + dose exposure incubation (24 hrs) =Final incubation (96 hrs).

5)Phe. Hya PAS	Mes	++	++	++	++	++	++	++	Increased color intensity	++	++	++	++	++	++	++	Increased color intensity
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
6) Phe-Deace PAS	Mes	++	++	++	++	++	++	++	Regained color reaction	++	++	++	++	++	++	++	Regained color reaction
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
7) Ace-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity at all sites	++	++	++	++	++	++	++	Blocked regained with equal intensity at all sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
8) Dace-PAS	Mes	±	±	±	±	±	±	±	Blocked Regained with equal intensity in all sites	±	±	±	±	±	±	±	Blocked regained with equal intensity at all sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9) Sulfur induced metachromacia	Mes	++	++	++	++	++	++	++	Increased intensity of coloration at all the sites	++	++	++	++	++	++	++	Increased intensity of coloration at all the sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	

Table no. I. b b Initial incubation (72 hrs) + exposure incubation (48 hrs) =Final incubation (120 hrs).

Staining Tech.	Brain zone	0.5 mM H_2O_2 + 3mg vitamin C treated								Control 3mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1) PAS	Mes	++	++	++	++	++	++	++	Trace reac.at all brain regions	±	±	±	±	±	±	±	Weak reac.at cyto, Ics, PM, ML
	Met	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Mye	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Die	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Tel	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
2) Amy Dig PAS	Mes	++	++	++	++	++	++	++	No histo Chemically detectable glycogen at any of the sites	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites
	Met	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Mye	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Die	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Tel	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
3)Dias dig -PAS	Mes	++	++	++	++	++	++	++	No histo Chemically detectable glycogen at any of the sites	+	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites
	Met	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Mye	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Die	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Tel	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
4)Amy dig. Sap-PAS	Mes	+++	+++	+++	+++	+++	+++	+++	Regained with equal intensity in all sites	++	++	++	++	++	++	++	Regained with equal intensity in all sites
	Met	+++	+++	+++	+++	+++	+++	+++		++	++	++	++	++	++	++	
	Mye	+++	+++	+++	+++	+++	+++	+++		++	++	++	++	++	++	++	
	Die	+++	+++	+++	+++	+++	+++	+++		++	++	++	++	++	++	++	
	Tel	+++	+++	+++	+++	+++	+++	+++		++	++	++	++	++	++	++	

Table no. I.b Initial incubation (72 hrs) + exposure incubation (48 hrs) =Final incubation (120 hrs).

Staining Tech.	Brain zones	Normal								0.5mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)PAS	Mes	±	±	±	±	±	±	±	Trace reaction at all brain regions.	+	+++	++	+	+++	++	+++	Weak reac. at cyto, Moderat Reac.at Ics,Intesnse rea at PM, ML
	Met	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
	Mye	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
	Die	±	±	+	±	±	±	±		+	+++	++	+	+++	++	+++	
	Tel	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
2)Amy Dig PAS	Mes	±	±	±	±	±	±	±	No histo-Chemically detectable glycogen at any of the sites	+	+++	++	+	+++	++	+++	No histo-Chemically detectable glycogn at any of the sites
	Met	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
	Mye	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
	Die	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
	Tel	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
3)Dias digPAS	Mes	±	±	±	±	±	±	±	No histo-Chemically detectable glycogen at any of the sites	+	+++	++	+	+++	++	+++	No histo-chemically detectable glycogn at any of the sites
	Met	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
	Mye	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
	Die	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
	Tel	±	±	±	±	±	±	±		+	+++	++	+	+++	++	+++	
4) Amy dig.-Sap-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity in all sites	+++	+++	+++	+++	+++	+++	+++	Regained with equal intensity in all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no I.b Initial incubation (72 hrs) + exposure incubation (48 hrs) =Final incubation (120 hrs). continued.....

5)Phe hyd-PAS	Mes	++	++	++	++	++	++	++	Increased color intensity	+++	+++	+++	+++	+++	+++	+++	Increase color intensity
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
6) Phe-Deace PAS	Mes	++	++	++	++	++	++	++	Regained color reaction	+++	+++	+++	+++	+++	+++	+++	Regaine color reaction
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
7) Ace-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity at all sites	+++	+++	+++	+++	+++	+++	+++	Blocked regained with eq intensity all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
8) Dace-PAS	Mes	±	±	±	±	±	±	±	Blocked Regained with equal intensity in all sites	±	±	±	±	±	±	±	Blocked regained with eq intensity all sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9) Sulfer induced metachro macia	Mes	++	++	++	++	++	++	++	Increased color intensity	+++	+++	+++	+++	+++	+++	+++	Increase color intensity
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no. I. b b Initial incubation (72 hrs) + exposure incubation (48 hrs) =Final incubation (120 hrs).

Staining Tech.	Brain zone	0.5 mM H_2O_2 + 3mg vitamin C treated								Control 3mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1) PAS	Mes	++	++	++	++	++	++	++	Trace reac.at all brain regions	±	±	±	±	±	±	±	Weak reac.at cyto, Ics, PM, ML
	Met	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Mye	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Die	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Tel	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
2) Amy Dig PAS	Mes	++	++	++	++	++	++	++	No histo Chemically detectable glycogen at any of the sites	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites
	Met	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Mye	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Die	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Tel	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
3)Dias dig -PAS	Mes	++	++	++	++	++	++	++	No histo Chemically detectable glycogen at any of the sites	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites
	Met	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Mye	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Die	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
	Tel	++	++	++	++	++	++	++		±	±	±	±	±	±	±	
4)Amy dig. Sap-PAS	Mes	+++	+++	+++	+++	+++	+++	+++	Regainecd with equal intensity in all sites	++	++	++	++	++	++	++	Regained with equal intensity in all sites
	Met	+++	+++	+++	+++	+++	+++	+++		++	++	++	++	++	++	++	
	Mye	+++	+++	+++	+++	+++	+++	+++		++	++	++	++	++	++	++	
	Die	+++	+++	+++	+++	+++	+++	+++		++	++	++	++	++	++	++	
	Tel	+++	+++	+++	+++	+++	+++	+++		++	++	++	++	++	++	++	

Table no. I. b b Initial incubation (72 hrs) + exposure incubation (48 hrs) =Final incubation (120 hrs). continued.....

4)Phe. Hya PAS	Mes	++	++	++	++	++	++	++	Increased color intensity	++	++	++	++	++	++	++	Increase color intensity
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
6) Phe-Deace PAS	Mes	++	++	++	++	++	++	++	Regained color reaction	++	++	++	++	++	++	++	Regaine color reaction
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
7) Ace-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity at all sites	++	++	++	++	++	++	++	Blocked regained with eq intensity all sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
8) Dace-PAS	Mes	±	±	±	±	±	±	±	Blocked Regained with equal intensity in all sites	±	±	±	±	±	±	±	Blocked regained with eq intensity all sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9) Sulfer induced metachro macia	Mes	++	++	++	++	++	++	++	Increased color intensity	++	++	++	++	++	++	++	Increase color intensity
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	

Table no. VI. a Initial incubation (96 hrs) + exposure incubation (24 hrs) =Final incubation (120 hrs)

Staning techn	Brain zone	Normal								0.5 mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)PAS	Mes	±	±	±	±	±	±	±	Trace reaction at all brain regions	++	+++	++	++	+++	++	+++	Moderat Reac. at cyto Ics, Intesnse rea at PM, ML
	Met	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Mye	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Die	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Tel	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
2)Amy Dig PAS	Mes	±	±	±	±	±	±	±	No histo-Chemically detectable glycogen at any of the sites	++	+++	++	++	+++	++	+++	No histo-Chemically detectable glycogn at any of the sites
	Met	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Mye	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Die	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Tel	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
3)Dias dig - PAS	Mes	±	±	±	±	±	±	±	No histo-Chemically detectable glycogen at any of the sites	++	+++	++	++	+++	++	+++	No histo-chemically detectable glycogn at any of the sites
	Met	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Mye	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Die	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Tel	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
4)Amyd igSap-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity in all sites	+++	+++	+++	+++	+++	+++	+++	Regained with equal intensity in all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no. VI. a Initial incubation (96 hrs) + exposure incubation (24 hrs) =Final incubation (120 hrs)

4)Phe hyd-PAS	Mes	++	++	++	++	++	++	++	Increased color intensity	+++	+++	+++	+++	+++	+++	+++	Increased color intensity
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
6) Phe-Deace PAS	Mes	++	++	++	++	++	++	++	Regained color reaction	+++	+++	+++	+++	+++	+++	+++	Regained color reaction
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
7) Ace-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity at all sites	+++	+++	+++	+++	+++	+++	+++	Blocked regained with equal intensity at all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
8) Dace-PAS	Mes	±	±	±	±	±	±	±	Blocked Regained with equal intensity in all sites	±	±	±	±	±	±	±	Blocked regained with equal intensity at all sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9) sulfer induced metachromacia	Mes	++	++	++	++	++	++	++	Increased intensity of coloration at all sites.	+++	+++	+++	+++	+++	+++	+++	Increased intensity of coloration at all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no. VI. a Initial incubation (96 hrs) + exposure incubation (24 hrs) =Final incubation (120 hrs)

Staining Tech.	Brain zones	0.5 mM H_2O_2 + 3 mg vitamin C treated								Control 3 mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1) PAS	Mes	±	±	±	±	±	±	±	Trace reac.at all brain regions	±	±	±	±	±	±	±	Weak reac.at cyto, Moderat Reac.at Ics,Intesnse rea at PM, ML
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
2)Amy Dig PAS	Mes	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
3)Dias dig -PAS	Mes	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of thesites	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
4) Amy dig-Sap-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity in all sites	++	++	++	++	++	++	++	Regained with equal intensity in all sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	

Table no. VI. a Initial incubation (96 hrs) + exposure incubation (24 hrs) =Final incubation (120 hrs)

5)Phe. Hya PAS	Mes	++	++	++	++	++	++	++	Increased color intensity	++	++	++	++	++	++	++	Increased color intensity
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
6) Phe-Deace PAS	Mes	++	++	++	++	++	++	++	Regained color reaction	++	++	++	++	++	++	++	Regained color reaction
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
7) Ace-PAS	Mes	++	++	++	++	++	++	++	Regained with cqual intensity at all sites	++	++	++	++	++	++	++	Blocked regained with equal intensity at all sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
8) Dace-PAS	Mes	±	±	±	±	±	±	±	Blocked Regained with equal intensity in all sites	±	±	±	±	±	±	±	Blocked regained with equal intensity at all sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9) Sulfur induced metachromacia	Mes	++	++	++	++	++	++	++	Increased intensity of coloration at all the sites	++	++	++	++	++	++	++	Increased intensity of coloration at all the sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	

Table no. VI. a Initial incubation (120 hrs) + exposure incubation (24 hrs) =Final incubation (144 hrs).

Staning techn	Brain zone	Normal								0.5 mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)PAS	Mes	±	±	±	±	±	±	±	Trace reaction at all brain regions	++	+++	++	++	+++	++	+++	Moderat Reac. at cyto Ics, Intesnse rea at PM, ML
	Met	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Mye	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Die	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Tel	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
2)Amy Dig PAS	Mes	±	±	±	±	±	±	±	No histo-Chemically detectable glycogen at any of the sites	++	+++	++	++	+++	++	+++	No histo-Chemically detectable glycogn at any of the sites
	Met	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Mye	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Die	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Tel	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
3)Dias dig - PAS	Mes	±	±	±	±	±	±	±	No histo-Chemically detectable glycogen at any of the sites	++	+++	++	++	+++	++	+++	No histo-chemically detectable glycogn at any of the sites
	Met	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Mye	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Die	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
	Tel	±	±	±	±	±	±	±		++	+++	++	++	+++	++	+++	
4)Amy digSap-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity in all sites	+++	+++	+++	+++	+++	+++	+++	Regained with equal intensity in all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no. VI. a Initial incubation (120 hrs) + exposure incubation (24 hrs) =Final incubation (144 hrs).

4)Phe hyd-PAS	Mes	++	++	++	++	++	++	++	Increased color intensity	+++	+++	+++	+++	+++	+++	+++	Increased color intensity
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
6) Phe-Deace PAS	Mes	++	++	++	++	++	++	++	Regained color reaction	+++	+++	+++	+++	+++	+++	+++	Regained color reaction
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
7) Ace-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity at all sites	+++	+++	+++	+++	+++	+++	+++	Blocked regained with equal intensity at all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
8) Dace-PAS	Mes	±	±	±	±	±	±	±	Blocked Regained with equal intensity in all sites	±	±	±	±	±	±	±	Blocked regained with equal intensity at all sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9) sulfer induced metachromacia	Mes	++	++	++	++	++	++	++	Increased intensity of coloration at all sites.	+++	+++	+++	+++	+++	+++	+++	Increased intensity of coloration at all sites
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no. VI. a Initial incubation (120hrs) + exposure incubation (24 hrs) =Final incubation (144 hrs).

Staining Tech.	Brain zones	0.5 mM H_2O_2 + 3 mg vitamin C treated								Control 3 mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1) PAS	Mes	±	±	±	±	±	±	±	Trace reac.at all brain regions	±	±	±	±	±	±	±	Weak reac.at cyto, Moderat Reac.at Ics,Intesnse rea at PM, ML
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tcl	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
2)Amy Dig PAS	Mes	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
3)Dias dig -PAS	Mes	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of thesites	±	±	±	±	±	±	±	No histo chemically detectable glycogen at any of the sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
4) Amy dig-Sap-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity in all sites	++	++	++	++	++	++	++	Regained with equal intensity in all sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	

Table no. VI. a Initial incubation (120 hrs) + exposure incubation (24 hrs) =Final incubation (144 hrs).

5)Phe. Hya PAS	Mes	++	++	++	++	++	++	++	Increased color intensity	++	++	++	++	++	++	++	Increased color intensity
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
6) Phe-Deace PAS	Mes	++	++	++	++	++	++	++	Regained color reaction	++	++	++	++	++	++	++	Regained color reaction
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
7) Ace-PAS	Mes	++	++	++	++	++	++	++	Regained with equal intensity at all sites	++	++	++	++	++	++	++	Blocked regained with equal intensity at all sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
8) Dace-PAS	Mes	±	±	±	±	±	±	±	Blocked Regained with equal intensity in all sites	±	±	±	±	±	±	±	Blocked regained with equal intensity at all sites
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9) Sulfur induced metachro macia	Mes	++	++	++	++	++	++	++	Increased intensity of coloration at all the sites	++	++	++	++	++	++	++	Increased intensity of coloration at all the sites
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	

PLATE I

PLATE I- Initial incubation (24 hrs) + Dose exposure incubation (24 hrs) = Final development (48 hrs). PAS

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen .- Neuroepithelial layer present. Compactly arranged cells without cell surfaces, intensely stained basophilic nuclei. with faint coloration

Fig. 2 - Meten. - Cell compactly arranged without intercellular spaces, basophilic nuclei. with faint coloration

Fig. 3 - Myelen - Cells compactly arranged without intercellular spaces intensely stained basophilic nuclei. with faint coloration

Fig. 4 - Dien - Compactly arranged cells without intercellular spaces. with faint color

Fig. 5 - Telen. - Compactly arranged cell without intercellular spaces. with faint color

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000.

Fig. 6 - Mesen. - Cells compactly arranged with little intercellular spaces. Foggy cells towards the luminal side. Magenta color increased

Fig. 7 - Meten. - Cells compactly arranged, foggy cell towards the luminal side.

Fig. 8 - Myelen - Cells compactly arranged, foggy cells towards the luminal side

Fig. 9 - Dien - Cells compactly arranged without intercellular spaces, foggy cell towards the luminal side.

Fig. 10 - Telen- Cells compactly arranged with foggy appearance.

Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000. faint color

Fig. 11 - Mesen - Cells compactly arranged without intensely stained nuclei.

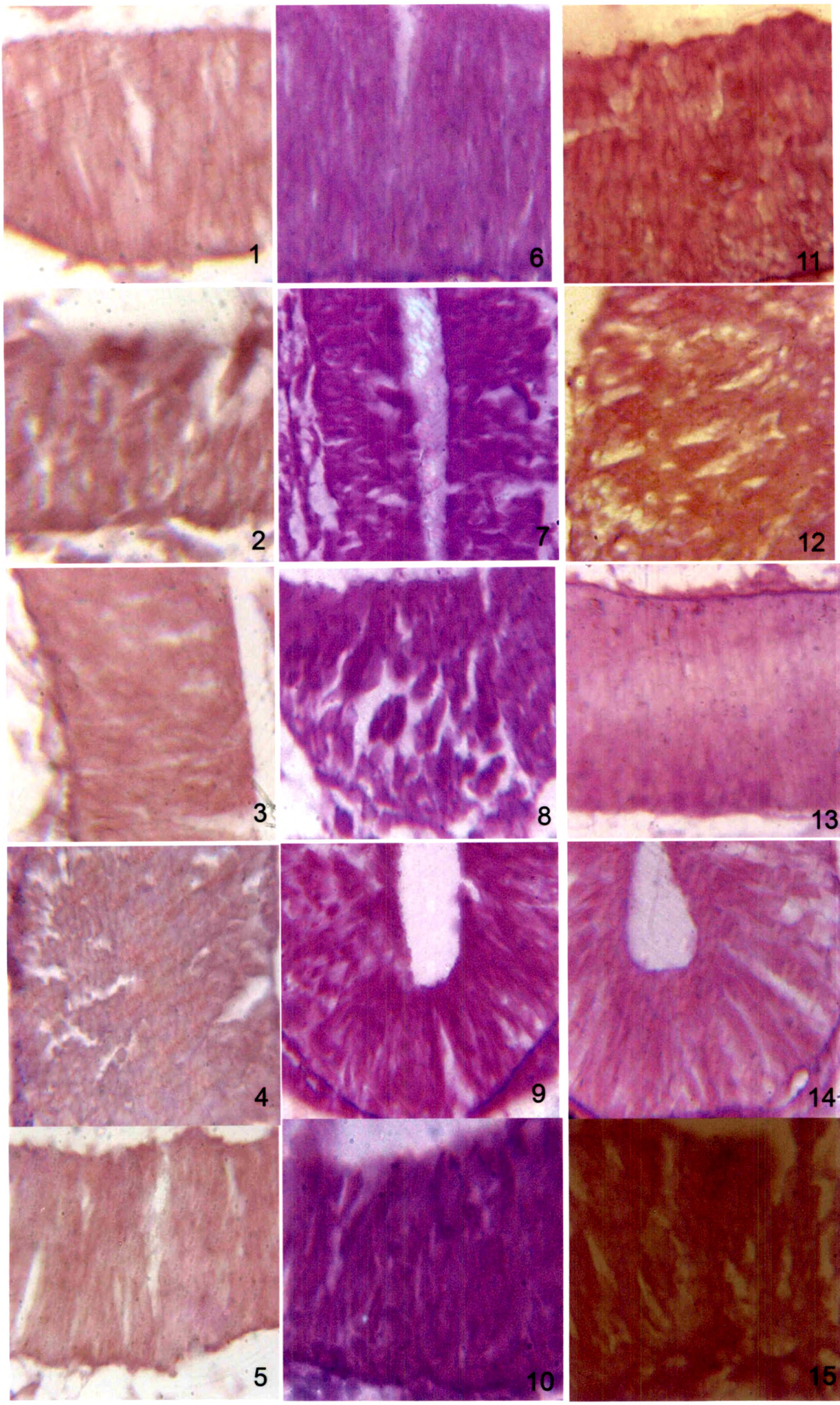
Fig. 12 - Meten. - Cells compactly arranged not well marked cell boundaries.

Fig. 13 - Myelen - Cells compactly arranged without cell boundaries.

Fig. 14 - Dien - Cells compactly arranged without well stained cells.

Fig. 15 - Telen. - Cells compactly arranged without intercellular spaces. No well marked cell boundaries.

PLATE I



ependymal and mantle layer while marginal layer was free of cell bodies. Therefore, distribution was localized within cytoplasm of neurons/neuroepithelial cells intercellular spaces on at all surfaces these being the main components. These zones are extended in all the three regions and very few alterations were observed within the regions. But contents altered at localizations stated above and weighted quantitatively as described in Table A. The distributions of NGAG in different histological elements under different experimental conditions are presented as follows.

Normal:

i) 48 hours of development

The NGAGs distributed in five brain regions viz. mesencephalon, metencephalon, myelencephalon, diencephalon and telencephalon did not show any variation. Neutral GAGs were weak in concentration in cytoplasm, cell coat and the intercellular space.

ii) 58 hrs of development

NGAGs were in traces in all the histologically observed elements viz. cytoplasm, cell coat and intracellular space of neuroepithelial regions.

iii) 64 hrs of development

Distribution of NGAGs in concentration similar in all the five regions of brain. The NGAGs content was in traced amount in cellular elements and intercellular space of ependymal, mantle and marginal zonal elements.

iv) 72 hrs of development

Similar traced content and distribution of NGAG was observed in all the zones of five brain regions.

v) 82 hrs development-

The distribution and concentration was similar to that was noted at 72 hrs (trace).

vi) 88 hrs development-

No alterations in NGAG content and distribution were observed as compared to the brain of 72 hrs of development.

vii) 96 hrs development-

NGAG content and distribution was as observed in brain of 88 hrs embryo.

viii) 106 hrs development-

NAGAG content remained in traced amount at the same histological elements.

ix) 112 hrs development-

The concentration of NGAG content was marginally increased at the localizations reported at 106 hrs of development.

x) 120 hrs development-

The content and distribution of NGAG remained same as observed in 112 hrs embryo.

xi) 130 hrs development-

The content and distribution of NGAG was similar to that was noted in brain of embryo at 120 hrs of development.

xi) 136 hrs development-

NGAG content and distribution in brain was similar to that was observed at 120 hrs of development.

xii) 144 hrs development

NGAG content and distribution in brain did not alter after 136 hrs of development and remained same at 144 hrs of development.

Control HBSS:

NGAG content and distribution of NGAG remained at all the receptive hrs of development as observed in normal embryo brains.

Control 3 mg vitamin C:

NGAG distribution and content in brain of embryos of different hrs of development (corresponding to normal) did not alter.

0.5 mM H_2O_2 treated:

Initiation at 24 hrs of development

i) Initial incubation (24 hrs) + Dose exposure incubation (24 hrs) = Final development (48 hrs) –

The exposure of embryos to H_2O_2 resulted in significantly increased concentration of NGAG at all the localizations observed in brain of embryo.

ii) Initial incubation (24 hrs) + Dose exposure incubation (48 hrs) = Final development (72 hrs) –

The content of NGAG was marginally depleted in cytoplasm and extracellular spaces but remained as intense as noted in 24 hrs initiated and 24 hrs treated group embryos at cell surfaces

PLATE II

PLATE I- Initial incubation (24 hrs) + Dose exposure incubation (96 hrs) = Final development (120 hrs).

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen .- Neuroepithelial layer present. Compactly arranged cells without cell surfaces, intensely stained basophilic nuclei.

Fig. 2 - Meten. - Cell compactly arranged without intercellular spaces, basophilic nuclei.

Fig. 3 - Myelen - Cells compactly arranged without intercellular spaces intensely stained basophilic nuclei.

Fig. 4 - Dien - Compactly arranged cells without intercellular spaces.

Fig. 5 - Telen. - Compactly arranged cell without intercellular spaces.

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000. Increased magenta color

Fig. 6 - Mesen. - Cells compactly arranged with little intercellular spaces. Foggy cells towards the luminal side.

Fig. 7 - Meten. - Cells compactly arranged, foggy cell towards the luminal side.

Fig. 8 - Myelen - Cells compactly arranged, foggy cells towards the luminal side

Fig. 9 - Dien - Cells compactly arranged without intercellular spaces, foggy cell towards the luminal side.

Fig. 10 - Telen- Cells compactly arranged with foggy appearance.

Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen - Cells compactly arranged without intensely stained nuclei.

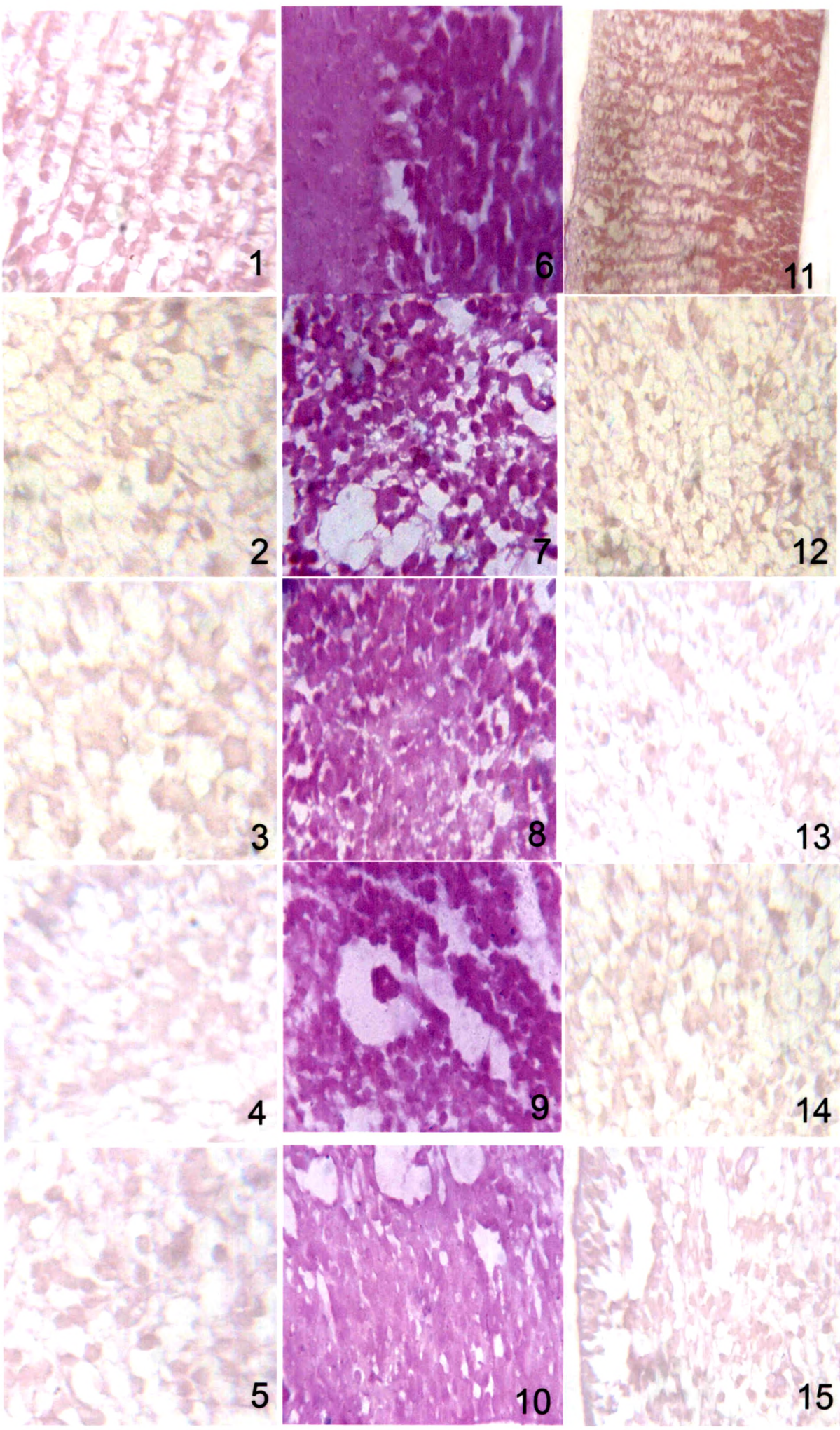
Fig. 12 - Meten. - Cells compactly arranged not well marked cell boundaries.

Fig. 13 - Myelen - Cells compactly arranged without cell boundaries.

Fig. 14 - Dien - Cells compactly arranged without well stained cells.

Fig. 15 - Telen. - Cells compactly arranged without intercellular spaces. No well marked cell boundaries.

PLATE II



iii) Initial incubation (24 hrs) + dose exposure incubation (72 hrs) = Final development (96 hrs)-

The NGAG content was significantly increased (as compared to normal) at cell surfaces and intracellular spaces but not in the cytoplasm.

iv) Initial incubation (24 hrs) + Dose exposure incubation (96 hrs) = Final development (120 hrs) -

The NGAG content at cell surfaces, cytoplasm and intercellular spaces remained significantly high as compared to its content noted in normal brain (corresponding development hrs).

Initiation at 34 hrs of development

v) Initial incubation (34 hrs) + Dose exposure incubation (24 hrs) = Final development (58 hrs) –

The NGAG distribution and content remained unaltered as compared to the to the corresponding normal embryo brain.

vi) Initial incubation (34 hrs) + Dose exposure incubation (48 hrs) = Final development (82 hrs) –

There were no alterations in NGAG contents and distribution as compared to the NGAG contents and localizations in normal embryo brain at corresponding developing hrs.

vii) Initial incubation (34 hrs) + Dose exposure incubation (72 hrs) = Final development (106 hrs) –

NGAG remained unaltered in its distribution and content as compared to its corresponding normal embryo a: given hrs of development.

viii) Initial incubation (34 hrs) + Dose exposure incubation (96 hrs) = Final development (130 hrs) –

The observed contents of NGAG at the different elements studied remained unaltered as compared to the NGAG content and distribution noted in normal embryo t 130 hrs of development.

Initiation at 40 hrs of development

ix) Initial incubation (40 hrs) + Dose exposure incubation (40 hrs) = Final development (64 hrs) –

There were no significant changes in NGAG content and distribution as compared to NGAG contents and distribution as compared to NGAG contents and distributions observed in brain of normal embryo at 64 hrs of development.

x) Initial incubation (40 hrs) + Dose exposure incubation (48 hrs) = Final development (88 hrs) –

Exposure of H_2O_2 initiated at 40 hrs and continued at 48 hrs did not alter the NGAG content and distribution in brain of normal embryo at 88 hrs of development.

xi) Initial incubation (40 hrs) + Dose exposure incubation (72 hrs) = Final development (112 hrs)

Treatment of H_2O_2 initiated at 40 hrs and continued for 72 hrs did not alter the NGAG content at resultant hrs of experiment in brain of embryo corresponding hrs of development.

xii) Initial incubation (40 hrs) + Dose exposure incubation (96 hrs) = Final development (136 hrs) –

The prolong exposure of 96 hrs on H_2O_2 treatment given at 40 hrs of development did not altered the NGAG distribution and the content as compared to the NGAG distribution and the contents that were observed in brain of embryo at 136 hrs of development.

Initiation at 48 hrs of development

xiii) Initial incubation (48 hrs) + Dose exposure incubation (24 hrs) = Final development (72 hrs) –

Trace content of NGAG in brain of embryo of 72 hrs on exposure of H_2O_2 initiated at 48 hrs and prolonged further for 24 hrs were increased significantly at the same localizations reported in brain of embryo of 72 hrs of development.

xiv) Initial incubation (48 hrs) + Dose exposure incubation (48 hrs) = Final development (96 hrs) –

As compared to the NGAG content of brain reported at 96 hrs of developing embryo were significantly increased by the stared treatment of H_2O_2 at the same localizations.

xv) Initial incubation (48 hrs) + Dose exposure incubation (72 hrs) = final development (120 hrs)-

The above stated experimental conditions increased the NGAG contents significantly as compared to NGAG content reported in brain of 120 hrs developed normal embryo at the same localizations.

PLATE III

PLATE I- Initial incubation (48 hrs) + Dose exposure incubation (24 hrs) = Final development (72 hrs).

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen .- Neuroepithelial layer present. Compactly arranged cells without cell surfaces, intensely stained basophilic nuclei.

Fig. 2 - Meten. - Cell compactly arranged without intercellular spaces, basophilic nuclei.

Fig. 3 - Myelen - Cells compactly arranged without intercellular spaces intensely stained basophilic nuclei.

Fig. 4 - Dien - Compactly arranged cells without intercellular spaces.

Fig. 5 - Telen. - Compactly arranged cell without intercellular spaces.

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000. Increased magenta color

Fig. 6 - Mesen. - Cells compactly arranged with little intercellular spaces. Foggy cells towards the luminal side.

Fig. 7 - Meten. - Cells compactly arranged, foggy cell towards the luminal side.

Fig. 8 - Myelen - Cells compactly arranged, foggy cells towards the luminal side

Fig. 9 - Dien - Cells compactly arranged without intercellular spaces, foggy cell towards the luminal side.

Fig. 10 - Telen- Cells compactly arranged with foggy appearance.

Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen - Cells compactly arranged without intensely stained nuclei.

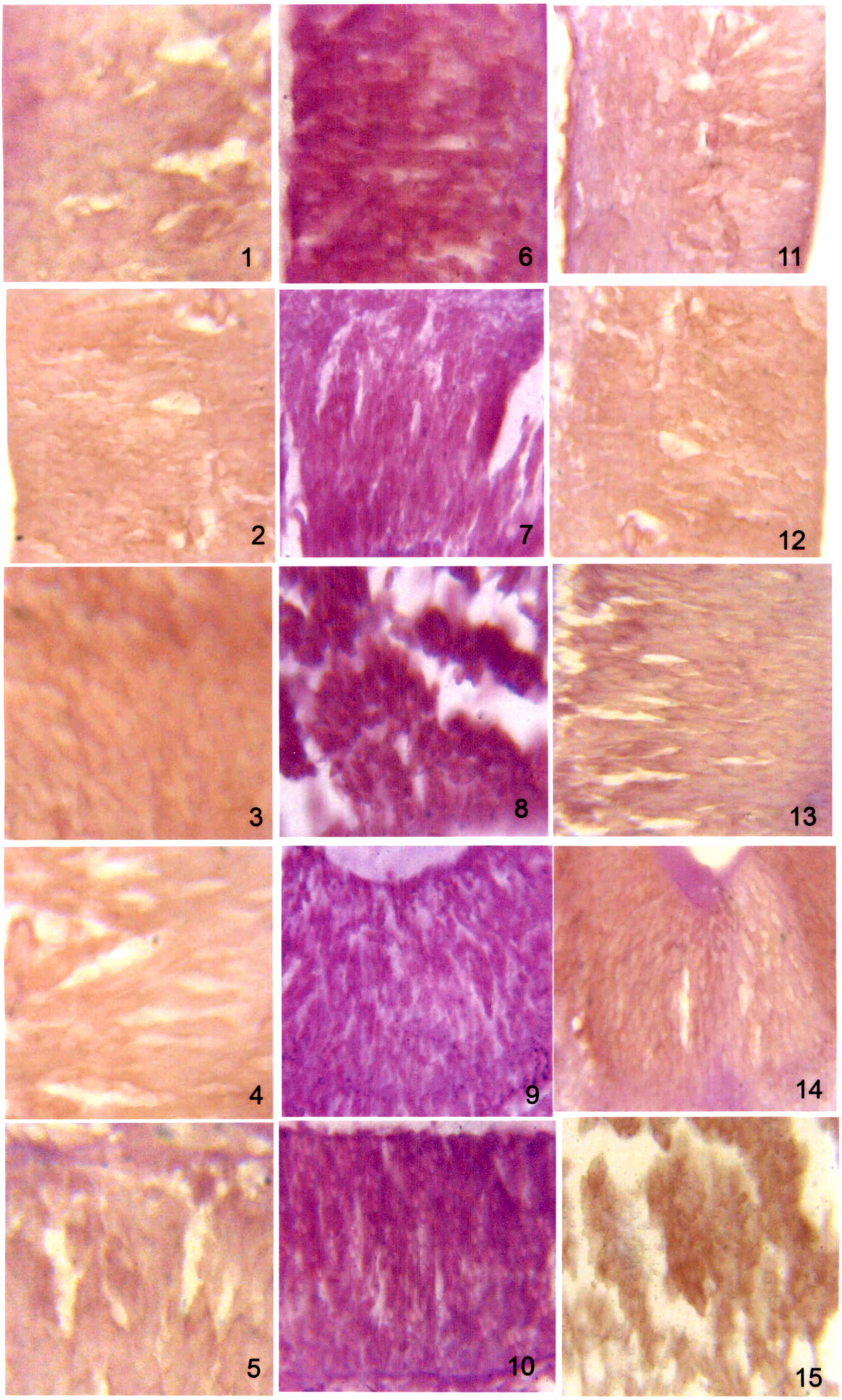
Fig. 12 - Meten. - Cells compactly arranged not well marked cell boundaries.

Fig. 13 - Myelen - Cells compactly arranged without cell boundaries.

Fig. 14 - Dien - Cells compactly arranged without well stained cells.

Fig. 15 - Telen. - Cells compactly arranged without intercellular spaces. No well marked cell boundaries.

PLATE III



Initiation at 72 hrs of development

xvi) Initial incubation (72 hrs) + Dose exposure incubation (24 hrs) = Final development (96 hrs) –

This treatment marginally increased the content of NGAG in cytoplasm while significantly increased content was noted at cell surfaces of all the three zones as compared to NGAG content and distribution o brain of 96 hrs developed normal embryo.

xvii) Initial incubation (72 hrs) + dose exposure incubation (48 hrs) = final development (120 hrs)-

The treatment stated in title resulted to alter the NGAG content. The content was increased moderately in cytoplasm and intercellular spaces while significantly high content was observed at cell surfaces in all the three zones of brain as compared to NGAG contents of brain of normal animals at 120 hrs of development.

Initiation at 96 hrs of development

xviii) Initial incubation (96 hrs) + Dose exposure incubation (24 hrs) = final development (120 hrs)-

NGAG content of cytoplasm and intercellular spaces and at the cell surfaces in all three zones of brain as compared to the NGAG content observed in brain of 120 hrs developed normal embryo.

Initiation at 120 hrs of development

ix) Initial incubation (120 hrs) + Dose exposure incubation (24 hrs) = Final development (144 hrs)-

The above stated treatment increased the content of NGAG significantly in cytoplasm of cells in ependymal and mantle layer. But traced content remained at cell surface and intracellular spaces of ependymal and mantle layer and marginal layer as compared to the NGAG contents reported in brain of normal embryo at 144 hrs of development.

0.5 mM H_2O_2 + 3 mg vitamin C treated:

i) Initial incubation (24 hrs) + Dose exposure incubation (24 hrs) = Final development (48 hrs)-

Moderate content of NGAGs was noted in the cytoplasm and intercellular space of all the regions of the brain. Similarly cell surface of neuroepithelial cells.

ii) Initial incubation (24 hrs) + Dose exposure incubation (48 hrs) = Final development (72 hrs)-

The result obtained showed the moderate content of NGAG in cytoplasm in the three zones of intercellular space and at cell surface of the cells distributed.

iii) Initial incubation (24 hrs) + Dose exposure incubation (72 hrs) = Final development (96 hrs)-

Weak content of NGAGs were obtained in the cytoplasm, intercellular space and in the cell surface of the three zones of different five regions of brain.

iv) Initial incubation (24 hrs) + Dose exposure incubation (96 hrs) = Final development (120 hrs)-

Staining technique in the Table A iv) confirmed the weak content of NGAGs both in the cytoplasm and intercellular space along with the distribution at the cell surface in cell coat.

Initiation at 34 hrs of development

v) Initial incubation (34 hrs) + Dose exposure incubation (24 hrs) = Final development (58 hrs) –

Moderate NGAGs content was noted in cytoplasm and intercellular spaces within the three zones of different five regions of brain. Similarly, the content of animal cell surface was significantly increased.

vi) Initial incubation (34 hrs) + Dose exposure incubation (48 hrs) = Final development (82 hrs) –

Moderate amount NGAGs was recorded in cytoplasm intercellular space and in cell surfaces. This was true incase of the three zones of the five different regions of brain.

vii) Initial incubation (34 hrs) + Dose exposure incubation (72 hrs = Final development (106 hrs) –

Trace amount of NGAGs were noted at the three different sites i. e. cytoplasm, intercellular space and at the cell surface in the cell coat. All these distribution sites in three different zones of five brain regions showed similarly in their content.

viii) Initial incubation (34 hrs) + Dose exposure incubation (96 hrs) = Final development (130 hrs) –

There was no alteration in content and distribution of NGAGs that were observed in the different zones of five brain regions on normal embryo at 130 hrs of development.

PLATE IV

PLATE I- Initial incubation (48 hrs) + Dose exposure incubation (72 hrs) = Final development (120 hrs).

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen .- Neuroepithelial layer present. Compactly arranged cells without cell surfaces, intensely stained basophilic nuclei.

Fig. 2 - Meten. - Cell compactly arranged without intercellular spaces, basophilic nuclei.

Fig. 3 - Myelen - Cells compactly arranged without intercellular spaces intensely stained basophilic nuclei.

Fig. 4 - Dien - Compactly arranged cells without intercellular spaces.

Fig. 5 - Telen. - Compactly arranged cell without intercellular spaces.

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000.

Fig. 6 - Mesen. - Cells compactly arranged with little intercellular spaces. Foggy cells towards the luminal side.

Fig. 7 - Meten. - Cells compactly arranged, foggy cell towards the luminal side.

Fig. 8 - Myelen - Cells compactly arranged, foggy cells towards the luminal side

Fig. 9 - Dien - Cells compactly arranged without intercellular spaces, foggy cell towards the luminal side.

Fig. 10 - Telen- Cells compactly arranged with foggy appearance.

Fig. 10-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen - Cells compactly arranged without intensely stained nuclei.

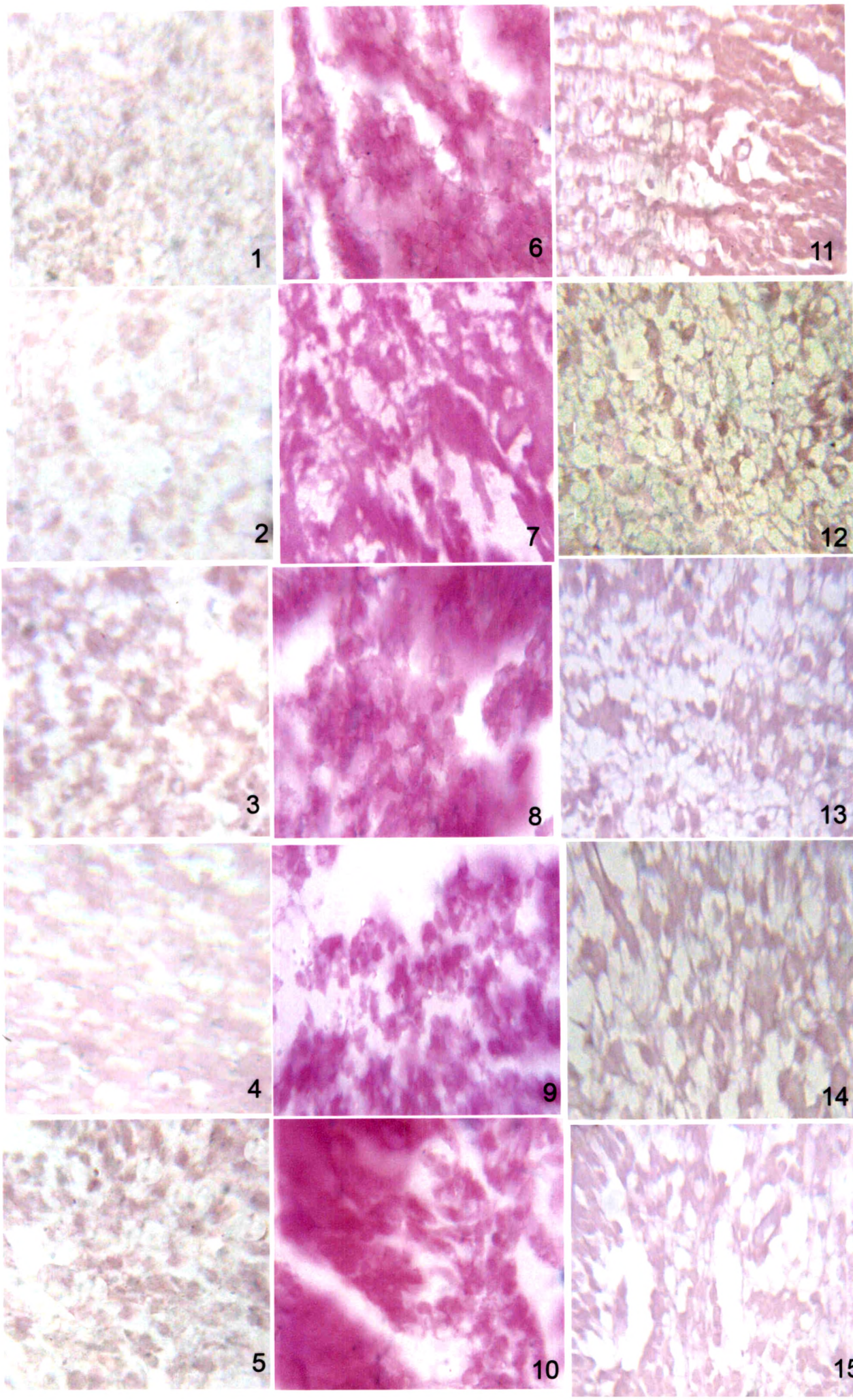
Fig. 12 - Meten. - Cells compactly arranged not well marked cell boundaries.

Fig. 13 - Myelen - Cells compactly arranged without cell boundaries.

Fig. 14 - Dien - Cells compactly arranged without well stained cells.

Fig. 15 - Telen. - Cells compactly arranged without intercellular spaces. No well marked cell boundaries.

PLATE IV



Initiation at 40 hrs of development

ix) Initial incubation 40 hrs + Dose exposure incubation 40 hrs = Final development (64 hrs) –

Moderate content of NGAGs was observed at cell surface but in remaining two sites i. e. cytoplasm and intercellular spaces, the content was in trace amount.

x) Initial incubation 40 hrs + Dose exposure incubation 48 hrs = Final development (88 hrs) –

Weak NGAGs content was noted at all the sites of their presence that was marginally high over. The NGAGs content reported at these sites in normal embryo in brain at 88 hrs development.

xi) Initial incubation (40 hrs) + Dose exposure incubation (72 hrs) = Final development (112 hrs)-

The amount of NGAGs did not alter from the content that was reported in the brain regional zones of normal embryo at 112 hrs of development.

xii) Initial incubation (40 hrs) + Dose exposure incubation (96 hrs) = Final developmental (136 hrs) –

The treatment schedule given to the normal developing embryo did not change the distribution of NGAGs in the three localizations where they used to find and also the amount as compared to the NGAGs content noted in normal embryo brain at 136 hrs of development.

Initiation at 48 hrs of development

xii) Initial incubation (48 hrs) + Dose exposure incubation (24 hrs) = Final development (72 hrs)-

Marginal high content of NGAGs was noted in cytoplasm, intercellular spaces and in the cell surface were observed in all the three zones of brain regions as compared to NGAGs content at the same localization in brain of normal embryo in 72 hrs development.

xiv) Initial incubation (48 hrs) + Dose exposure incubation (48 hrs) = Final development (96 hrs)-

The distribution of NGAGs at different localization viz. cytoplasm, intercellular space and cell surfaces in three zones of five regions of embryonic brain was similar to the contents of NGAGs at corresponding localizations of brain in embryo of 96 hrs of development.

xv) Initial incubation (48 hrs) + Dose exposure incubation (72 hrs) = Final development (120 hrs)-

The content and distribution of NGAGs at different localization observed in brain of normal embryo at 120 hrs of development were not altered under the experimental schedule stated (xv).

Initiation at 72 hrs of development

xvi) Initial incubation (72 hrs) + Dose exposure incubation (24 hrs) = Final development (96 hrs)-

The content of NGAGs was marginally increased at the localizations stated earlier over the contents of NGAG reported in the corresponding sites in brain of embryo at 96 hrs of development.

xvii) Initial incubation (72 hrs) + Dose exposure incubation (48 hrs) = Final development (120 hrs)-

The content of NGAGs observed in three zones of five brain regions of normal embryo at 120 hrs of development remained red in the brain of embryos treated under above stated experimental schedule.

Initiation at 96 hrs of development

xviii) Initial incubation (96 hrs) + Dose exposure incubation (24 hrs) = Final development 120 hrs-

Marginally increased NGAGs content were noted in the cytoplasm, intercellular space and at the cell surface as compared to the NGAGs contents of zones and regions of brain of chick embryo at 120 hrs of development.

Initiation at 120 hrs of development

xix) Initial incubation 120 hrs + Dose exposure incubation 24 hrs = Final development (144 hrs) –

The content and distribution remained unaltered in the different localizations in brain of embryo at 144 hrs of development under above stated experimental schedule at 144 hrs of development.

Discussion:

Following intervals (48 hrs) showed deplete in content while more prolong intervals (72 hrs, 96 hrs), showed sustained increased content indicating acute cellular responses to sustained stress.

Thus, cells have behaved independently producing NGAGs so that they can protect themselves from increased free radicals.

PLATE V

PLATE I- Initial incubation (72 hrs) + Dose exposure incubation (24 hrs) = Final development (96 hrs).

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen .- Neuroepithelial layer present. Compactly arranged cells without cell surfaces, intensely stained basophilic nuclei.

Fig. 2 - Meten. - Cell compactly arranged without intercellular spaces, basophilic nuclei.

Fig. 3 - Myelen - Cells compactly arranged without intercellular spaces intensely **stained basophilic nuclei.**

Fig. 4 - Dien - Compactly arranged cells without intercellular spaces.

Fig. 5 - Telen. - Compactly arranged cell without intercellular spaces.

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000. Increased magenta color

Fig. 6 - Mesen. - Cells compactly arranged with little intercellular spaces. Foggy cells towards the luminal side.

Fig. 7 - Meten. - Cells compactly arranged, foggy cell towards the luminal side.

Fig. 8 - Myelen - Cells compactly arranged, foggy cells towards the luminal side

Fig. 9 - Dien - Cells compactly arranged without intercellular spaces, foggy cell towards the luminal side.

Fig. 10 - Telen- Cells compactly arranged with foggy appearance.

Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen - Cells compactly arranged without intensely stained nuclei.

Fig. 12 - Meten. - Cells compactly arranged not well marked cell boundaries.

Fig. 13 - Myelen - Cells compactly arranged without cell boundaries.

Fig. 14 - Dien - Cells compactly arranged without well stained cells.

Fig. 15 - Telen. - Cells compactly arranged without intercellular spaces. No well marked cell boundaries.

PLATE V

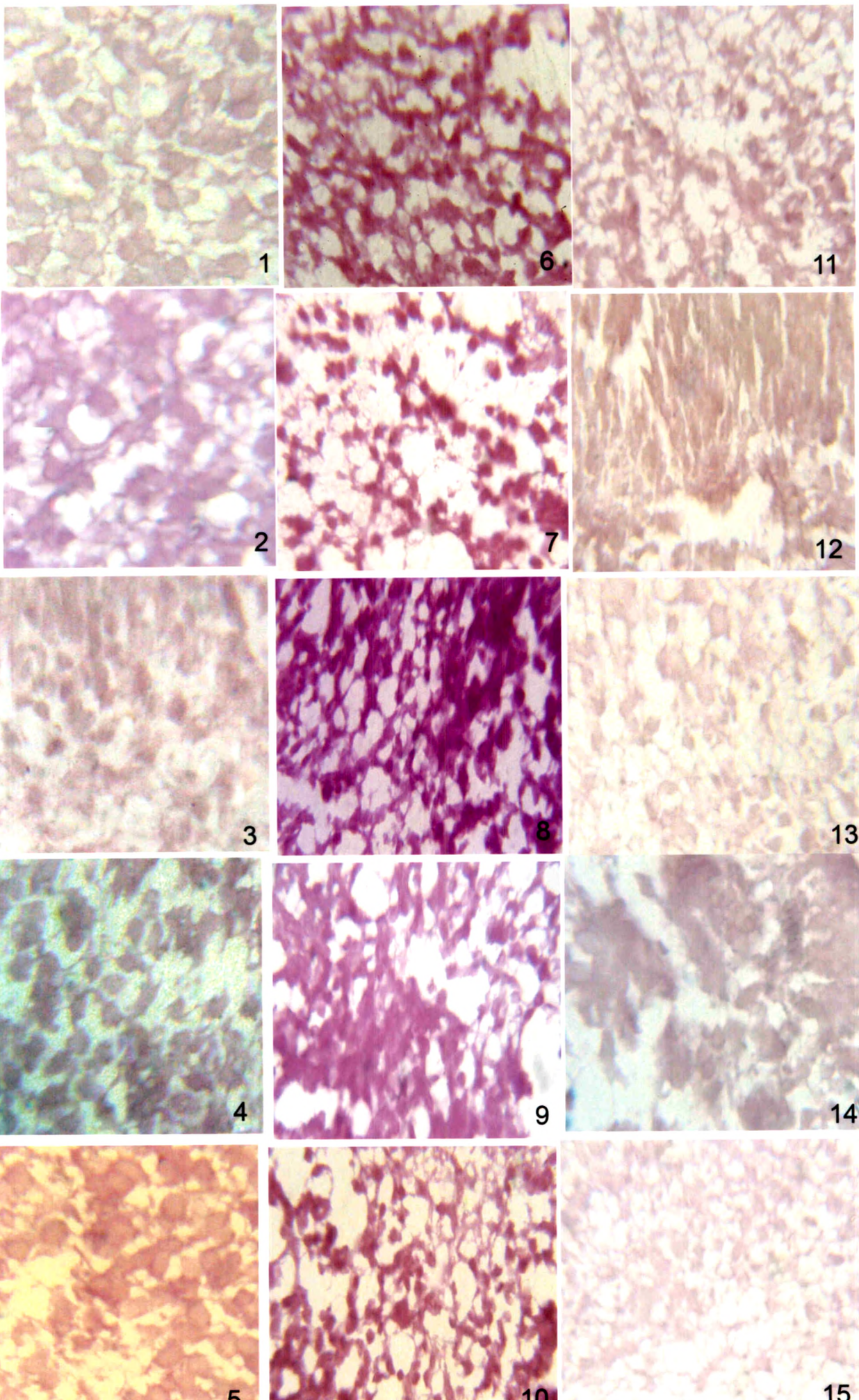


PLATE VI

PLATE VI- Initial incubation (72 hrs) + Dose exposure incubation (48 hrs) = Final development (120 hrs).

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen .- Neuroepithelial layer present. Compactly arranged cells without cell surfaces, intensely stained basophilic nuclei.

Fig. 2 - Meten. - Cell compactly arranged without intercellular spaces, basophilic nuclei.

Fig. 3 - Myelen - Cells compactly arranged without intercellular spaces intensely stained basophilic nuclei.

Fig. 4 - Dien - Compactly arranged cells without intercellular spaces.

Fig. 5 - Telen. - Compactly arranged cell without intercellular spaces.

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000.

Fig. 6 - Mesen. - Cells compactly arranged with little intercellular spaces. Foggy cells towards the luminal side.

Fig. 7 - Meten. - Cells compactly arranged, foggy cell towards the luminal side.

Fig. 8 - Myelen - Cells compactly arranged, foggy cells towards the luminal side

Fig. 9 - Dien - Cells compactly arranged without intercellular spaces, foggy cell towards the luminal side.

Fig. 10 - Telen- Cells compactly arranged with foggy appearance.

Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen - Cells compactly arranged without intensely stained nuclei.

Fig. 12 - Meten. - Cells compactly arranged not well marked cell boundaries.

Fig. 13 - Myelen - Cells compactly arranged without cell boundaries.

Fig. 14 - Dien - Cells compactly arranged without well stained cells.

Fig. 15 - Telen. - Cells compactly arranged without intercellular spaces. No well marked cell boundaries.

PLATE VI

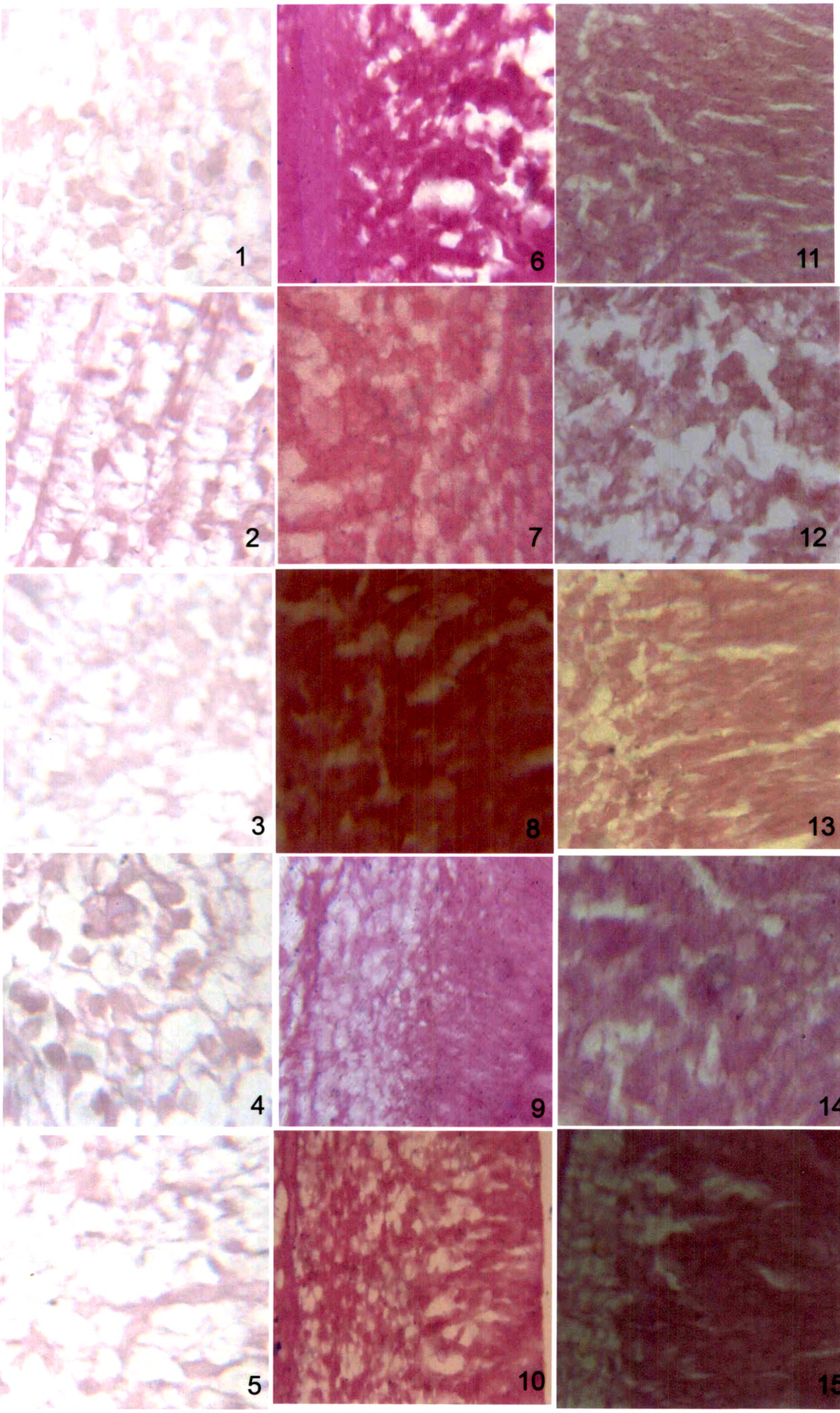


PLATE VII

PLATE VII- Initial incubation (96 hrs) + Dose exposure incubation (24 hrs) = Final development (120 hrs).

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen .- Neuroepithelial layer present. Compactly arranged cells without cell surfaces, intensely stained basophilic nuclei.

Fig. 2 - Meten. - Cell compactly arranged without intercellular spaces, basophilic nuclei.

Fig. 3 - Myelen - Cells compactly arranged without intercellular spaces intensely stained basophilic nuclei.

Fig. 4 - Dien - Compactly arranged cells without intercellular spaces.

Fig. 5 - Telen. - Compactly arranged cell without intercellular spaces.

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000. increased vacuolation

Fig. 6 - Mesen. - Cells compactly arranged with little intercellular spaces. Foggy cells towards the luminal side.

Fig. 7 - Meten. - Cells compactly arranged, foggy cell towards the luminal side.

Fig. 8 - Myelen - Cells compactly arranged, foggy cells towards the luminal side

Fig. 9 - Dien - Cells compactly arranged without intercellular spaces, foggy cell towards the luminal side.

Fig. 10 - Telen- Cells compactly arranged with foggy appearance.

Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen - Cells compactly arranged without intensely stained nuclei.

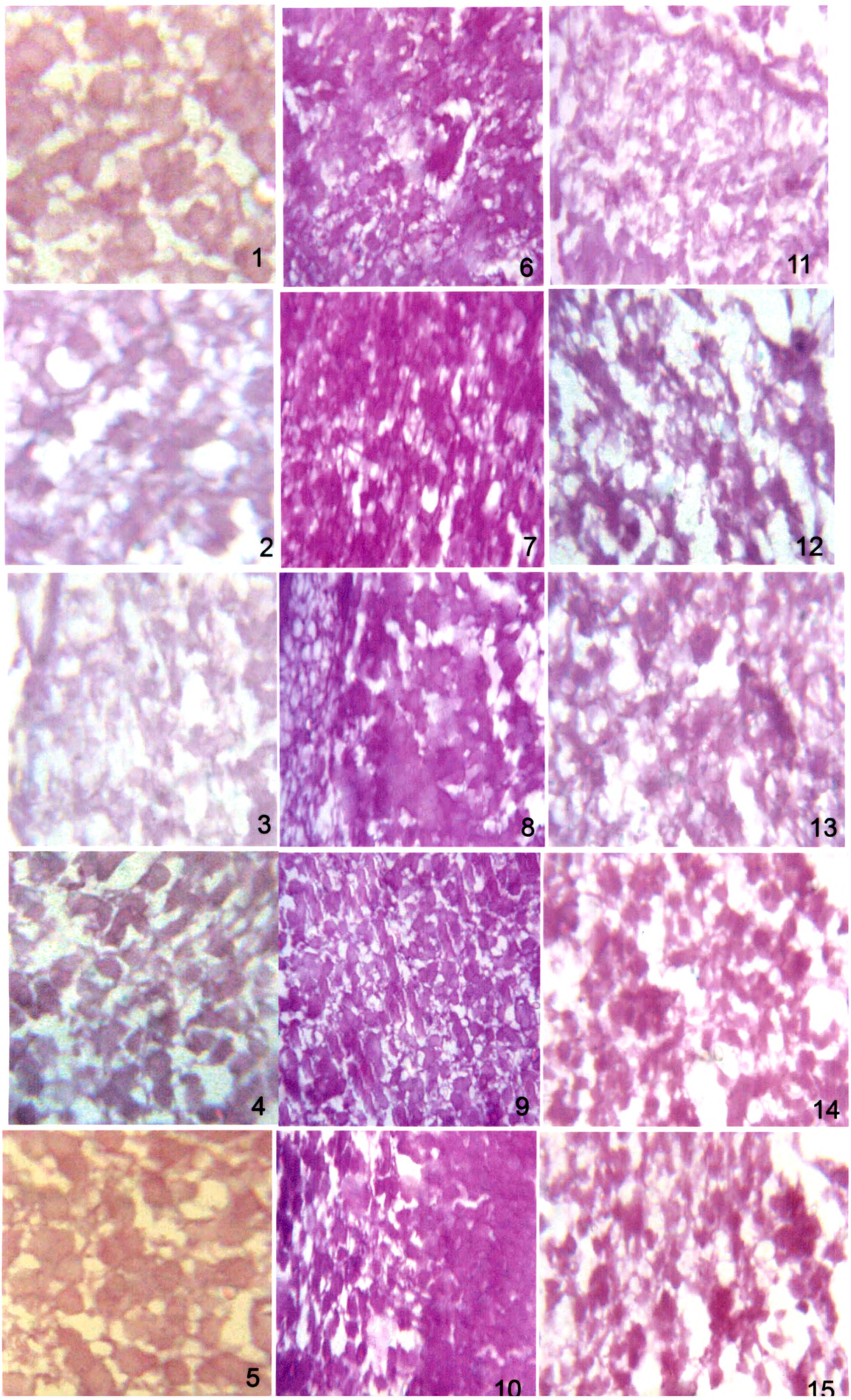
Fig. 12 - Meten. - Cells compactly arranged not well marked cell boundaries.

Fig. 13 - Myelen - Cells compactly arranged without cell boundaries.

Fig. 14 - Dien - Cells compactly arranged without well stained cells.

Fig. 15 - Telen. - Cells compactly arranged without intercellular spaces. No well marked cell boundaries.

PLATE VII



Besides cell may have been ready with the substrates required for the further development i.e. heparin sulfates, chondratin sulfates which are present in developing chick embryo during brain development (Sandalis Spyross *et. al.*, 2003 and Sachiko Aono *et. a*l. 2004), in all parts of the developing brain and in the intercellular spaces, cell surface an cytoplasm (ER and Golgi apparatus). Because the sulfation step in biosynthesis of the sulfated glycosaminoglycans involves N-deacetylation and N-sulfation of N-acetylglucosamine residues and 2-o sulfation of adjacent iduronic acid residues (Habunehi *et. al.* 1992, Brickmane *et. al.* , 1998) and also 6-0 sulfation of N-sulfated glucosamine residues (Kan *et. al.* 1993). These sulfation that occure by substitutions in ER and Golgi because of the enzymes involved are their residential proteins NGAG or their fractions which may be in the process of formation of these GAGs and therefore cell may have been ready with it and it is immediately available immediately after exposure and then may have been synthesized on prolong exposure. Sulfation induced metachromacia at all sites of NGAG distribution have correspondingly weighed metachromacia (Sulfur induced metachromacia) in cytoplasm (ER and Golgi may be in process of maturation and synthesis and sulfation).

At 144 hrs, weak content of NGAG (analyzed by 1-9 techniques _Table no. I) was confirmed at different localizations in cytoplasm, intercellular spaces and at cell surfaces. It was increased to mode rate distribution on Phenylhydrazine block hydrolysis by saponification , acetylation block followed by deacetylation and only saponification indicating in addition to weak content of acetylated NGAG were present since on saponification Amylase digestion-PAS reaction was enhanced. This was true in cells distributed in ependymal, mantle zones and cell surfaces in marginal zone. The distribution and content was present at all the five brain regions viz. mesencephalon, metencephalon, myelencephalon, diencephalons and telencephalon.

In H_2O_2 treated embryos irrespective of the initiation of treatment at hrs the content of NGAG was increased of treatment after 24 hrs of exposure intervals at all three sites observed in normal conditions. The reaction intensities weighted indicating 50% increase in content at all the sites and under all the above stated experimental schedules.

Similarly irrespective of the initiation hrs of the H_2O_2 treatment when exposure given was of 48 hrs the reaction was obliterated with 50% decrease in

content (as weighted visually) in cytoplasm and with 25% decrease in content in at cell surfaces and intercellular spaces.

On 72 hrs exposure interval and irrespective of the H_2O_2 dose initiation the NGAG content was increased at all the three sites observed by 75% (as weighted visually) and which remained with same intensity (Content) at remaining prolong intervals.

These results indicated that the content of NGAG was increased in immediate hrs of an exposure indicating immediate stress response
(Campo G M *et. a*l. 2004).

Simultaneously given 3 mg vitamin C managed the alterations in NGAG irrespective of the initiation hrs of H_2O_2 treatment (along with 3 mg vitamin C) or the length of hrs of exposure time except H_2O_2 induced observed effects were observed after treatment of 24 hrs which may have been cleared in successive steps of development.

B) Sulfated glycosaminoglycans:

Observations:

Alterations in SGAGs are depicted in Plate no I- VII and Table no I a-Id

Normal:

48 hrs development:

Trace amount of sulfated GAGs was present at the cytoplasm and intercellular spaces of the neruoepithelial cells; weak amount was noted at the cell surface of the five brain regions.

58 hrs development-

Sulfated GAGs was noted in trace amount in the cytoplasm, weak at the cell surface and moderate amount was noted at the intercellular space of the neuroepithelial cells of the five brain regions.

64 hrs development-

Trace amount of sulfated GAGs was noted in the cytoplasm, weak at the cell surfaces and moderate at the intercellular spaces of the ependymal, mantle, and marginal zone of the five brain regions.

72 hrs development-

Trace amount of sulfated GAGs was noted in the cytoplasm while moderate amount was noted at the cell surface, intercellular spaces and marginal zone of the

brain regions viz. mesencephalon, metencephalon, myelencephalon, diencephalons, and telencephalon.

82 hrs development-

Trace amount was noted in the cytoplasm, moderate was noted at the cell surface, intercellular space and the marginal layer of the ependymal, mantle and marginal zone of the five brain regions.

88 hrs development-

The amount of sulfated GAGs was noted in trace amount in the cytoplasm while the moderate amount was noted at the cell surface, intercellular space and marginal zone of the three regions considered for the five brain regions.

96 hrs development-

Cytoplasm of the neuroepithelial cells of ependymal and mantle zone showed the trace amount of sulfated GAGs while the cell surface, intercellular space and the marginal zone observed with the moderate amount of sulfated GAGs.

106 hrs development-

Sulfated GAGs was present in the cytoplasm of the neuroepithelial cells of the three neuronal cells showed trace amount. The cell surface, intercellular space and marginal zone showed the moderate amount of GAGs in all five-brain regions studied.

112 hrs development-

Moderate amount of sulfated GAGs was observed at the cell surface, intercellular space and the marginal zone of the five brain regions. Cytoplasm of the ependymal, mantle zone contains the trace amount of sulfated GAGs.

120 hrs development-

The amount of sulfated GAGs was present in the cytoplasm of the neuronal lass was trace while at the cell surface, intercellular space and marginal layer contains the moderate amount of sulfated GAGs in the five brain regions.

130 hrs development-

The amount of sulfated GAGs was similar to that was noted visually for the 1210 hrs of normal development. Trace amount was noted in the cytoplasm, moderate at the cell surface, intercellular space and the marginal zone of the five brain vesicles.

136 hrs development-

Trace amount was noted in the cytoplasm, moderate at the cell surface, intercellular space and marginal zone of the five brain vesicles.

144 hrs development-

Weak amount was noted in the cytoplasm which was increased moderately than the other developmental hours considered while the cell surface, intercellular space and the marginal zone of the five brain vesicles.

Control: HBSS-

The amount of sulfated GAGs was similar to that was noted in the normal developmental hour studied for the normal or untreated group of embryos. So the data in not presented here.

Control: 3 mg vitamin C-

Control 3 mg vitamin C studied for the considered developmental group of embryos showed equal amount of sulfated GAGs as noted in the normal developmental hour. So the data is not presented here.

0.5 mM H_2O_2 treated

Initiation at 24 hrs of development

i) Initial incubation (24 hrs) + Dose exposure incubation (24 hrs)= Final development (48 hrs) -

Moderate increase n the amount of sulfated GAGs was noted at the cell surface, intercellular space and marginal zone of the brain regions. While the amount of cytoplasm remains unchanged.

ii) Initial incubation (24 hrs) + Dose exposure incubation (48 hrs) = Final development (72 hrs) –

Trace amount of sulfated GAGs was noted in the cytoplasm, moderate amount was noted at the cell surface while intercellular space and marginal layer showed the significantly increased amount of the sulfated GAGs was noted.

iii) Initial incubation (24 hrs) + Dose exposure incubation (72 hrs) = Final developmental (96 hrs) –

Moderate amount of sulfated GAGs was noted in the cytoplasm, significant amount was increased at the cell surface, intercellular space and marginal zone of the five brain vesicles.

iv) Initial incubation (24 hrs) + Dose exposure incubation (96 hrs) = Final development (120 hrs) –

The result of the group iii remain s same with moderate increase in the cytoplasm and significant increase at the cell surface, intercellular space of the ependymal, mantle and marginal zone of the five brain vesicles.

Sulfated GAGs.

Table no. I .a Initial incubation (24 hrs) + exposure incubation (24 hrs) =Final incubation (48 hrs).

Staining technique	Brain Region	Normal				0.5 mM H_2O_2 treated			
		Neuroepithelial layer				Neuroepithelial layer			
		Cyt	Cs	Ics	Inference to observations	Cyt	Cs	Ics	Inference to observations
1)AB pH 1	Mesen	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces.	+	++	++	Weak coloration was noted in the Cyt, moderate intensity of coloration was noted at the Cs and Ics
	Meten	±	+	±		+	++	++	
	Myelan	±	+	±		+	++	++	
	Dien	±	+	±		+	++	++	
	Telcn	±	+	±		+	++	++	
2) AF	Mesen	+	±	±	Weak coloration was noted at the Cs and Ics while weak color intensity was noted in Cyt	+	++	++	Weak coloration was noted in the Cyt, moderate intensity of coloration was noted at the Cs and Ics
	Meten	+	±	±		+	++	++	
	Myelan	+	±	±		+	++	++	
	Dien	+	±	±		+	++	++	
	Telen	+	±	±		+	++	++	
3) AF-AB 2.5	Mesen	+P	+B	±P	Purple weak intensity of coloration was noted in Cyt, weak blue coloration at Cs and trace purple at Ics	+P	++B	++P	Weak purple in Cyt. Moderate blue at Cs and purple coloration at the Ics
	Meten	+P	+B	±P		+P	++B	++P	
	Myelan	+P	+B	±P		+P	++B	++P	
	Dien	+P	+B	±P		+P	++B	++P	
	Telen	+P	+B	±P		+P	++B	++P	
4)Alcian blue at pH5.6 With graded concentrations of $MgCl_2$									
i) 0.0 M	Mesen	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces.	+	++	++	Weak coloration was noted in the Cyt, moderate intensity of coloration was noted at the Cs and Ics
	Meten	±	+	±		+	++	++	
	Myelan	±	+	±		+	++	++	
	Dien	±	+	±		+	++	++	
	Telen	±	+	±		+	++	++	
ii) 0.1 M	Mesen	+	±	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces.	+	++	++	Weak coloration was noted in the Cyt, moderate intensity of coloration was noted at the Cs and Ics
	Meten	±	+	±		+	++	++	
	Myelan	±	+	±		+	++	++	
	Dien	±	+	±		+	++	++	
	Telen	±	+	±		+	++	++	

Table no. I. a Initial incubation (24 hrs) + exposure incubation (24 hrs) =Final incubation (48 hrs) continued.....

Staining technique	Brain zones	Normal				0.5 mM H2O2			
		Neuroepithelial layer				Neuroepithelial layer			
		Cyt	Cs	Ics	Inference to observations	Cyt	Cs	Ics	Inference to observations
iii) 0.2 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
iv) 0.4 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
v) 0.5 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
vi) 0.6 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
vii) 0.8 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	

Table no.I.a Initial incubation (24 hrs) + Dose re incubation (24 hrs) =Final incubation (48 hrs) continued.....

viii) 1.0 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
5) Azure A metachromecia at different pH levels									
i)pH 0.5	Mesen	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces	+	++	++	Weak coloration was noted in the Cyt, moderate intensity of coloration was noted at the Cs and Ics
	Meten	±	+	±		+	++	++	
	Myelan	±	+	±		+	++	++	
	Dien	±	+	±		+	++	++	
	Telen	±	+	±		+	++	++	
ii)pH 1.0	Mesen	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces	+	++	++	Weak coloration was noted in the Cyt, moderate intensity of coloration was noted at the Cs and Ics
	Meten	±	+	±		+	++	++	
	Myelan	±	+	±		+	++	++	
	Dien	±	+	±		+	++	++	
	Telen	±	+	±		+	++	++	
iii) pH 1.5	Mesen	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces	+	++	++	Weak coloration was noted in the Cyt, moderate intensity of coloration was noted at the Cs and Ics
	Meten	±	+	±		+	++	++	
	Myelan	±	+	±		+	++	++	
	Dien	±	+	±		+	++	++	
	Telen	±	+	±		+	++	++	
6) AZ 4.5	Mesen	-	±	±	Absence color intensity in Cyt, Trace coloration at Cs and Ics	±	++	++	Increased color intensity at Cs and Ics
	Meten	-	±	±		±	++	++	
	Myelan	-	±	±		±	++	++	
	Dien	-	±	±		±	++	++	
	Telen	-	±	±		±	++	++	

Table no. I a Initial incubation (24 hrs) + Dose re incubation (24 hrs) =Final incubation (48 hrs) CONTINUED.....

Staining technique	Brain Region	0.5 mM H_2O_2 treated +3mg vitamin C treated				Control 3mg vitamin c treated			
		Neuroepithelial layer				Neuroepithelial layer			
		Cyt	Cs	Ics	Inference to observations	Cyt	Cs	Ics	Inference to observations
1)AB pH 1	Mesen	±	++	++	Trace reaction was noted in the Cyt, moderate coloration was noted at Cs and Ics	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces.
	Meten	±	++	++		±	+	±	
	Myelan	±	++	++		±	+	±	
	Dien	±	++	++		±	+	±	
	Telen	±	++	++		±	+	±	
2) AF	Mesen	±	++	++	Trace reaction was noted in the Cyt, moderate coloration was noted at Cs and Ics	+	±	±	Weak coloration was noted at the Cs and Ics while weak color intensity was noted in Cyt
	Meten	±	++	++		+	±	±	
	Myelan	±	++	++		+	±	±	
	Dien	±	++	++		+	±	±	
	Telen	±	++	++		+	±	±	
3) AF-AB 2.5	Mesen	±P	++B	++P	Weak purple in Cyt. Moderate blue at Cs and purple coloration at the Ics	+P	+B	±P	Purple weak intensity of coloration was noted in Cyt, weak blue coloration at Cs and trace purple at Ics
	Meten	±P	++B	++P		+P	+B	±P	
	Myelan	±P	++B	++P		+P	+B	±P	
	Dien	±P	++B	++P		+P	+B	±P	
	Telen	±P	++B	++P		+P	+B	±P	
4)Alcian blue at pH5.6 With graded concentrations of $MgCl_2$									
i) 0.0 M	Mesen	±	++	+++	Trace reaction was noted in the Cyt, moderate coloration was noted at Cs and Ics	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces.
	Meten	±	++	+++		±	+	±	
	Myelan	±	++	+++		±	+	±	
	Dien	±	++	+++		±	+	±	
	Telen	±	++	+++		±	+	±	
ii) 0.1 M	Mesen	±	++	+++	Trace reaction was noted in the Cyt, moderate coloration was noted at Cs and Ics	+	±	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces.
	Meten	±	++	+++		±	+	±	
	Myelan	±	++	+++		±	+	±	
	Dien	±	++	+++		±	+	±	
	Telen	±	++	+++		±	+	±	

Table no. I a Initial incubation (24 hrs) + Dose re incubation (24 hrs) =Final incubation (48 hrs) continued .....

Staining technique	Brain zones	0.5 mM H_2O_2 treated +3mg vitamin C treated				Control 3mg vitamin c treated			
		Neuroepithelial layer				Neuroepithelial layer			
		Cyt	Cs	Ics	Inference to observations	Cyt	Cs	Ics	Inference to observations
iii) 0.2 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
iv) 0.4 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
v) 0.5 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
vi) 0.6 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
vii) 0.8 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	

Table no. I a Initial incubation (24 hrs) + Dose re incubation (24 hrs) =Final incubation (48 hrs) continued .....

viii) 1.0 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
5) Azure A metachromecia at different pH levels									
i)pH 0.5	Mesen	±	++	++	Trace reaction was noted in the Cyt, moderate coloration was noted at Cs and Ics	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces
	Meten	±	++	++		±	+	±	
	Myelan	±	++	++		±	+	±	
	Dien	±	++	++		±	+	±	
	Telen	±	++	++		±	+	±	
ii)pH 1.0	Mesen	±	++	++	Trace reaction was noted in the Cyt, moderate coloration was noted at Cs and Ics	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces
	Meten	±	++	++		±	+	±	
	Myelan	±	++	++		±	+	±	
	Dien	±	++	++		±	+	±	
	Telen	±	++	++		±	+	±	
iii) pH 1.5	Mesen	±	++	++	Trace reaction was noted in the Cyt, moderate coloration was noted at Cs and Ics	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces
	Meten	±	++	++		±	+	±	
	Myelan	±	++	++		±	+	±	
	Dien	±	++	++		±	+	±	
	Telen	±	++	++		±	+	±	
6)AZ 4.5	Mesen	-	±	±	Absence color intensity in Cyt, Trace coloration at Cs and Ics	-	±	±	Absence color intensity in Cyt, Trace coloration at Cs and Ics
	Meten	-	±	±		-	±	±	
	Myelan	-	±	±		-	±	±	
	Dien	-	±	±		-	±	±	
	Telen	-	±	±		-	±	±	

Table no. I. b Initial incubation (24 hrs) + Dose exposure (48 hrs) =Final incubation (72 hrs).

Staining Tech.	Brain region	Normal								0.5mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 1	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	++	±	++	++	++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Mye	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Die	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Tel	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
2) AF	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	++	±	++	++	++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Mye	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Die	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Tel	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
3) AF-AB 2.5	Mes	±P	+B	±P	±P	+B	±P	+B	Trace purple in Cyt, trace purple Ics and weak blue at ICs	±P	++B	++P	±P	++B	++P	++P	Trace purple in cyt, moderate blue at Cs purple at Ics ML
	Met	±B	+B	±P	±P	+B	±P	+B		±P	++B	++P	±P	++B	++P	++P	
	Mye	±P	+B	±P	±P	+B	±P	+B		±P	++B	++P	±P	++B	++P	++P	
	Die	±P	+B	±P	±P	+B	±P	+B		±P	++B	++P	±P	++B	++P	++P	
	Tel	±P	+B	±P	±P	+B	±P	+B		±P	++B	++P	±P	++B	++P	++P	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2																	
i) 0.0M	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	++	±	++	++	++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Mye	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Die	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Tel	±	+	±	±	+	±	+		±	++	++	±	++	++	++	

Table no. I. b Initial incubation (24 hrs) + Dose exposure (48 hrs) =Final incubation (72 hrs). continued.....

ii) 0.1 M	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	++	±	++	++	++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Mye	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Die	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Tel	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
iii) 0.2M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iv) 0.4M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
v)0.5M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
vi)0.6M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. I. b Initial incubation (24 hrs) + Dose exposure (48 hrs) =Final incubation (72 hrs). continued.....

ii) 0.8 M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iii) 1.0M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
5) Azure A metachromecia at different pH levels																	
i)pH 0.5	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	++	±	++	++	++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Mye	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Die	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Tel	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
ii)pH 1.0	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	++	±	++	++	++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Mye	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Die	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Tel	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
iii) pH 1.5	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	++	±	++	++	++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Mye	±	+	±	±	+	⊥	+		±	++	++	±	++	++	++	
	Die	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Tel	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
6) AZ 4.5	Mes	±	+	+	±	+	+	+	Trce color intensity in Cyt, weak at Cs and ics	±	++	++	±	++	++	++	Trace color in Cyt ,moderate intensity at Cs and Ics
	Met	±	+	+	±	+	+	+		±	++	++	±	++	++	++	
	Mye	±	+	+	±	+	+	+		±	++	++	±	++	++	++	
	Die	±	+	+	±	+	+	+		±	++	++	⊥	++	++	++	
	Tel	±	+	+	±	+	+	+		±	++	++	±	++	++	++	

Table no. I. b Initial incubation (24 hrs) + Dose exposure (48 hrs) =Final incubation (72 hrs). continued.....

Initial incubation (24 hrs) + exposure incubation (48 hrs) =Final incubation (72 hrs).																	
Staining Tech.	Brain region	0.5mM H_2O_2 x+ 3mg vitamin C treated								Control 3mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 1	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
2) AF	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
3) AF-AB 2.5	Mes	±P	+B	±P	±P	+B	±P	+B	Trace purple in Cyt, trace purple Ics and weak blue at ICs	±P	+B	±P	±P	+B	±P	+B	Trace purple in Cyt, trace purple Ics and weak blue at ICs
	Met	±B	+B	±P	±P	+B	±P	+B		±B	+B	±P	±P	+B	±P	+B	
	Mye	±P	+B	±P	±P	+B	±P	+B		±P	+B	±P	±P	+B	±P	+B	
	Die	±P	+B	±P	±P	+B	±P	+B		±P	+B	±P	±P	+B	±P	+B	
	Tel	±P	+B	±P	±P	+B	±P	+B		±P	+B	±P	±P	+B	±P	+B	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2																	
i) 0.0 M	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	

Table no. I. b Initial incubation (24 hrs) + Dose exposure (48 hrs) =Final incubation (72 hrs). continued.....

ii) 0.1 M	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
iii) 0.2M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Myc	-		-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iv) 0.4M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
v)0.5M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
vi)0.6M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. I. b Initial incubation (24 hrs) + Dose exposure (48 hrs) =Final incubation (72 hrs). continued.....

ii) 0.8 M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iii) 1.0M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
5) Azure A metachromecia at different pH levels																	
i)pH 0.5	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
ii)pH 1.0	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
iii) pH 1.5	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	+	+	±	+		±	+	±	±	+	±	+	
6) AZ 4.5	Mes	±	+	+	±	+	+	+	Trace to weak coloration observed	±	+	+	±	+	+	+	Trace to weak coloration observed
	Met	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Mye	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Die	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Tel	±	+	+	±	+	+	+		±	+	+	±	+	+	+	

Table no. I. c Initial incubation (24 hrs) + exposure incubation (72 hrs) =Final incubation (96 hrs).

Staining Tech.	Brain region	Normal								0.5mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 1	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	++	+++	+++	++	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
2) AF	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit	++	+++	+++	++	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
3) AF-AB 2.5	Mes	±P	++B	±P	±P	++B	±P	++B	Trace purple in Cyt. Moderate blue at Cs and Ml	++P	+3B	+3B	++	+3B	+3B	+3B	Staining increases in intensity at all sites considered
	Met	±P	++B	±P	±P	++B	±P	++B		++P	+3B	+3B	++	+3B	+3B	+3B	
	Mye	±P	++B	±P	±P	++B	±P	++B		++P	+3B	+3B	++	+3B	+3B	+3B	
	Die	±P	++B	±P	±P	++B	±P	++B		++P	+3B	+3B	++	+3B	+3B	+3B	
	Tel	±P	++B	±P	±P	++B	±P	++B		++P	+3B	+3B	++	+3B	+3B	+3B	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2																	
i) 0.0 M	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit	++	+++	+++	++	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	

Table no. I. c Initial incubation (24 hrs) + exposure incubation (72 hrs) =Final incubation (96 hrs) continued .....

ii) 0.1 M	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit	++	+++	+++	++	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
iii) 0.2M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iv) 0.4M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
v)0.5M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
vi)0.6M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tcl	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. I. c Initial incubation (24 hrs) + exposure incubation (72 hrs) =Final incubation (96 hrs) continued .....

ii) 0.8 M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iii) 1.0 M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
5) Azure A metachromecia at different pH levels																	
i)pH 0.5	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	++	+++	+++	++	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
ii)pH 1.0	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	++	+++	+++	++	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
iii) pH 1.5	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	++	+++	+++	++	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
6) AZ 4.5	Mes	±	+	+	±	+	+	+	Weak to moderate coloration at each sites	±	++	++	±	++	++	++	Moderately increased color insity at sites
	Met	±	+	+	±	+	+	+		±	++	++	±	++	++	++	
	Mye	±	+	+	±	+	+	+		±	++	++	±	++	++	++	
	Die	±	+	+	±	+	+	+		±	++	++	±	++	++	++	
	Tel	±	+	+	±	+	+	+		±	++	++	±	++	++	++	

Table no. I. c Initial incubation (24 hrs) + exposure incubation (72 hrs) =Final incubation (96 hrs). continued.....

Initial incubation (24 hrs) + exposure incubation (72 hrs) =Final incubation (96 hrs).																		
Staining Tech.	Brain region	0.5mM H_2O_2 x+ 3mg vitamin C treated								Control 3mg vitamin C treated								
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB I	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
2) AF	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
3) AF-AB 2.5	Mes	±P	++B	±P	±P	++B	±P	++B	Trace purple in Cyt. Moderate blue at Cs and Ml	±P	++B	±P	±P	++B	±P	++B	Trace purple in Cyt. Moderate blue at Cs and Ml
	Met	±P	++B	±P	±P	++B	±P	++B		±P	++B	±P	±P	++B	±P	++B	
	Mye	±P	++B	±P	±P	++B	±P	++B		±P	++B	±P	±P	++B	±P	++B	
	Die	±P	++B	±P	±P	++B	±P	++B		±P	++B	±P	±P	++B	±P	++B	
	Tel	±P	++B	±P	±P	++B	±P	++B		±P	++B	±P	±P	++B	±P	++B	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2																		
i) 0.0 M	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	

Table no. I. c Initial incubation (24 hrs) + exposure incubation (72 hrs) =Final incubation (96 hrs) continued.....

ii) 0.1 M	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
iii) 0.2M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iv) 0.4M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
v)0.5M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
vi)0.6M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. I. c Initial incubation (24 hrs) + exposure incubation (72 hrs) =Final incubation (96 hrs) continued.....

ii) 0.8 M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iii) 1.0M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
5) Azure A metachromecia at different pH levels																	
i)pH 0.5	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
ii)pH 1.0	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
iii) pH 1.5	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
6) AZ 4.5	Mes	±	+	+	±	+	+	+	Weak to moderate coloration at cach sites	±	++	++	±	++	++	++	Moderat ncrease in the coloration
	Met	±	+	+	±	+	+	+		±	++	++	±	++	++	++	
	Mye	±	+	+	±	+	+	+		±	++	++	±	++	++	++	
	Die	±	+	+	⊥	+	I	+		±	++	++	±	++	++	++	
	Tel	±	+	+	±	+	+	+		±	++	++	±	++	++	++	

Table noI d Initial incubation (24 hrs) + exposure incubation (96 hrs) =Final incubation (120hrs).

Staining tech.	Brain region	Normal								0.5mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 1	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	+++	+++	+++	+++	+++	+++	+++	Intense coloration was onserved at all sites studied
	Met	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
2) AF	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	+++	+++	+++	+++	+++	+++	+++	Intense coloration was onserved at all sites studied
	Met	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
3) AF-AB 2.5	Mes	±P	++B	++P	±P	++B	++P	++B	Trace purple in Cyt moderate blue and purple at Cs and Ics	+++	+++	+++	+++	+++	+++	+++	Intense coloration was onserved at all sites studied
	Met	±P	++B	++P	±P	++B	++P	++B		+++	+++	+++	+++	+++	+++	+++	
	Mye	±P	++B	++P	±P	++B	++P	++B		+++	+++	+++	+++	+++	+++	+++	
	Die	±P	++B	++P	±P	++B	++P	++B		+++	+++	+++	+++	+++	+++	+++	
	Tel	±P	++B	++P	±P	++B	++P	++B		+++	+++	+++	+++	+++	+++	+++	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2																	
i) 0.0 M	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	+++	+++	+++	+++	+++	+++	+++	Intense coloration was onserved at all sites studied
	Met	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table noI d Initial incubation (24 hrs) + exposure incubation (96 hrs) =Final incubation (120hrs) continued.....

ii) 0.1 M	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	+++	+++	+++	+++	+++	+++	+++	Intense coloration was onserved at all sites studied
	Met	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
iii) 0. 2 M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iv) 0.4M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
v)0.5M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
vi)0.6M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table noI d Initial incubation (24 hrs) + exposure incubation (96 hrs) =Final incubation (120hrs) continued.....

ii) 0.8 M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iii)1.0 M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Myc	-		-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
5) Azure A metachromecia at different pH levels																	
i)pH 0.5	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	+++	+++	+++	+++	+++	+++	+++	Intense coloration was onserved at all sites studied
	Met	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
ii)pH 1.0	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	+++	+++	+++	+++	+++	+++	+++	Intense coloration was onserved at all sites studied
	Met	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
iii) pH 1.5	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	+++	+++	+++	+++	+++	+++	+++	Intense coloration was onserved at all sites studied
	Met	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
6) AZ 4.5	Mes	±	+	+	±	+	+	+	Trace to weak intensity of coloration	+	++	++	+	++	++	++	Moderate increase in the coloration
	Met	±	+	+	±	+	+	+		+	++	++	+	++	++	++	
	Mye	±	+	+	±	+	+	+		+	++	++	+	++	++	++	
	Die	±	+	+	±	+	+	+		+	++	++	+	++	++	++	
	Tel	±	+	+	±	+	+	+		+	++	++	+	++	++	++	

Table noI d Initial incubation (24 hrs) + exposure incubation (96 hrs) =Final incubation (120hrs) continued.....

Staining Tech.	Brain region	0.5mM H_2O_2 x+ 3mg vitamin C treated								Control 3mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 1	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered
	Met	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Mye	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Die	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Tel	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
2) AF	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered
	Met	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Mye	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Die	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Tel	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
3) AF-AB 2.5	Mes	±P	++B	++P	±P	++B	++P	++B	Trace purple in Cyt moderate blue and purple at Cs and Ics	±P	++B	++P	±P	++B	++P	++B	Intense coloration was onserved at all sites studied
	Met	±P	++B	++P	±P	++B	++P	++B		±P	++B	++P	±P	++B	++P	++B	
	Mye	±P	++B	++P	±P	++B	++P	++B		±P	++B	++P	±P	++B	++P	++B	
	Die	±P	++B	++P	±P	++B	++P	++B		±P	++B	++P	±P	++B	++P	++B	
	Tel	±P	++B	++P	±P	++B	++P	++B		±P	++B	++P	±P	++B	++P	++B	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2																	
i) 0.0 M	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered
	Met	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Mye	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Die	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Tel	±	++	++	±	++	++	++		±	++	++	±	++	++	++	

Table noI d Initial incubation (24 hrs) + exposure incubation (96 hrs) =Final incubation (120hrs) continued.....

ii) 0.1 M	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered
	Met	±	++	++	±	++	++	++		±	++	++	±	++	++	++		
	Mye	±	++	++	±	++	++	++		±	++	++	±	++	++	++		
	Die	±	++	++	±	++	++	++		±	++	++	±	++	++	++		
	Tel	±	++	++	±	++	++	++		±	++	++	±	++	++	++		
iii) 0.2 M	Mes	-	-	-	-	-	-	-	No coloration was observed	-	-	-	-	-	-	-	No coloration was observed	
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
iv) 0.4M	Mes	-	-	-	-	-	-	-	No coloration was observed	-	-	-	-	-	-	-	No coloration was observed	
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
v)0.5M	Mes	-	-	-	-	-	-	-	No coloration was observed	-	-	-	-	-	-	-	No coloration was observed	
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
vi)0.6M	Mes	-	-	-	-	-	-	-	No coloration was observed	-	-	-	-	-	-	-	No coloration was observed	
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Tcl	-	-	-	-	-	-	-		-	-	-	-	-	-	-		

Table noI d Initial incubation (24 hrs) + exposure incubation (96 hrs) =Final incubation (120hrs) continued.....

ii) 0.8 M	Mes	-	-	-	-	-	-	-	No coloration was observed	-	-	-	-	-	-	-	No coloration was observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iii)1.0 M	Mes	-	-	-	-	-	-	-	No coloration was observed	-	-	-	-	-	-	-	No coloration was observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
5) Azurc A metachromecia at different pH levels																	
i)pH 0.5	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered
	Met	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Mye	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Die	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Tel	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
ii)pH 1.0	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites onsidered	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered
	Met	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Mye	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Die	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Tel	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
iii) pH 1.5	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites onsidered	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered
	Met	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Mye	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Dic	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Tel	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
6) AZ 4.5	Mes	±	+	+	±	+	+	+	Trace to weak intensity of coloration	±	+	+	±	+	+	+	Trace to weak intensity of coloration
	Met	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Mye	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Die	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Tel	±	+	+	±	+	+	+		±	+	+	±	+	+	+	

Table no. IV. a Initial incubation (48hrs) + Dose exposure incubation (24 hrs) + Final development (72 hrs).

Staining technique	Brain Region	Normal				0.5 mM H_2O_2 treated			
		Neuroepithelial layer				Neuroepithelial layer			
		Cyt	Cs	Ics	Inference to observations	Cyt	Cs	Ics	Inference to observations
1)AB pH 1	Mesen	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces.	+	++	++	Weak coloration was noted in the Cyt, moderate intensity of coloration was noted at the Cs and Ics
	Meten	±	+	±		+	++	++	
	Myelan	±	+	±		+	++	++	
	Dien	±	+	±		+	++	++	
	Telen	±	+	±		+	++	++	
2) AF	Mesen	+	±	±	Weak coloration was noted at the Cs and Ics while weak color intensity was noted in Cyt	+	++	++	Weak coloration was noted in the Cyt, moderate intensity of coloration was noted at the Cs and Ics
	Meten	+	±	±		+	++	++	
	Myelan	+	±	±		+	++	++	
	Dien	+	±	±		+	++	++	
	Telen	+	±	±		+	++	++	
3) AF-AB 2.5	Mesen	+P	+B	±P	Purple weak intensity of coloration was noted in Cyt, weak blue coloration at Cs and trace purple at Ics	+P	++B	++P	Weak purple in Cyt. Moderate blue at Cs and purple coloration at the Ics
	Meten	+P	+B	±P		+P	++B	++P	
	Myelan	+P	+B	±P		+P	++B	++P	
	Dien	+P	+B	±P		+P	++B	++P	
	Telen	+P	+B	±P		+P	++B	++P	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2									
i) 0.0 M	Mesen	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces.	+	++	++	Weak coloration was noted in the Cyt, moderate intensity of coloration was noted at the Cs and Ics
	Meten	±	+	±		+	++	++	
	Myelan	±	+	±		+	++	++	
	Dien	±	+	±		+	++	++	
	Telen	±	+	±		+	++	++	
ii) 0.1 M	Mesen	+	±	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces.	+	++	++	Weak coloration was noted in the Cyt, moderate intensity of coloration was noted at the Cs and Ics
	Meten	±	+	±		+	++	++	
	Myelan	±	+	±		+	++	++	
	Dien	±	+	±		+	++	++	
	Telen	±	+	±		+	++	++	

Table no. IV. a Initial incubation (48hrs) + Dose exposure incubation (24 hrs) + Final development (72 hrs) continued.....

Staining technique	Brain zones	Normal				0.5 mM H2O2			
		Neuroepithelial layer				Neuroepithelial layer			
		Cyt	Cs	Ics	Inference to observations	Cyt	Cs	Ics	Inference to observations
iii) 0.2 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
iv) 0.4 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
v) 0.5 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
vi) 0.6 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
vii) 0.8 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	

Table no. IV. a Initial incubation (48hrs) + Dose exposure incubation (24 hrs) + Final development (72 hrs) continued.....

viii) 1.0 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
5) Azure A metachromecia at different pH levels									
i)pH 0.5	Mesen	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces	+	++	++	Weak coloration was noted in the Cyt, moderate intensity of coloration was noted at the Cs and Ics
	Meten	±	+	±		+	++	++	
	Myelan	±	+	±		+	++	++	
	Dien	±	+	±		+	++	++	
	Telen	±	+	±		+	++	++	
ii)pH 1.0	Mesen	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces	+	++	++	Weak coloration was noted in the Cyt, moderate intensity of coloration was noted at the Cs and Ics
	Meten	±	+	±		+	++	++	
	Myelan	±	+	±		+	++	++	
	Dien	±	+	±		+	++	++	
	Telen	±	+	±		+	++	++	
iii) pH 1.5	Mesen	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces	+	++	++	Weak coloration was noted in the Cyt, moderate intensity of coloration was noted at the Cs and Ics
	Meten	±	+	±		+	++	++	
	Myelan	±	+	±		+	++	++	
	Dien	±	+	±		+	++	++	
	Telen	±	+	±		+	++	++	

Table no. IV. a Initial incubation (48hrs) + Dose exposure incubation (24 hrs) + Final development (72 hrs) continued.....

Staining technique	Brain Region	0.5 mM H_2O_2 treated +3mg vitamin C treated				Control 3mg vitamin c treated			
		Neuroepithelial layer				Neuroepithelial layer			
		Cyt	Cs	Ics	Inference to observations	Cyt	Cs	Ics	Inference to observations
1)AB pH 1	Mesen	±	++	++	Trace reaction was noted in the Cyt, moderate coloration was noted at Cs and Ics	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces.
	Meten	±	++	++		±	+	±	
	Myelan	±	++	++		±	+	±	
	Dien	±	++	++		±	+	±	
	Telen	±	++	++		±	+	±	
2) AF	Mesen	±	++	++	Trace reaction was noted in the Cyt, moderate coloration was noted at Cs and Ics	+	±	±	Weak coloration was noted at the Cs and Ics while weak color intensity was noted in Cyt
	Meten	±	++	++		+	±	±	
	Myelan	±	++	++		+	±	±	
	Dien	±	++	++		+	±	±	
	Telen	±	++	++		+	±	±	
3) AF-AB 2.5	Mesen	±P	++B	++P	Weak purple in Cyt. Moderate blue at Cs and purple coloration at the Ics	+P	+B	±P	Purple weak intensity of coloration was noted in Cyt, weak blue coloration at Cs and trace purple at Ics
	Meten	±P	++B	++P		+P	+B	±P	
	Myelan	±P	++B	++P		+P	+B	±P	
	Dien	±P	++B	++P		+P	+B	±P	
	Telen	±P	++B	++P		+P	+B	±P	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2									
i) 0.0 M	Mesen	±	++	+++	Trace reaction was noted in the Cyt, moderate coloration was noted at Cs and Ics	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces.
	Meten	±	++	+++		±	+	±	
	Myelan	±	++	+++		±	+	±	
	Dien	±	++	+++		±	+	±	
	Telen	±	++	+++		±	+	±	
ii) 0.1 M	Mesen	±	++	+++	Trace reaction was noted in the Cyt, moderate coloration was noted at Cs and Ics	+	±	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces.
	Meten	±	++	+++		±	+	±	
	Myelan	±	++	+++		±	+	±	
	Dien	±	++	+++		±	+	±	
	Telen	±	++	+++		±	+	±	

Table no. IV. a Initial incubation (48hrs) + Dose exposure incubation (24 hrs) + Final development (72 hrs) continued.....

Staining technique	Brain zones	0.5 mM H_2O_2 treated +3mg vitamin C treated				Control 3mg vitamin c treated			
		Neuroepithelial layer				Neuroepithelial layer			
		Cyt	Cs	Ics	Inference to observations	Cyt	Cs	Ics	Inference to observations
iii) 0.2 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
iv) 0.4 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
v) 0.5 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
vi) 0.6 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
vii) 0.8 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	

Table no. IV. a Initial incubation (48hrs) + Dose exposure incubation (24 hrs) + Final development (72 hrs) continued.....

viii) 1.0 M	Mesen	-	-	-	No coloration was observed	-	-	-	No coloration was observed
	Meten	-	-	-		-	-	-	
	Myelan	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	
5) Azure A metachromecia at different pH levels									
i)pH 0.5	Mesen	±	++	++	Trace reaction was noted in the Cyt, moderate coloration was noted at Cs and Ics	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces
	Meten	±	++	++		±	+	±	
	Myelan	±	++	++		±	+	±	
	Dien	±	++	++		±	+	±	
	Telen	±	++	++		±	+	±	
ii)pH 1.0	Mesen	±	++	++	Trace reaction was noted in the Cyt, moderate coloration was noted at Cs and Ics	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces
	Meten	±	++	++		±	+	±	
	Myelan	±	++	++		±	+	±	
	Dien	±	++	++		±	+	±	
	Telen	±	++	++		±	+	±	
iii) pH 1.5	Mesen	±	++	++	Trace reaction was noted in the Cyt, moderate coloration was noted at Cs and Ics	±	+	±	Trace intensity at the Ics and in the Cyt. Weak coloration was noted at the cell surfaces
	Meten	±	++	++		±	+	±	
	Myelan	±	++	++		±	+	±	
	Dien	±	++	++		±	+	±	
	Telen	±	++	++		±	+	±	

Table no. IV. bInitial incubation (48 hrs) + Dose exposure incubation (48 hrs) =Final development (96 hrs)

Staining Tech.	Brain region	Normal								0.5mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 1	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	++	±	++	++	++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Mye	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Die	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Tel	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
2) AF	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	++	±	++	++	++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Mye	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Die	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Tel	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
3) AF-AB 2.5	Mes	±P	+B	±P	±P	+B	±P	+B	Trace purple in Cyt, trace purple Ics and weak blue at ICs	±P	++B	++P	±P	++B	++P	++P	Trace purple in cyt, moderate blue at Cs purple at Ics ML
	Met	±B	+B	±P	±P	+B	±P	+B		±P	++B	++P	±P	++B	++P	++P	
	Mye	±P	+B	±P	±P	+B	±P	+B		±P	++B	++P	±P	++B	++P	++P	
	Die	±P	+B	±P	±P	+B	±P	+B		±P	++B	++P	±P	++B	++P	++P	
	Tel	±P	+B	±P	±P	+B	±P	+B		±P	++B	++P	±P	++B	++P	++P	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2																	
i) 0.0M	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	++	±	++	++	++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Mye	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Die	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Tel	±	+	±	±	+	±	+		±	++	++	±	++	++	++	

Table no. IV. bInitial incubation (48 hrs) + Dose exposure incubation (48 hrs) =Final development (96 hrs) continued.....

ii) 0.1 M	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	++	±	++	++	++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Mye	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Die	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Tel	±	+	±	±	+	±	+		±.	++	++	±	++	++	++	
iii) 0.2M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iv) 0.4M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
v)0.5M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
vi)0.6M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. IV. bInitial incubation (48 hrs) + Dose exposure incubation (48 hrs) =Final development (96 hrs) continued.....

ii) 0.8 M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iii) 1.0M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
5) Azure A metachromecia at different pH levels																	
i)pH 0.5	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	++	±	++	++	++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Mye	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Die	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Tel	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
ii)pH 1.0	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	++	±	++	++	++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Mye	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Die	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Tel	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
iii) pH 1.5	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	++	±	++	++	++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Mye	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Die	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Tel	±	+	±	±	+	±	+		±	++	++	±	++	++	++	

Table no. IV. bInitial incubation (48 hrs) + Dose exposure incubation (48 hrs) =Final development (96 hrs) continued.....

Staining Tech.	Brain region	0.5mM H_2O_2 x+ 3mg vitamin C treated								Control 3mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 1	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
2) AF	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
3) AF-AB 2.5	Mes	±P	+B	±P	±P	+B	±P	+B	Trace purple in Cyt, trace purple Ics and weak blue at ICs	±P	+B	±P	±P	+B	±P	+B	Trace purple in Cyt, trace purple Ics and weak blue at ICs
	Met	±B	+B	±P	±P	+B	±P	+B		±B	+B	±P	±P	+B	±P	+B	
	Mye	±P	+B	±P	±P	+B	±P	+B		±P	+B	±P	±P	+B	±P	+B	
	Die	±P	+B	±P	±P	+B	±P	+B		±P	+B	±P	±P	+B	±P	+B	
	Tel	±P	+B	±P	±P	+B	±P	+B		±P	+B	±P	±P	+B	±P	+B	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2																	
i) 0.0 M	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	

Table no. IV. bInitial incubation (48 hrs) + Dose exposure incubation (48 hrs) =Final development (96 hrs) continued.....

ii) 0.1 M	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
iii) 0.2M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iv) 0.4M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
v)0.5M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
vi)0.6M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. IV. bInitial incubation (48 hrs) + Dose exposure incubation (48 hrs) =Final development (96 hrs) continued.....

ii) 0.8 M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iii) 1.0M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
5) Azure A metachromecia at different pH levels																	
i)pH 0.5	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
ii)pH 1.0	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
iii) pH 1.5	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	

Table no. IV. c Initial incubation (48 hrs) + Dose exposure Incubation (72 hrs) =Final development (120 hrs)

Staining Tech.	Brain region	Normal								0.5mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 1	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	+++	+++	+++	+++	+++	+++	+++	Significant increase in the intensity of coloration
	Met	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
2) AF	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit	+++	+++	+++	+++	+++	+++	+++	Significant increase in the intensity of coloration
	Met	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
3) AF-AB 2.5	Mes	±P	++B	±P	±P	++B	±P	++B	Trace purple in Cyt. Moderate blue at Cs and Ml	+3P	+3B	+3B	+3P	+3B	+3B	+3B	Significant increase in the intensity of coloration
	Met	±P	++B	±P	±P	++B	±P	++B		+3P	+3B	+3B	+3P	+3B	+3B	+3B	
	Mye	±P	++B	±P	±P	++B	±P	++B		+3P	+3B	+3B	+3P	+3B	+3B	+3B	
	Die	±P	++B	±P	±P	++B	±P	++B		+3P	+3B	+3B	+3P	+3B	+3B	+3B	
	Tel	±P	++B	±P	±P	++B	±P	++B		+3P	+3B	+3B	+3P	+3B	+3B	+3B	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2																	
i) 0.0 M	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit	+++	+++	+++	+++	+++	+++	+++	Significant increase in the intensity of coloration d
	Met	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	

Table no. IV. c Initial incubation (48 hrs) + Dose exposure Incubation (72 hrs) =Final development (120) hrs continued.....

ii) 0.1 M	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit	+++	+++	+++	+++	+++	+++	+++	Significant increase in the intensity of coloration
	Met	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
iii) 0.2M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iv) 0.4M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
v)0.5M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
vi)0.6M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. IV. c Initial incubation (48 hrs) + Dose exposure Incubation (72 hrs) =Final development (120) continued.....

hrs.

ii) 0.8 M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iii) 1.0M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
5) Azure A metachromecia at different pH levels																	
i)pH 0.5	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	++	+++	+++	++	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
ii)pH 1.0	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	++	+++	+++	++	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
iii) pH 1.5	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	++	+++	+++	++	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	

Table no. IV. c Initial incubation (48 hrs) + Dose exposure Incubation (72 hrs) =Final development (120) continued.....

Staining Tech.	Brain region	0.5mM H_2O_2 x+ 3mg vitamin C treated								Control 3mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 1	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
2) AF	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
3) AF-AB 2.5	Mes	±P	++B	±P	±P	++B	±P	++B	Trace purple in Cyt. Moderate blue at Cs and Ml	±P	++B	±P	±P	++B	±P	++B	Trace purple in Cyt. Moderate blue at Cs and Ml
	Met	±P	++B	±P	±P	++B	±P	++B		±P	++B	±P	±P	++B	±P	++B	
	Mye	±P	++B	±P	±P	++B	±P	++B		±P	++B	±P	±P	++B	±P	++B	
	Die	±P	++B	±P	±P	++B	±P	++B		±P	++B	±P	±P	++B	±P	++B	
	Tel	±P	++B	±P	±P	++B	±P	++B		±P	++B	±P	±P	++B	±P	++B	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2																	
i) 0.0 M	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	

Table no. IV. c Initial incubation (48 hrs) + Dose exposure Incubation (72 hrs) =Final development (120) continued.....

ii) 0.1 M	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
iii) 0.2M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-		-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iv) 0.4M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
v)0.5M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
vi)0.6M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. IV. c Initial incubation (48 hrs) + Dose exposure Incubation (72 hrs) =Final development (120) continued.....

ii) 0.8 M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iii) 1.0M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
5) Azure A metachromecia at different pH levels																	
i)pH 0.5	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
ii)pH 1.0	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
iii) pH 1.5	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	

Table no V. a Initial incubation (72 hrs) + Dose exposure incubation (24 hrs) + Final development (96 hrs)

Staining Tech.	Brain region	Normal								0.5mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 1	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	++	+++	+++	++	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
2) AF	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit	++	+++	+++	++	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
3) AF-AB 2.5	Mes	±P	++B	±P	±P	++B	±P	++B	Trace purple in Cyt. Moderate blue at Cs and MI	++P	+3B	+3B	++	+3B	+3B	+3B	Staining increases in intensity at all sites considered
	Met	±P	++B	±P	±P	++B	±P	++B		++P	+3B	+3B	++	+3B	+3B	+3B	
	Mye	±P	++B	±P	±P	++B	±P	++B		++P	+3B	+3B	++	+3B	+3B	+3B	
	Die	±P	++B	±P	±P	++B	±P	++B		++P	+3B	+3B	++	+3B	+3B	+3B	
	Tel	±P	++B	±P	±P	++B	±P	++B		++P	+3B	+3B	++	+3B	+3B	+3B	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2																	
i) 0.0 M	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit	++	+++	+++	++	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	

Table no V. a Initial incubation (72 hrs) + Dose exposure incubation (24 hrs) + Final development (96 hrs) continued.....

ii) 0.1 M	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit	++	+++	+++	++	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
iii) 0.2M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iv) 0.4M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
v)0.5M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
vi)0.6M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no V. a Initial incubation (72 hrs) + Dose exposure incubation (24 hrs) + Final development (96 hrs) continued.....

ii) 0.8 M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iii) 1.0M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
5) Azure A metachromecia at different pH levels																	
i)pH 0.5	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	++	+++	+++	++	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
ii)pH 1.0	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	++	+++	+++	++	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
iii) pH 1.5	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	++	+++	+++	++	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		++	+++	+++	++	+++	+++	+++	

Table no V. a Initial incubation (72 hrs) + Dose exposure incubation (24 hrs) + Final development (96 hrs) continued.....

Staining Tech.	Brain region	0.5mM H_2O_2 x+ 3mg vitamin C treated								Control 3mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 1	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
2) AF	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Myc	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
3) AF-AB 2.5	Mes	±P	++B	±P	±P	++B	±P	++B	Trace purple in Cyt. Moderate blue at Cs and Ml	±P	++B	±P	±P	++B	±P	++B	Trace purple in Cyt. Moderate blue at Cs and Ml
	Met	±P	++B	±P	±P	++B	±P	++B		±P	++B	±P	±P	++B	±P	++B	
	Mye	±P	++B	±P	±P	++B	±P	++B		±P	++B	±P	±P	++B	±P	++B	
	Die	±P	++B	±P	±P	++B	±P	++B		±P	++B	±P	±P	++B	±P	++B	
	Tel	±P	++B	±P	±P	++B	±P	++B		±P	++B	±P	±P	++B	±P	++B	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2																	
i) 0.0 M	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	

Table no V. a Initial incubation (72 hrs) + Dose exposure incubation (24 hrs) + Final development (96 hrs) continued.....

ii) 0.1 M	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
iii) 0.2M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iv) 0.4M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
v)0.5M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
vi)0.6M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no V. a Initial incubation (72 hrs) + Dose exposure incubation (24 hrs) + Final development (96 hrs) continued.....

ii) 0.8 M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iii) 1.0M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
5) Azure A metachromecia at different pH levels																	
i)pH 0.5	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
ii)pH 1.0	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
iii) pH 1.5	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	

Table no V. b Initial incubation (72 hrs) + Dose exposure incubation (48 hrs) = Final development (120 hrs).

Staining tech.	Brain region	Normal								0.5mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 1	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	+	+++	+++	+	+++	+++	+++	Moderate staining in Cyt remaining sies intensely stained
	Met	±	++	++	±	++	++	++		+	+++	+++	+	+++	+++	+++	
	Mye	±	++	++	±	++	++	++		+	+++	+++	+	+++	+++	+++	
	Die	±	++	++	±	++	++	++		+	+++	+++	+	+++	+++	+++	
	Tel	±	++	++	±	++	++	++		+	+++	+++	+	+++	+++	+++	
2) AF	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	+	+++	+++	+	+++	+++	+++	Moderate staining in Cyt remaining sies intensely stained
	Met	±	++	++	±	++	++	++		+	+++	+++	+	+++	+++	+++	
	Mye	±	++	++	±	++	++	++		+	+++	+++	+	+++	+++	+++	
	Die	±	++	++	±	++	++	++		+	+++	+++	+	+++	+++	+++	
	Tel	±	++	++	±	++	++	++		+	+++	+++	+	+++	+++	+++	
3) AF-AB 2.5	Mes	±P	++B	++P	±P	++B	++P	++B	Trace purple in Cyt moderate blue and purple at Cs and Ics	+	+++	+++	+	+++	+++	+++	Moderate staining in Cyt remaining sies intensely stained
	Met	±P	++B	++P	±P	++B	++P	++B		+	+++	+++	+	+++	+++	+++	
	Mye	±P	++B	++P	±P	++B	++P	++B		+	+++	+++	+	+++	+++	+++	
	Die	±P	++B	++P	±P	++B	++P	++B		+	+++	+++	+	+++	+++	+++	
	Tel	±P	++B	++P	±P	++B	++P	++B		+	+++	+++	+	+++	+++	+++	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2																	
i) 0.0 M	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	+	+++	+++	+	+++	+++	+++	Intense coloration was onserved at all sites studied
	Met	±	++	++	±	++	++	++		+	+++	+++	+	+++	+++	+++	
	Mye	±	++	++	±	++	++	++		+	+++	+++	+	+++	+++	+++	
	Die	±	++	++	±	++	++	++		+	+++	+++	+	+++	+++	+++	
	Tel	±	++	++	±	++	++	++		+	+++	+++	+	+++	+++	+++	

Table no V. b Initial incubation (72 hrs) + Dose exposure incubation (48 hrs) = Final development (120 hrs) continued.....

ii) 0.1 M	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	+	+++	+++	+	+++	+++	+++	Moderate staining in Cyt remaining sies intensely stained
	Met	±	++	++	±	++	++	++		+	+++	+++	+	+++	+++	+++	
	Mye	±	++	++	±	++	++	++		+	+++	+++	+	+++	+++	+++	
	Die	±	++	++	±	++	++	++		+	+++	+++	+	+++	+++	+++	
	Tel	±	++	++	±	++	++	++		+	+++	+++	+	+++	+++	+++	
iii) 0.2 M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iv) 0.4M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
v)0.5M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
vi)0.6M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no V. b Initial incubation (72 hrs) + Dose exposure incubation (48 hrs) = Final development (120 hrs) continued.....

ii) 0.8 M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iii)1.0 M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
5) Azure A metachromecia at different pH levels																	
i)pH 0.5	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	+++	+++	+++	+++	+++	+++	+++	Intense coloration was onserved at all sites studied
	Met	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
ii)pH 1.0	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	+++	+++	+++	+++	+++	+++	+++	Intense coloration was onserved at all sites studied
	Met	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
iii) pH 1.5	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	+++	+++	+++	+++	+++	+++	+++	Intense coloration was onserved at all sites studied
	Met	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	++	±	++	++	++		+++	+++	+++	+++	+++	+++	+++	

Table no V. b Initial incubation (72 hrs) + Dose exposure incubation (48 hrs) = Final development (120 hrs) continued.....

Staining Tech.	Brain region	0.5mM H_2O_2 x+ 3mg vitamin C treated								Control 3mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 1	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered
	Met	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Mye	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Die	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Tel	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
2) AF	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered
	Met	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Mye	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Die	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Tel	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
3) AF-AB 2.5	Mes	±P	++B	++P	±P	++B	++P	++B	Trace purple in Cyt moderate blue and purple at Cs and Ics	±P	++B	++P	±P	++B	++P	++B	Intense coloration was onserved at all sites studied
	Met	±P	++B	++P	±P	++B	++P	++B		±P	++B	++P	±P	++B	++P	++B	
	Mye	±P	++B	++P	±P	++B	++P	++B		±P	++B	++P	±P	++B	++P	++B	
	Die	±P	++B	++P	±P	++B	++P	++B		±P	++B	++P	±P	++B	++P	++B	
	Tel	±P	++B	++P	±P	++B	++P	++B		±P	++B	++P	±P	++B	++P	++B	
4)Alcian blue at pII5.6 With graded concentrations of MgCl2																	
i) 0.0 M	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered
	Met	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Mye	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Die	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Tel	±	++	++	±	++	++	++		±	++	++	±	++	++	++	

Table no V. b Initial incubation (72 hrs) + Dose exposure incubation (48 hrs) = Final development (120 hrs) continued.....

ii) 0.1 M	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered
	Met	±	++	++	±	++	++	++		±	++	++	±	++	++	++		
	Mye	±	++	++	±	++	++	++		±	++	++	±	++	++	++		
	Die	±	++	++	±	++	++	++		±	++	++	±	++	++	++		
	Tel	±	++	++	±	++	++	++		±	++	++	±	++	++	++		
iii) 0.2 M	Mes	-	-	-	-	-	-	-	No coloration was observed	-	-	-	-	-	-	-	No coloration was observed	
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
iv) 0.4M	Mes	-	-	-	-	-	-	-	No coloration was observed	-	-	-	-	-	-	-	No coloration was observed	
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
v)0.5M	Mes	-	-	-	-	-	-	-	No coloration was observed	-	-	-	-	-	-	-	No coloration was observed	
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
vi)0.6M	Mes	-	-	-	-	-	-	-	No coloration was observed	-	-	-	-	-	-	-	No coloration was observed	
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-		
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-		

Table no V. b Initial incubation (72 hrs) + Dose exposure incubation (48 hrs) = Final development (120 hrs) continued.....

ii) 0.8 M	Mes	-	-	-	-	-	-	-	No coloration was observed	-	-	-	-	-	-	-	No coloration was observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iii)1.0 M	Mes	-	-	-	-	-	-	-	No coloration was observed	-	-	-	-	-	-	-	No coloration was observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
5) Azure A metachromecia at different pH levels																	
i)pH 0.5	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	±	++	++	±	++	++	++	Wcak color intensity in Cyt, moderate at all other sites considered
	Met	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Mye	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Die	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Tel	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
ii)pH 1.0	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered
	Met	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Mye	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Die	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Tel	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
iii) pH 1.5	Mes	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered	±	++	++	±	++	++	++	Weak color intensity in Cyt, moderate at all other sites considered
	Met	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Mye	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Die	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Tel	±	++	++	±	++	++	++		±	++	++	±	++	++	++	

Table no VI a Initial incubation (96 hrs) + Dose exposure incubation (24 hrs) = Final development (120 hrs)

Staining Tech.	Brain region	Normal								0.5mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 1	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	+++	+++	±	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Die	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
2) AF	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit	±	+++	+++	±	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Die	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
3) AF-AB 2.5	Mes	±P	++B	±P	±P	++B	±P	++B	Trace purple in Cyt. Moderate blue at Cs and Ml	±P	+3B	+3B	±P	+3B	+3B	+3B	Staining increases in intensity at all sites considered
	Met	±P	++B	±P	±P	++B	±P	++B		±P	+3B	+3B	±P	+3B	+3B	+3B	
	Mye	±P	++B	±P	±P	++B	±P	++B		±P	+3B	+3B	±P	+3B	+3B	+3B	
	Die	±P	++B	±P	±P	++B	±P	++B		±P	+3B	+3B	±P	+3B	+3B	+3B	
	Tel	±P	++B	±P	±P	++B	±P	++B		±P	+3B	+3B	±P	+3B	+3B	+3B	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2																	
i) 0.0 M	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit	±	+++	+++	±	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Die	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	

Table no VI a Initial incubation (96 hrs) + Dose exposure incubation (24 hrs) = Final development (120 hrs) continued.....

ii) 0.1 M	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit	±	+++	+++	±	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Die	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
iii) 0.2M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iv) 0.4M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
v)0.5M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
vi)0.6M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no VI a Initial incubation (96 hrs) + Dose exposure incubation (24 hrs) = Final development (120 hrs) continued.....

ii) 0.8 M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iii) 1.0M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
5) Azure A metachromecia at different pH levels																	
i)pH 0.5	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	+++	+++	±	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Die	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
ii)pH 1.0	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	+++	+++	±	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Die	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
iii) pH 1.5	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	+++	+++	±	+++	+++	+++	Moderate intensity in Cyt, intense at all other sited
	Met	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Die	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		±	+++	+++	±	+++	+++	+++	

Table no VI a Initial incubation (96 hrs) + Dose exposure incubation (24 hrs) = Final development (120 hrs) continued.....

Staining Tech.	Brain region	0.5mM H_2O_2 x+ 3mg vitamin C treated								Control 3mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 1	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
2) AF	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sit
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
3) AF-AB 2.5	Mes	±P	++B	±P	±P	++B	±P	++B	Trace purple in Cyt. Moderate blue at Cs and Ml	±P	++B	±P	±P	++B	±P	++B	Trace purple in Cyt. Moderate blue at Cs and Ml
	Met	±P	++B	±P	±P	++B	±P	++B		±P	++B	±P	±P	++B	±P	++B	
	Mye	±P	++B	±P	±P	++B	±P	++B		±P	++B	±P	±P	++B	±P	++B	
	Die	±P	++B	±P	±P	++B	±P	++B		±P	++B	±P	±P	++B	±P	++B	
	Tel	±P	++B	±P	±P	++B	±P	++B		±P	++B	±P	±P	++B	±P	++B	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2																	
i) 0.0 M	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	

Table no VI a Initial incubation (96 hrs) + Dose exposure incubation (24 hrs) = Final development (120 hrs) continued.....

ii) 0.1 M	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
iii) 0.2M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iv) 0.4M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
v)0.5M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
vi)0.6M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. I. c Initial incubation (96 hrs) + exposure incubation (24 hrs) =Final incubation (120 hrs) continued.....

ii) 0.8 M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iii) 1.0M	Mes	-	-	-	-	-	-	-	No coloration observed	-	-	-	-	-	-	-	No coloration observed
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
5) Azure A metachromecia at different pH levels																	
i)pH 0.5	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
ii)pH 1.0	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
iii) pH 1.5	Mes	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites	±	++	±	±	++	±	++	Trace intensity in Cyt, moderate insity at other sites
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	

Table no. VII. A Initial incubation (120 hrs) + Dose exposure incubation (24 hrs) = Final development (144 hrs).

Staining Tech.	Brain region	Normal								0.5mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 1	Mes	±	++	++	±	++	++	±	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	+++	±	++	+++	+++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	++	++	±	++	++	±		±	++	+++	±	++	+++	+++	
	Mye	±	++	++	±	++	++	±		±	++	+++	±	++	+++	+++	
	Die	±	++	++	±	++	++	±		±	++	+++	±	++	+++	+++	
	Tel	±	++	++	±	++	++	±		±	++	+++	±	++	+++	+++	
2) AF	Mes	±	++	++	±	++	++	±	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	+++	±	++	+++	+++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	++	++	±	++	++	±		±	++	+++	±	++	+++	+++	
	Mye	±	++	++	±	++	++	±		±	++	+++	±	++	+++	+++	
	Die	±	++	++	±	++	++	±		±	++	+++	±	++	+++	+++	
	Tel	±	++	++	±	++	++	±		±	++	+++	±	++	+++	+++	
3) AF-AB 2.5	Mes	±P	+B	±P	±P	+B	±P	+B	Trace purple in Cyt, trace purple Ics and weak blue at ICs	±P	++B	+3B	±P	+3B	+3B	+3P	Trace purple in cyt, moderate blue at Cs purple at Ics ML
	Met	±B	+B	±P	±P	+B	±P	+B		±P	++B	+3B	±P	+3B	+3B	+3P	
	Mye	±P	+B	±P	±P	+B	±P	+B		±P	++B	+3B	±P	+3B	+3B	+3P	
	Die	±P	+B	±P	±P	+B	±P	+B		±P	++B	+3B	±P	+3B	+3B	+3P	
	Tel	±P	+B	±P	±P	+B	±P	+B		±P	++B	+3B	±P	+3B	+3B	+3P	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2																	
i) 0.0M	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	+++	±	++	+++	+++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	+++	±	++	+++	+++	
	Mye	±	+	±	±	+	±	+		±	++	+++	±	++	+++	+++	
	Die	±	+	±	±	+	±	+		±	++	+++	±	++	+++	+++	
	Tel	±	+	±	±	+	±	+		±	++	+++	±	++	+++	+++	

Table no. VII. A Initial incubation (120 hrs) + Dose exposure incubation (24 hrs) = Final development (144 hrs).

ii) 0.1 M	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	++	±	++	++	++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Mye	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Die	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Tel	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
iii) 0.2M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iv) 0.4M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
v)0.5M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
vi)0.6M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. VII. A Initial incubation (120 hrs) + Dose exposure incubation (24 hrs) = Final development (144 hrs).

ii) 0.8 M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iii) 1.0M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
5) Azure A metachromecia at different pH levels																	
i)pH 0.5	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	++	±	++	++	++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Mye	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Die	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Tel	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
ii)pH 1.0	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	++	±	++	++	++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Mye	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Die	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Tel	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
iii) pH 1.5	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	++	++	±	++	++	++	Trace amount of intensity in Cyt, moderate intensity at Cs and Ics
	Met	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Mye	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Die	±	+	±	±	+	±	+		±	++	++	±	++	++	++	
	Tel	±	+	±	±	+	±	+		±	++	++	±	++	++	++	

Table no. VII. A Initial incubation (120 hrs) + Dose exposure incubation (24 hrs) = Final development (144 hrs).

Initial incubation (24 hrs) + exposure incubation (48 hrs) =Final incubation (72 hrs).																	
Staining Tech.	Brain region	0.5mM H_2O_2 x+ 3mg vitamin C treated								Control 3mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 1	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
2) AF	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
3) AF-AB 2.5	Mes	±P	+B	±P	±P	+B	±P	+B	Trace purple in Cyt, trace purple Ics and weak blue at ICs	±P	+B	±P	±P	+B	±P	+B	Trace purple in Cyt, trace purple Ics and weak blue at ICs
	Met	±B	+B	±P	±P	+B	±P	+B		±B	+B	±P	±P	+B	±P	+B	
	Mye	±P	+B	±P	±P	+B	±P	+B		±P	+B	±P	±P	+B	±P	+B	
	Die	±P	+B	±P	±P	+B	±P	+B		±P	+B	±P	±P	+B	±P	+B	
	Tel	±P	+B	±P	±P	+B	±P	+B		±P	+B	±P	±P	+B	±P	+B	
4)Alcian blue at pH5.6 With graded concentrations of MgCl2																	
i) 0.0 M	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	

Table no. VII. A Initial incubation (120 hrs) + Dose exposure incubation (24 hrs) = Final development (144 hrs).

ii) 0.1 M	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
iii) 0.2M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iv) 0.4M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
v)0.5M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
vi)0.6M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. VII. A Initial incubation (120 hrs) + Dose exposure incubation (24 hrs) = Final development (144 hrs).

ii) 0.8 M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
iii) 1.0M	Mes	-	-	-	-	-	-	-	No coloration	-	-	-	-	-	-	-	No coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
5) Azure A metachromecia at different pH levels																	
i)pH 0.5	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
ii)pH 1.0	Mes	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
iii) pH 1.5	Mcs	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML	±	+	±	±	+	±	+	Trace intensity in Cyt and at Ics weak intensity at Cs and ML
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+	
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+	

PLATE I

PLATE I- Initial incubation (24 hrs) + Dose exposure incubation (24 hrs) = Final development (48 hrs). a- AB pH -1 and b-AF-AB pH 2.5

Fig 1-5- Normal embryonic brain X 1000.

Fig. 1a - Mesen .- cells stained with weak ab pH 1 staining mostly at cell surfces

1b-Mesen- Cell surfaces are AB pH 2.5 +ve, while cytoplasm AF positive

Fig. 2a - Meten. – Cells stained with weak ab pH 1 staining mostly at cell surfces

2b- Meten - Cell surfaces are AB pH 2.5 +ve, while cytoplasm AF positive

Fig. 3a - Myelen – cells stained with weak ab pH 1 staining mostly at cell surfces

3b- Myel. – Cell surfaces are AB pH 2.5 +ve, while cytoplasm AF positive

Fig. 4a - Dien – cells stained with weak ab pH 1 staining mostly at cell surfces

4b- Dien- Cell surfaces are AB pH 2.5 +ve, while cytoplasm AF positive

Fig. 5a - Telen. – cells stained with weak ab pH 1 staining mostly at cell surfces

5b –Telen - Cell surfaces are AB pH 2.5 +ve, while cytoplasm AF positive

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000.

Fig. 6a - Mesen .- Colour innsity increases significantly

6b-Mesen- Colour innsity increases significantly

Fig. 7a - Meten. – Colour innsity increases signific

7b- Meten- Colour innsity increases significantly

Fig. 8a - Myelen – Colour innsity increases significantly

8b- Myel. – Colour innsity increases significantly

Fig. 9a - Dien – Colour innsity increases significantly

9b- Dien- Colour innsity increases significantly

Fig. 10a - Telen. – Colour innsity increases significantly

10b –Telen - Colour innsity increases significantly

Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11a - Mesen .- Color intensity decreases but not equal to normal.

11b-Mesen- Color intensity decreases but not equal to normal

Fig. 12a - Meten. – Color intensity decreases but not equal to normal

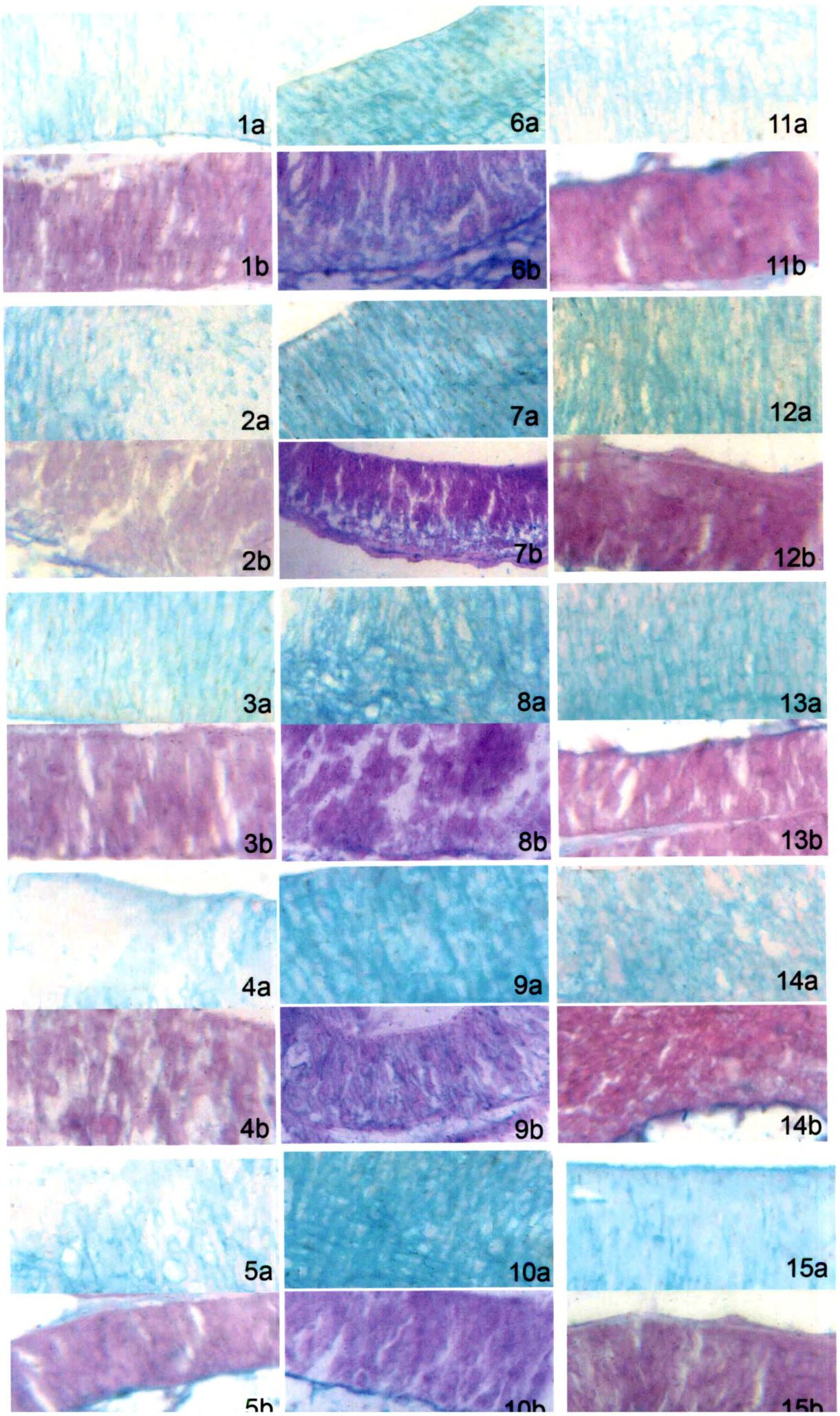
12b- Meten- Color intensity decreases but not equal to normal

Fig. 13a - Myelen – Color intensity decreases but not equal to normal

13b- Myel. – Color intensity decreases but not equal to normal

Fig. 14a - Dien – Color intensity decreases but not equal to normal

PLATE I


1a
1b
2a
2b
3a
3b
4a
4b
5a
5b
6a
6b
7a
7b
8a
8b
9a
9b
10a
10b
11a
11b
12a
12b
13a
13b
14a
14b
15a
15b

14b- Dien- Color intensity decreases but not equal to normal

Fig. 15a - Telen. – Color intensity decreases but not equal to normal

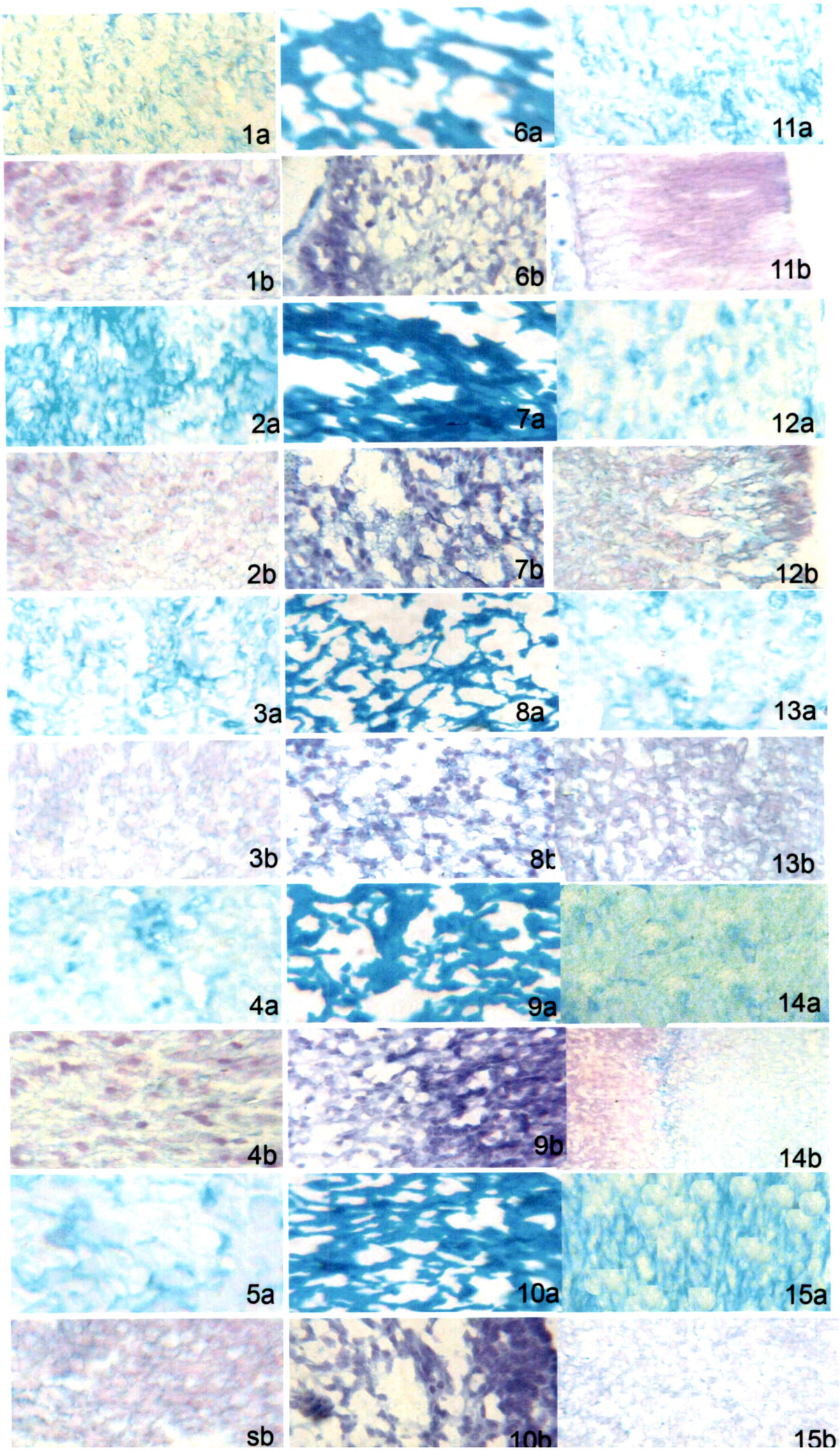
15b –Telen - Color intensity decreases but not equal to normal

PLATE II-

PLATE-II Initial incubation (24 hrs) + Dose exposure incubation (24 hrs) = Final development (48 hrs).

Fig 1-5- Normal embryonic brain X 1000.
Fig. 1a - Mesen .-Weak intensity of coloration at cell surfaces.
1b-Mesen- Weak intensity of coloration at cell surfaces.
Fig. 2a - Meten. – Weak intensity of coloration at cell surfaces.
2b- Meten - Weak intensity of coloration at cell surfaces.
Fig. 3a - Myelen – Weak intensity of coloration at cell surfaces.
3b- Myel. – Weak intensity of coloration at cell surfaces.
Fig. 4a - Dien – Weak intensity of coloration at cell surfaces.
4b- Dien- Weak intensity of coloration at cell surfaces.
Fig. 5a - Telen. – Weak intensity of coloration at cell surfaces.
5b –Telen - Weak intensity of coloration at cell surfaces.
Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000.
Fig. 6a - Mesen .- Color intensity increases significantly.
6b-Mesen- Color intensity increases significantly
Fig. 7a - Meten. – Color intensity increases significantly
7b- Meten- Color intensity increases significantly
Fig. 8a - Myelen – Color intensity increases significantly
8b- Myel. – Color intensity increases significantly
Fig. 9a - Dien – Color intensity increases significantly
9b- Dien- Color intensity increases significantly
Fig. 10a - Telen. – Color intensity increases significantly
10b –Telen - Color intensity increases significantly
Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.
Fig. 11a - Mesen .- Weak intensity of coloration at cell surfaces
11b-Mesen- Weak intensity of coloration at cell surfaces
Fig. 12a - Meten. – Weak intensity of coloration at cell surfaces
12b- Meten - Weak intensity of coloration at cell surfaces
Fig. 13a - Myelen – Weak intensity of coloration at cell surfaces
13b- Myel. – Weak intensity of coloration at cell surfaces
Fig. 14a - Dien – Weak intensity of coloration at cell surfaces
14b- Dien- Weak intensity of coloration at cell surfaces
Fig. 15a - Telen. – Weak intensity of coloration at cell surfaces
15b –Telen - Weak intensity of coloration at cell surfaces

PLATE II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Initiation at 34 hrs of development

v) Initial incubation (34 hrs) + (24 hrs) dose exposure incubation hrs = Final development (58 hrs) –

Cytoplasm showed weak amount of sulfated GAGs, cytoplasm with moderate amount of sulfated GAGs and the intercellular space of the neuroepithelial zone showed significant increase in the sulfated GAGs.

vi) Initial incubation (34 hrs) + Dose exposure incubation (48 hrs) = Final development (82 hrs) –

Weak increase of the sulfated GAGs was noted in the cytoplasm, moderate increase in the cell surface and the significantly increased sulfated GAGs at the intercellular space and the marginal zone of the five brain vesicles.

vii) Initial incubation (34 hrs) + Dose exposure incubation (72 hrs) = Final development (106 hrs) –

Moderate increase in the cytoplasm and significant increase at the intercellular space and marginal zone was noted for the sulfated GAGs

viii) Initial incubation hrs + Dose exposure incubation hrs = Final development hrs (34+ 96 = 130) –

The content of sulfated GAGs was moderately increased in the cytoplasm, significantly at the cell surface; intercellular space and the marginal zone of the five brain vesicles were noted.

Initiation at 40 hrs of development

ix) Initial incubation (40 hrs) + Dose exposure incubation (24 hrs) = Final development (64 hrs) –

Trace amount increased sulfated GAGs was noted in the cytoplasm, moderate increase was found at the cell surface, intercellular space and marginal zone of the five brain regions.

x) Initial incubation (40 hrs) + Dose exposure incubation (48 hrs) = Final development (88 hrs) –

Weak amount of increased sulfated GAGs was noted in the cytoplasm of the neuroepithelial cells moderate increase was noted in the cell surface, significant increase was observed in the intercellular space, and marginal zone of the sulfated GAGs was noted.

xi) Initial incubation (40 hrs) + Dose exposure incubation (72 hrs) = Final development (112 hrs) –

Moderate amount was noted in the cytoplasm; significantly increased amount of sulfated GAGs was noted at all the cellular structures considered for all five-brain regions.

xii) Initial incubation (40 hrs) + Dose exposure incubation (96 hrs) = Final development (136 hrs) –

The content of sulfated GAGs was significant increase at the cell surface, intercellular space and the marginal zone of the five brain regions. Cytoplasm showed moderately increased sulfated GAGs of the ependymal and mantle zone of the brain regions.

Initiation at 48 hrs of development

xiii) Initial incubation (48 hrs) + Dose exposure incubation (24 hrs) = Final development (72 hrs)-

Weak amount of sulfated GAGs was noted in the cytoplasm, while moderately increased sulfated GAGs was noted at the cell surface, intercellular space and the marginal zone of the five brain vesicles.

xiv) Initial incubation (48 hrs) + Dose exposure incubation (48 hrs) = Final developmental (96 hrs) –

Moderate increase of the sulfated GAGs was noted in the cytoplasm of the cells. Cell surface, intercellular space and marginal zone showed the significant increase for sulfated GAGs.

xv) Initial incubation (48 hrs) + Dose exposure incubation (72 hrs) = Final developmental (120 hrs) –

Significantly increased amount of sulfated GAGs was noted at the cell surfaces, intercellular space and the marginal zone of the ependymal, and mantle zone of the five brain regions of the brain, cytoplasm showed the moderate increase in the sulfated GAGs.

PLATE III

PLATE III- Initial incubation (48 hrs) + Dose exposure incubation (24 hrs) = Final development (72 hrs).

Fig 1-5- Normal embryonic brain X 1000.

Fig. 1a - Mesen. - Weak intensity of coloration at cell surfaces

1b-Mesen- Weak intensity of coloration at cell surfaces

Fig. 2a - Meten. – Weak intensity of coloration at cell surfaces

2b- Meten- Weak intensity of coloration at cell surfaces

Fig. 3a - Myelen – Weak intensity of coloration at cell surfaces

3b- Myel. – Weak intensity of coloration at cell surfaces

Fig. 4a - Dien – Weak intensity of coloration at cell surfaces

4b- Dien- Weak intensity of coloration at cell surfaces

Fig. 5a - Telen. – Weak intensity of coloration at cell surfaces

5b –Telen - Weak intensity of coloration at cell surfaces

Fig. 6 -11- 0.5 mM H_2O_2 treated embryonic brain X1000.

Fig. 6a - Mesen .- Color intensity increases significant

6b-Mesen- Color intensity increases significantly.

Fig. 7a - Meten. – Color intensity increases significantly.

7b- Meten- Color intensity increases significantly.

Fig. 8a - Myelen – Color intensity increases significantly.

8b- Myel. – Color intensity increases significantly.

Fig. 9a - Dien – Color intensity increases significantly.

9b- Dien- Color intensity increases significantly.

Fig. 10a - Telen. – Color intensity increases significantly.

10b –Telen - Color intensity increases significantly.

Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11a - Mesen .- Weak intensity of coloration at cell surfaces

11b-Mesen- Weak intensity of coloration at cell surfaces

Fig. 12a - Meten. – Weak intensity of coloration at cell surfaces

12b- Meten- Weak intensity of coloration at cell surfaces

Fig. 13a - Myelen – Weak intensity of coloration at cell surfaces

13b- Myel. – Weak intensity of coloration at cell surfaces

Fig. 14a - Dien – Weak intensity of coloration at cell surfaces

14b- Dien- Weak intensity of coloration at cell surfaces

Fig. 15a - Telen. – Weak intensity of coloration at cell surfaces

15b –Telen - Weak intensity of coloration at cell surfaces

PLATE III

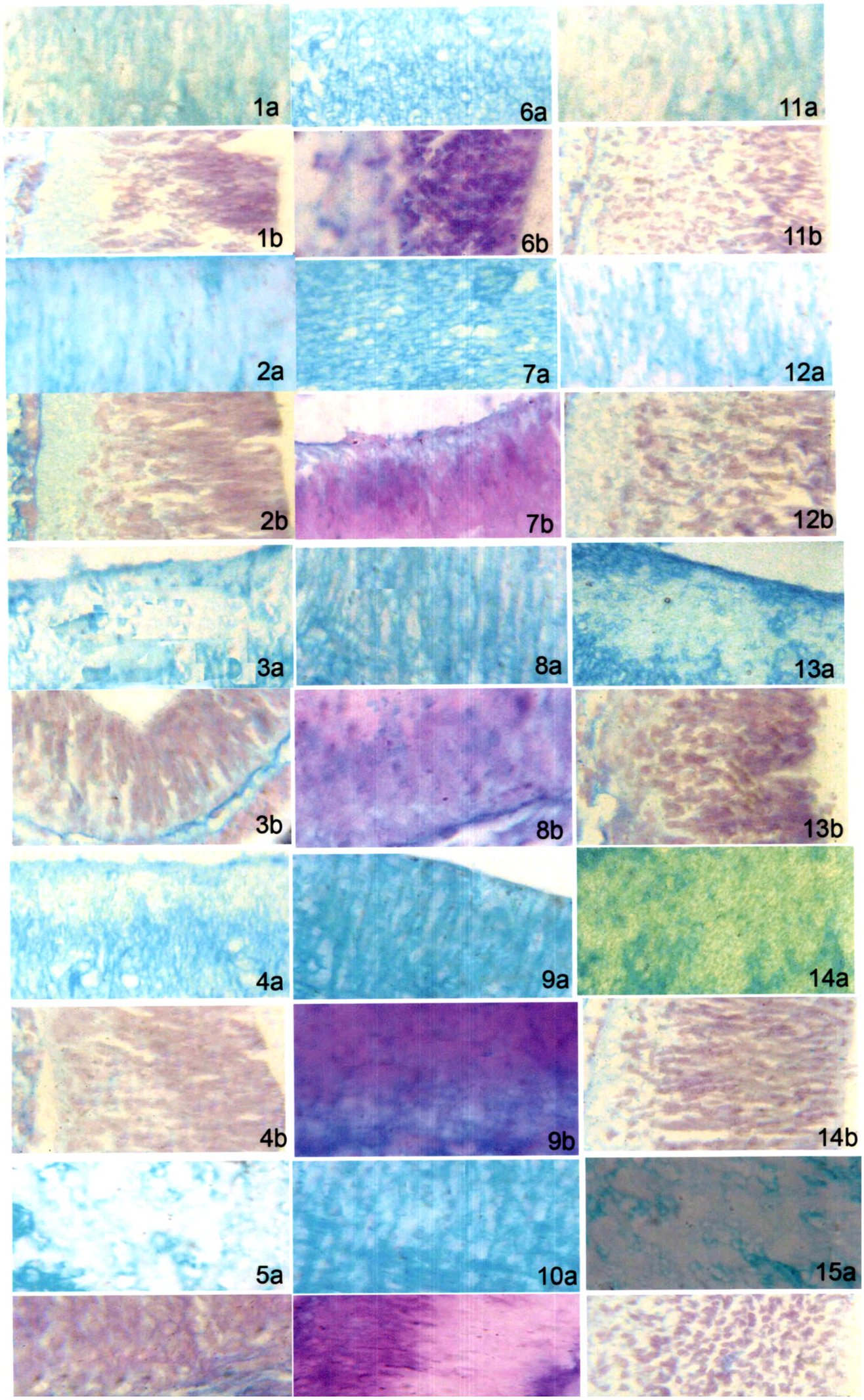
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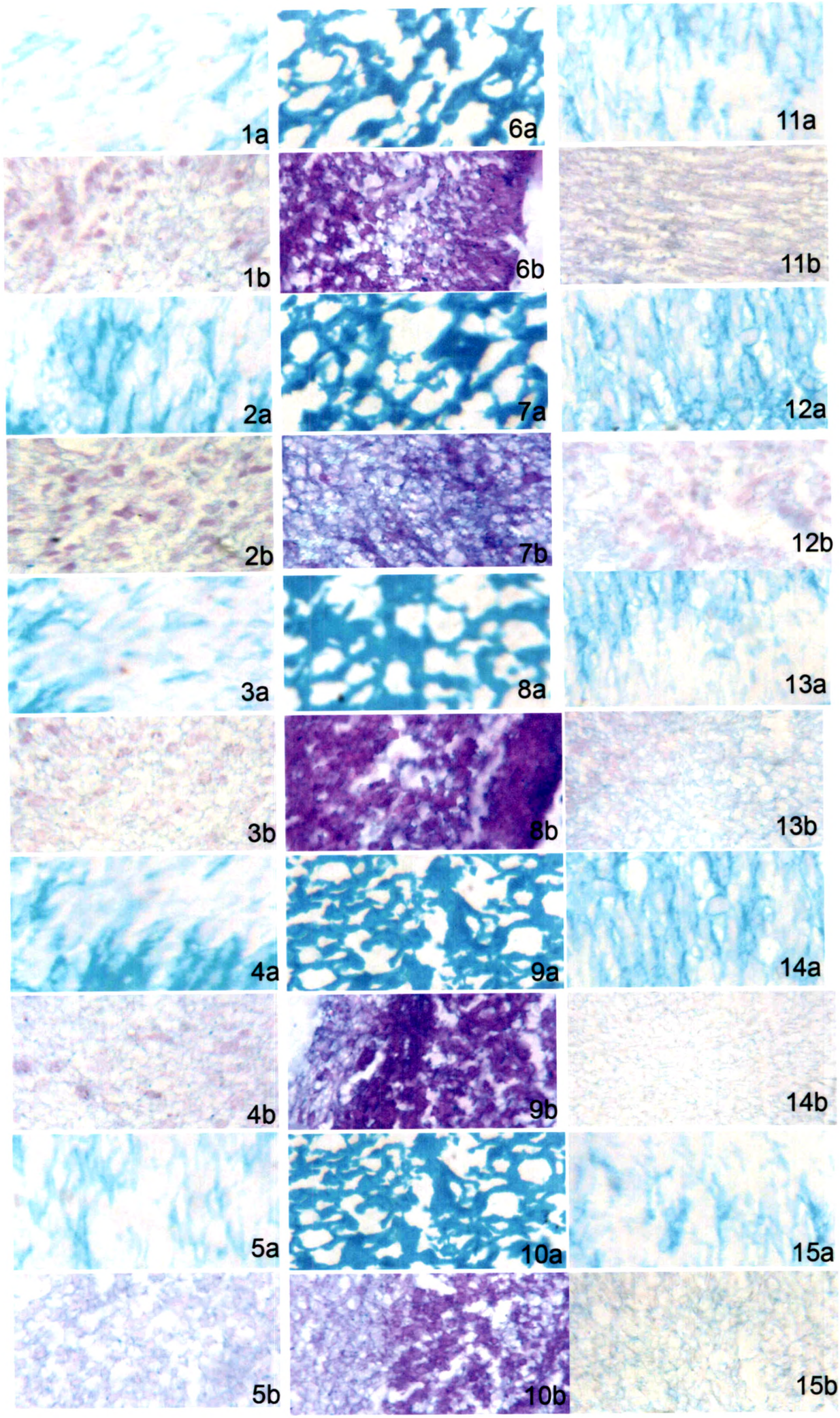
PLATE IV

PLATE IV- Initial incubation (48 hrs) + Dose exposure incubation (72 hrs) = Final development (120 hrs).

Fig. 1a - Mesen .- Weak intensity of coloration at cell surfaces
1b-Mesen- Weak intensity of coloration at cell surfaces
Fig. 2a - Meten. – Weak intensity of coloration at cell surfaces
2b- Meten- Weak intensity of coloration at cell surfaces
Fig. 3a - Myelen – Weak intensity of coloration at cell surfaces
3b- Myel. – Weak intensity of coloration at cell surfaces
Fig. 4a - Dien – Weak intensity of coloration at cell surfaces
4b- Dien- Weak intensity of coloration at cell surfaces
Fig. 5a - Telen. – Weak intensity of coloration at cell surfaces
5b –Telen - Weak intensity of coloration at cell surfaces
Fig. 6 -11- 0.5 mM H_2O_2 treated embryonic brain X1000.
Fig. 6a - Mesen .- Color intensity increases significant
6b-Mesen- Color intensity increases significantly.
Fig. 7a - Meten. – Color intensity increases significantly.
7b- Meten- Color intensity increases significantly.
Fig. 8a - Myelen – Color intensity increases significantly.
8b- Myel. – Color intensity increases significantly.
Fig. 9a - Dien – Color intensity increases significantly.
9b- Dien- Color intensity increases significantly.
Fig. 10a - Telen. – Color intensity increases significantly.
10b –Telen - Color intensity increases significantly.
Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.
Fig. 11a - Mesen .- Weak intensity of coloration at cell surfaces
11b-Mesen- Weak intensity of coloration at cell surfaces
Fig. 12a - Meten. – Weak intensity of coloration at cell surfaces
12b- Meten- Weak intensity of coloration at cell surfaces
Fig. 13a - Myelen – Weak intensity of coloration at cell surfaces
13b- Myel. – Weak intensity of coloration at cell surfaces
Fig. 14a - Dien – Weak intensity of coloration at cell surfaces
14b- Dien- Weak intensity of coloration at cell surfaces
Fig. 15a - Telen. – Weak intensity of coloration at cell surfaces
15b –Telen - Weak intensity of coloration at cell surfaces

PLATEIV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Initiation at 72 hrs of development

xvi) Initial incubation (72 hrs) + Dose exposure incubation (24 hrs) = Final development (96 hrs) –

Weak amount of sulfated GAGs was noted for the cytoplasm of the neuronal cells. Cell surface showed the moderate increase in the sulfated GAGs and intercellular space showed significant increase in the sulfated GAGs.

xvii) Initial incubation (72 hrs) + Dose exposure incubation (48 hrs) = Final development (120 hrs) –

Weak amount of sulfated GAGs was noted in the cytoplasm, significant amount of increased sulfated GAGs was noted at the cell surface, intercellular space and the marginal zone of the five brain vesicles.

Initiation at 96 hrs of development

xviii) Initial incubation (96 hrs) + Dose exposure incubation (24 hrs) = Final development (120 hrs) –

Trace amount of sulfated GAGs was noted in the cytoplasm, moderate at the cell surface and significant amount was present at the intercellular spaces of the ependymal, mantle and marginal zone of the brain regions.

Initiation at 120 hrs of development

xix) Initial incubation (120 hrs) + Dose exposure incubation (24 hrs) = Final development (144 hrs) –

Trace content in the cytoplasm as observed in the normal, moderate at the cell surface and significant increase at the intercellular space of the ependymal and mantle zone of the brain regions. Marginal layer also showed the significant amount of increase sulfated GAGs.

0.5 mM H_2O_2 + 3 mg vitamin C treated

Initiation at 24 hrs of development

i) Initial incubation (24 hrs) + Dose exposure incubation (24 hrs) = Final development (48 hrs) –

Moderate increased amount of sulfated GAGs was noted at the cell surface and intercellular space of the neuronal cells, cytoplasm showed trace amount of sulfated GAGs was noted.

ii) Initial incubation (24 hrs) + Dose exposure incubation (48 hrs) = Final development (72 hrs) –

The amount of sulfated GAGs was similar to that was observed in the normal hrs of development. cytoplasm showed the trace amount, cell surface, intercellular space and the marginal zone with the moderate amount of the sulfated GAGs.

iii) Initial incubation (24 hrs) + Dose exposure incubation (72 hrs) = Final development (96 hrs)-

Cytoplasm with trace amount of sulfated GAGs and cell surface, intercellular space and marginal zone with moderate amount of sulfated GAGs

iv) Initial incubation (24 hrs) + Dose exposure incubation (96 hrs) = Final development (120 hrs) –

The results was similar to the observed as in normal with trace amount in the cytoplasm, moderate at the cell surface, intercellular space and the marginal zone of the five brain regions.

Initiation at 34 hrs of development

v) Initial incubation (34 hrs) + Dose exposure incubation (24 hrs) = Final development (58 hrs) –

Weak amount was present at the cytoplasm, moderate was present the cell surface, intercellular space and marginal layer of the brain.

vi) Initial incubation (34 hrs) + Dose exposure incubation (48 hrs) = Final development (82 hrs) –Trace amount was noted in the cytoplasm, moderate was noted at the cell surface, intercellular space and the marginal layer of the ependymal, mantle and marginal zone of the five brain regions.

viii) Initial incubation (34 hrs) + Dose exposure incubation (72 hrs) = Final development (106 hrs) –

Sulfated GAGs in the cytoplasm was present in trace amount. The cell surface, intercellular space and marginal zone showed the moderate amount of GAGs in all five-brain regions studied

vii) Initial incubation (34 hrs) + Dose exposure incubation (96 hrs) = Final development (130 hrs) –

Sulfated GAGs was similar in amount that was noted visually for the 130 hrs of normal development. Cytoplasm with trace amount, moderate at the cell surface, intercellular space and the marginal zone of the five brain vesicles.

PLATE V

PLATE V- Initial incubation (72 hrs) + Dose exposure incubation (48 hrs) = Final development (120 hrs).

Fig. 1a - Mesen .- Weak intensity of coloration at cell surfaces
1b-Mesen- Weak intensity of coloration at cell surfaces
Fig. 2a - Meten. – Weak intensity of coloration at cell surfaces
2b- Meten- Weak intensity of coloration at cell surfaces
Fig. 3a - Myelen – Weak intensity of coloration at cell surfaces
3b- Myel. – Weak intensity of coloration at cell surfaces
Fig. 4a - Dien – Weak intensity of coloration at cell surfaces
4b- Dien- Weak intensity of coloration at cell surfaces
Fig. 5a - Telen. – Weak intensity of coloration at cell surfaces
5b –Telen - Weak intensity of coloration at cell surfaces

Fig. 6 -11- 0.5 mM H_2O_2 treated embryonic brain X1000.
Fig. 6a - Mesen .- Color intensity increases significant
6b-Mesen- Color intensity increases significantly.
Fig. 7a - Meten. – Color intensity increases significantly.
7b- Meten- Color intensity increases significantly.
Fig. 8a - Myelen – Color intensity increases significantly.
8b- Myel. – Color intensity increases significantly.
Fig. 9a - Dien – Color intensity increases significantly.
9b- Dien- Color intensity increases significantly.
Fig. 10a - Telen. – Color intensity increases significantly.
10b –Telen - Color intensity increases significantly.

Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.
Fig. 11a - Mesen .- Weak intensity of coloration at cell surfaces
11b-Mesen- Weak intensity of coloration at cell surfaces
Fig. 12a - Meten. – Weak intensity of coloration at cell surfaces
12b- Meten- Weak intensity of coloration at cell surfaces
Fig. 13a - Myelen – Weak intensity of coloration at cell surfaces
13b- Myel. – Weak intensity of coloration at cell surfaces
Fig. 14a - Dien – Weak intensity of coloration at cell surfaces
14b- Dien- Weak intensity of coloration at cell surfaces
Fig. 15a - Telen. – Weak intensity of coloration at cell surfaces
15b –Telen - Weak intensity of coloration at cell surfaces

PLATEV

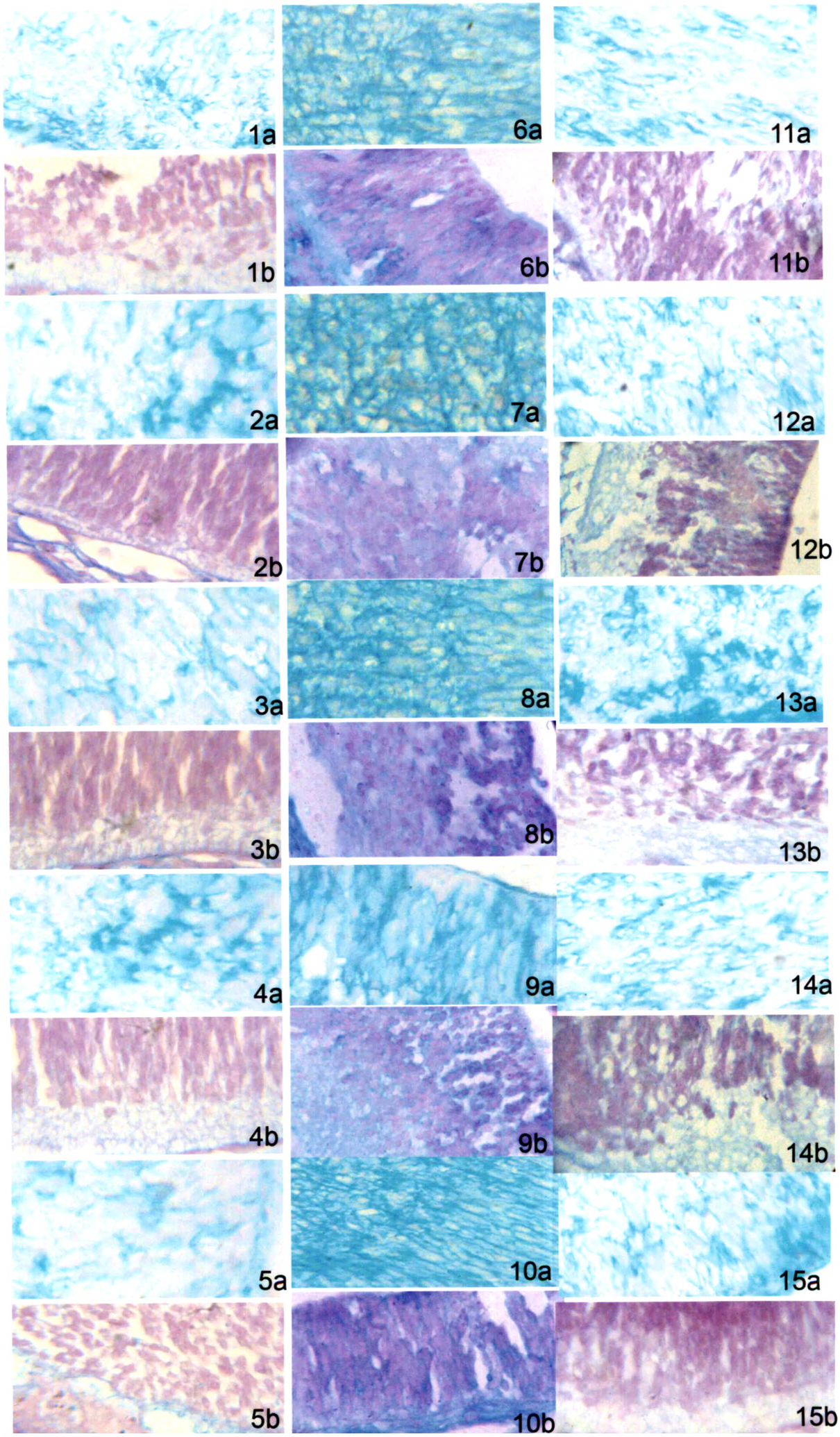
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PLATE VI

PLATE VI- Initial incubation (72 hrs) + Dose exposure incubation (24 hrs) = Final development (96 hrs).

Fig 1-5- Normal embryonic brain X 1000.

Fig. 1a - Mesen .- Weak intensity of coloration at cell surfaces
1b-Mesen- Weak intensity of coloration at cell surfaces
Fig. 2a - Meten. – Weak intensity of coloration at cell surfaces
2b- Meten- Weak intensity of coloration at cell surfaces
Fig. 3a - Myelen – Weak intensity of coloration at cell surfaces
3b- Myel. – Weak intensity of coloration at cell surfaces
Fig. 4a - Dien – Weak intensity of coloration at cell surfaces
4b- Dien- Weak intensity of coloration at cell surfaces
Fig. 5a - Telen. – Weak intensity of coloration at cell surfaces
5b –Telen - Weak intensity of coloration at cell surfaces

Fig. 6 -11- 0.5 mM H_2O_2 treated embryonic brain X1000.
Fig. 6a - Mesen .- Color intensity increases significant
6b-Mesen- Color intensity increases significantly.
Fig. 7a - Meten. – Color intensity increases significantly.
7b- Meten- Color intensity increases significantly.
Fig. 8a - Myelen – Color intensity increases significantly.
8b- Myel. – Color intensity increases significantly.
Fig. 9a - Dien – Color intensity increases significantly.
9b- Dien- Color intensity increases significantly.
Fig. 10a - Telen. – Color intensity increases significantly.
10b –Telen - Color intensity increases significantly.

Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.
Fig. 11a - Mesen .- Weak intensity of coloration at cell surfaces
11b-Mesen- Weak intensity of coloration at cell surfaces
Fig. 12a - Meten. – Weak intensity of coloration at cell surfaces
12b- Meten- Weak intensity of coloration at cell surfaces
Fig. 13a - Myelen – Weak intensity of coloration at cell surfaces
13b- Myel. – Weak intensity of coloration at cell surfaces
Fig. 14a - Dien – Weak intensity of coloration at cell surfaces
14b- Dien- Weak intensity of coloration at cell surfaces
Fig. 15a - Telen. – Weak intensity of coloration at cell surfaces
15b –Telen - Weak intensity of coloration at cell surfaces

PLATE VI

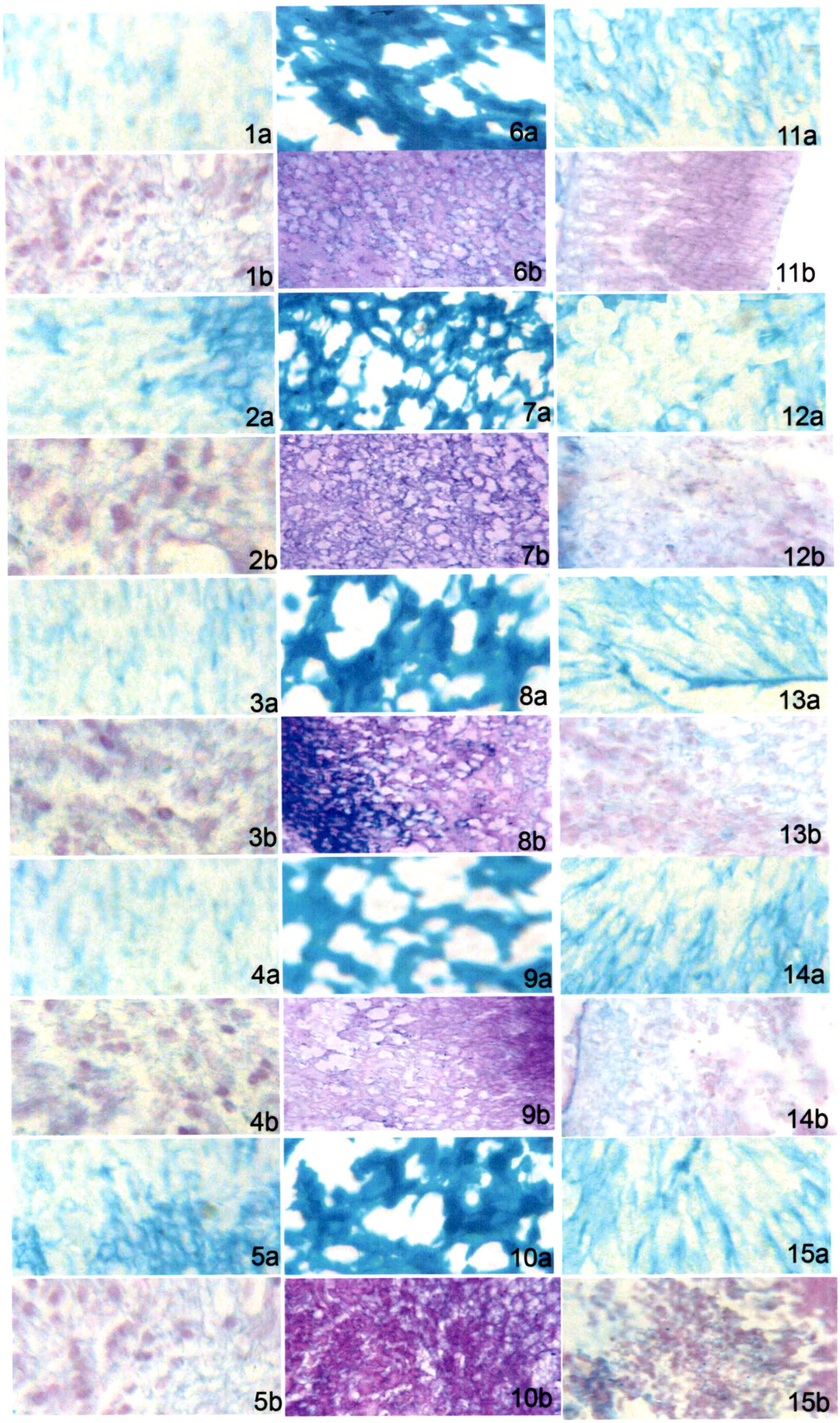
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PLATE VII

PLATE VII- Initial incubation (96 hrs) + Dose exposure incubation (24 hrs) = Final development (120 hrs).

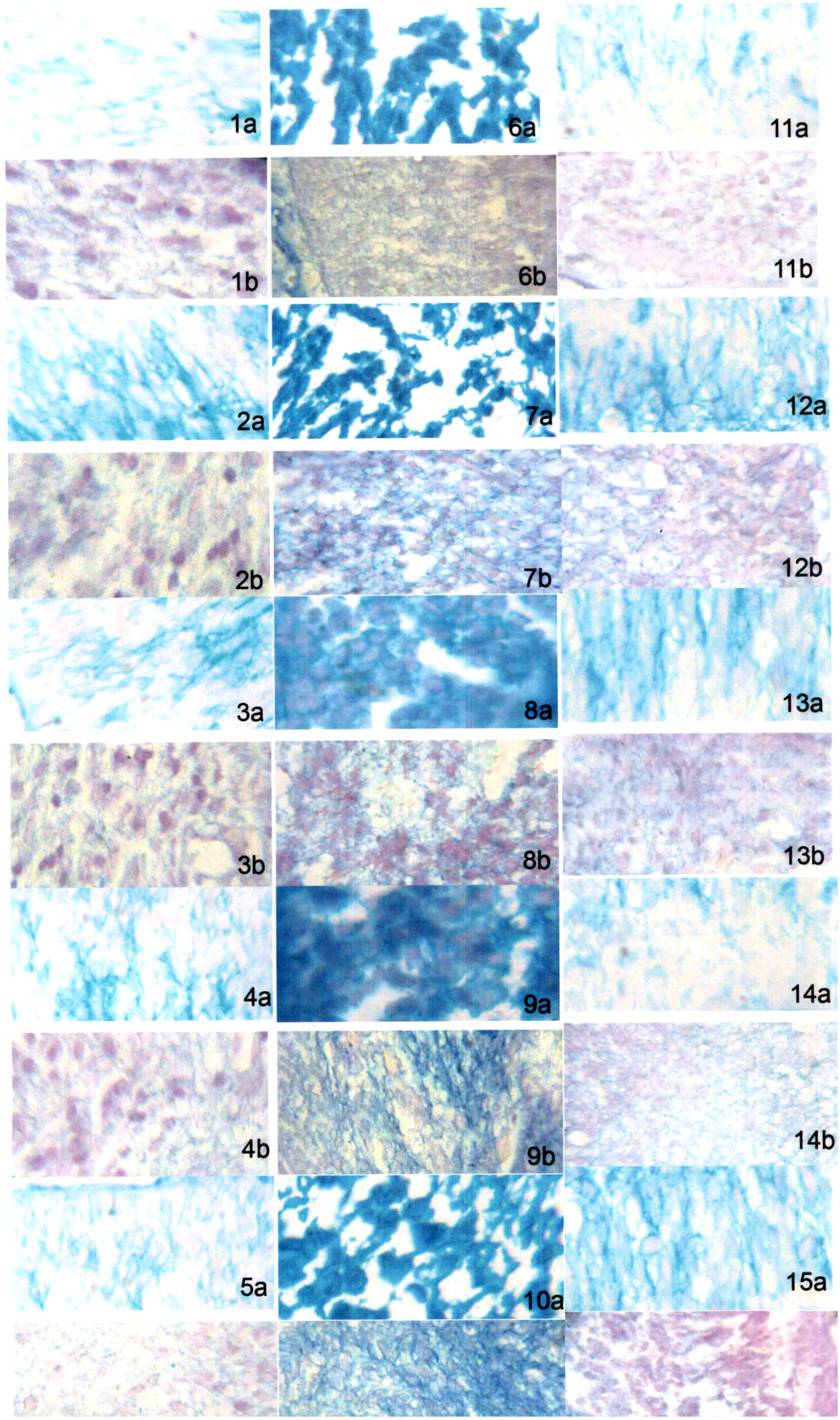
Fig 1-5- Normal embryonic brain X 1000.

Fig. 1a - Mesen .- Weak intensity of coloration at cell surfaces
1b-Mesen- Weak intensity of coloration at cell surfaces
Fig. 2a - Meten. – Weak intensity of coloration at cell surfaces
2b- Meten- Weak intensity of coloration at cell surfaces
Fig. 3a - Myelen – Weak intensity of coloration at cell surfaces
3b- Myel. – Weak intensity of coloration at cell surfaces
Fig. 4a - Dien – Weak intensity of coloration at cell surfaces
4b- Dien- Weak intensity of coloration at cell surfaces
Fig. 5a - Telen. – Weak intensity of coloration at cell surfaces
5b –Telen - Weak intensity of coloration at cell surfaces

Fig. 6 -11- 0.5 mM H_2O_2 treated embryonic brain X1000.
Fig. 6a - Mesen .- Color intensity increases significant
6b-Mesen- Color intensity increases significantly.
Fig. 7a - Meten. – Color intensity increases significantly.
7b- Meten- Color intensity increases significantly.
Fig. 8a - Myelen – Color intensity increases significantly.
8b- Myel. – Color intensity increases significantly.
Fig. 9a - Dien – Color intensity increases significantly.
9b- Dien- Color intensity increases significantly.
Fig. 10a - Telen. – Color intensity increases significantly.
10b –Telen - Color intensity increases significantly.

Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.
Fig. 11a - Mesen .- Weak intensity of coloration at cell surfaces
11b-Mesen- Weak intensity of coloration at cell surfaces
Fig. 12a - Meten. – Weak intensity of coloration at cell surfaces
12b- Meten- Weak intensity of coloration at cell surfaces
Fig. 13a - Myelen – Weak intensity of coloration at cell surfaces
13b- Myel. – Weak intensity of coloration at cell surfaces
Fig. 14a - Dien – Weak intensity of coloration at cell surfaces
14b- Dien- Weak intensity of coloration at cell surfaces
Fig. 15a - Telen. – Weak intensity of coloration at cell surfaces
15b –Telen - Weak intensity of coloration at cell surfaces

PLATE VII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Initiation at 40 hrs of development

ix) Initial incubation (40 hrs) + Dose exposure incubation (24 hrs) = Final developmental (64 hrs) –

Cytoplasm with trace amount of sulfated GAGs. Weak at the cell surfaces and moderate at the intercellular spaces of the ependymal, mantle, and marginal zone of the five brain regions.

x) Initial incubation (40 hrs) + Dose exposure incubation (48 hrs) = Final developmental (88 hrs) –

Trace amount of sulfated GAGs was noted in the cytoplasm while the moderate amount was noted at the cell surface, intercellular space and marginal zone of the three regions considered for the five brain regions.

xi) Initial incubation (40 hrs) + Dose exposure incubation (72 hrs) = Final development (112 hrs) –

Moderate amount of sulfated GAGs was observed at the cell surface, intercellular space and the marginal zone of the five brain regions. Cytoplasm of the ependymal, mantle zone contains the trace amount of sulfated GAGs.

xii) Initial incubation (40 hrs) + Dose exposure incubation (96 hrs) = Final development (136 hrs) –

Trace amount was noted in the cytoplasm, moderate at the cell surface, intercellular space and marginal zone of the five brain vesicles.

Initiation at 48 hrs of development

xiii) Initial incubation (48 hrs) + Dose exposure incubation (24 hrs) = Final development (72 hrs) –

Cytoplasm with weak amount of sulfated GAGs while cell surface, intercellular space and marginal zone with the moderate amount of sulfated GAGs

xiv) Initial incubation (48 hrs) + Dose exposure incubation (48 hrs) = Final development (96 hrs) –

Trace amount of sulfated GAGs was noted in cytoplasm of the neuroepithelial cells of ependymal and mantle zone showed the while the cell surface, intercellular space and the marginal zone observed with the moderate amount of sulfated GAGs.

xv) Initial incubation (48 hrs) + Dose exposure incubation (96 hrs) = Final development (120 hrs) –

The amount of sulfated GAGs was present in the cytoplasm of the neuronal lass was trace while at the cell surface, intercellular space and marginal layer contains the moderate amount of sulfated GAGs in the five brain regions

Initiation at 72 hrs of development

xvi) Initial incubation (72 hrs) + Dose exposure incubation (24 hrs) = Final development (96 hrs) –

Cytoplasm with weak amount of sulfated GAGs cell surface, intercellular space and marginal zone with moderate amount of GAGs.

xvii) Initial incubation (72 hrs) + Dose exposure incubation (48 hrs) = Final development (120 hrs) –

Cytoplasm of the neuronal cells contain the amount of sulfated GAGs was present in the was trace while at the cell surface, intercellular space and marginal layer contains the moderate amount of sulfated GAGs in the five brain regions

Initiation at 96 hrs of development

xviii) Initial incubation (96 hrs) + Dose exposure incubation (24 hrs) = Final development (120 hrs) –

Neuronal cells showed trace amount of sulfated GAGs in the cytoplasm, while at the cell surface, intercellular space and marginal layer contains the moderate amount of sulfated GAGs in the five brain regions

Initiation at 120 hrs of development

xix) Initial incubation (120 hrs) + Dose exposure incubation (24 hrs) = Final development (144 hrs) –

Sulfated GAGs in the cytoplasm of neuronal cells showed trace amount of, while at the cell surface, intercellular space and marginal layer contains the moderate amount of sulfated GAGs in the five brain regions

Discussion:

The results indicated that SGAG were observed on cell surfaces during early embryonic development (up to 96 hrs) in normal while in late hrs of development the reaction was in moderate (+3) concentration in cytoplasm and trace amount at cell surfaces. The reaction in intercellular spaces was not histologically detectable.

Hydrogen peroxide treatment had inhibited the migration of cells and neuroaxonal networking and hence associated cell surfaces reaction also showed

stumped aggregated masses but moderately stained in the embryos in early development.

The embryos that were treated with H_2O_2 and were observed for SGAG distribution in brain during 56-144 hrs of development showed moderate distribution of SGAG in cytoplasm of neuronal cells and cytoplasmic extensions of axons and dendrites, which were stunted. The aggregations of these cytoplasmic extensions showed high intensity possibly because of accumulation at the various regions rather than the actual increase in amount of SGAGs.

Treatment of H_2O_2 + vitamin C (3 mg) showed protection from the effects of H_2O_2 and normal distribution of SGAG was observed.

During early hrs of development the SGAG noted persisted the alcinophilia up to concentration 0.1 M $MgCl_2$ and Azure A (pH 0.5) metachromatia which was resistant to alcohol in these sites i.e. cell surfaces, indicating presence of SGAG.

In late hrs of development the neuronal cell cytoplasm lost alcinophilia at >0.2 M $MgCl_2$ indicating SGAG presence in cytoplasm. Azure A (pH 1) metachromatia was alcohol resisting, thus confirmed the presence of SGAG in cytoplasm weak at cell surfaces.

In normal embryo at the various stages of brain development up to 144 hrs when brain vesicles were completely formed. In the five regions of brain viz. mesencephalon , metencephalon , diencephalons and telencephalon and their three layers i.e. ependymal, mantle and marginal the distribution of sulfated glycosaminoglycans showed trace to weak amount in cytoplasm cell surfaces and was not histochemically detectable in intercellular spaces. The moderate amount was present at cell surfaces from 48 hrs to 96 hrs of development.

In the next phase of development i.e. 96 hrs-144 hrs, the distribution sites are cytoplasm and cell surfaces. The amount of sulfated GAG was increased at the different sites highest in normal distribution being at and cytoplasm and weak /traces at cell surfaces.

These results indicated that the SGAG at cell surfaces involved in the early brain vesicles formation and development of three layers of brain vesicles. Staining intensity of SGAG observed in early development of surfaces and in weak to moderate amount was also noted earlier (Tage *et al.*,1988).But in the later hrs of development neuronal cells cytoplasm was rich in SGAG and even in cytoplasm of cell extensions *viz.* axon and dendrites.

Treatment of H_2O_2 had interfered in the cell migration and axoneuronal network development and thus retarded the growth. Thus, the SGAG at cell surfaces in early hrs of development appeared to be involved in cell migration and axoneuronal networking. Since simultaneous treatment of 3 mg vitamin C, which is free radical scavengers and antioxidant, it had protected against H_2O_2 released free radicals, which maintained the normal distribution of SGAG and continued the migration of cells and cytoplasmic axon dendrite development to form the zones of brain.

Present results of SGAG in late hrs of development indicated its moderate distribution in cytoplasm and cytoplasmic extensions while H_2O_2 treatment at different initiation hrs had shown the stagnancy of the material and aggregation of growing ends of axoneuronal networking extensions. The dense staining seems to be due to aggregation but not due to increased amount SGAGs, which was protected by simultaneous treatment of vitamin C 3 mg.

In late hrs of development of brain, ventricular cavity is increased by raising the hydrostatic pressure within the vesicular cavities, which is created and regulated by the presence of SGAG at late hrs (Alonso, et. al. 1999) in chick neuronal secreted fluid dermatin sulphate, chondoitin sulphate. The cavity and tissue Kinetics studied in chick embryo during brain growth (24-120 hrs) showed brain enlargement 8.5 times, cavity expansion of 9.8 times and tissue growth 7.6 times (Pacheco *et al.,* 1985). This kinetics was retarded by 60% in H_2O_2 treated embryos but simultaneous treatment of vitamin C had altered this kinetics and remained similar to normal indicating there is abnormal growth of brain in presence of H_2O_2 + vitamin C (3 mg).

It has also been shown hat during 48-96 hrs of development brain cells were incapable to differentiate in culture (Tonzet *et al.,* 1975) and other growth factors in vivo. In present condition H_2O_2 mediated stability of differentiation was protected in presence of vitamin C (3 mg) indicating the potency of the cells to respond vitamin C (3 mg).

During early stages of development fibroblast growth factor -2 and other isoform of these factors from cerebrospinal fluid regulates the neuroepithelial cell behaviour i.e. cell protects and neurogenesis proliferation. *In vivo* and *in vitro* indicating complementary regulation by neuronal and extra-neuronal factors (Martin *et al. 1999,*). In present observations, also vitamin C (3 mg) treatment given simultaneously protected the normal development of brain and death of embryo. Since H_2O_2 induced abnormalities and survivals and death were observed (Section I). Thus

vitamin C directly or through its mediated metabolism stimulated and regulated the growth of brain under stressed condition in vivo indicating neuronal cells stimulatory potency of vitamin C. Heparin sulphate which creates the hydrostatic pressure smoothly and develops the ventricular cavities (Alonso, 1999). These are contributed by neuronal cells as well as by other cells. The cavity development begins after 144 hrs of development and requirements of above SGAGs is 120-144 onwards. Therefore during 96-144 hrs of development presence of cytoplasmic GAGs and normal and H_2O_2 + vitamin C (3 mg) treated embryos and also that in cytoplasmic extensions may be preparations of neuronal cells for production of SGAGs and hence are being in preparation of these materials in cytoplasm. So that, in following development hrs brain can enter ventricular expansion associated with growth.

C) Hyaluronan (HA):

Observations:

Alterations in HA are depicted in Plate no I- VII and Table no I a-Id

Normal:

48 hrs development-

HA was absent in the cytoplasm of the neuroepithelial cells and intercellular space of the brain regions, trace amount of HA was observed at the cell surfaces.

58 hrs development-

HA acid was present in trace amount at the cell surface, and was absent in the cell surfaces and intercellular spaces.

64 hrs development-

HA was absent in the cytoplasm of the neuroepithelial cells and intercellular spaces. Trace amount of HA was noted at the cell surface.

72 hrs development-

Trace amount of HA was present on cell surfaces, but was absent in the cytoplasm of the cells and intercellular spaces.

82 hrs development-

Trace amount of the HA was present at the cell surfaces, but was absent in cytoplasm and the intercellular spaces.

88 hrs development-

Trace amount of the HA was noted at the cell surfaces, and it was absent in cytoplasm and at intercellular space of the brain regions.

96 hrs development-

Very small amount of HA was present at the cell surface, but it was absent intercellular space and the marginal zone the brain.

106 hrs development-

The axonal outgrowths and the cell surface of the neuronal cells contains trace amount of the HA in the ependymal, mantle and marginal zone.

112 hrs development-

The extensions of the neuronal cells and the cell surfaces contained very trace amount of the HA, but was absent in the cytoplasm and intercellular regions.

120 hrs development-

Trace amount of HA was present at the cell surfaces, it was absent in the cytoplasm and intercellular space of the ependymal and mantle zone of the and marginal zone of the brain regions.

130 hrs development-

HA content was in amount trace at cell surface, intercellular spaces and cytoplasm was devoid of HA.

136 hrs development-

Trace amount of HA acid was present at the cell surfaces, and was absent in cytoplasm and intercellular spaces.

144 hrs development-

HA was present in the trace amount at the cell surfaces, intercellular spaces and cytoplasm was devoid of HA.

Control: HBSS-

In the brain of control embryo of HBSS showed the similar results which were observed in the brain of normal embryo of corresponding hours of development inthe brain regions.

Control: 3 mg vitamin C-

Treatment of 3 mg vitamin C given to normal embryo did not alter the HA content in brain of normal embryo.

PLATE VIII

PLATE I- Initial incubation (24 hrs) + Dose exposure incubation (96hrs) = Final development (120 hrs). HA

Fig 1-5- 0.5 mM H2O2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 1 - Mesen .- Well formed axonal outgrowths with distributed hyaluronan stained with AZ pH 4.5.

Fig. 2 - Meten. – Axonal outgrowths well formed.

Fig. 3 - Myelen - Axonal outgrowths well formed

Fig. 4 - Dien -. Axonal outgrowths well formed

Fig. 5 - Telen. – Axonal outgrowths well formed

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000.

Fig. 6 - Mesen. – Stunted axons , aggregated HA at the tips of axonal outgrowths.

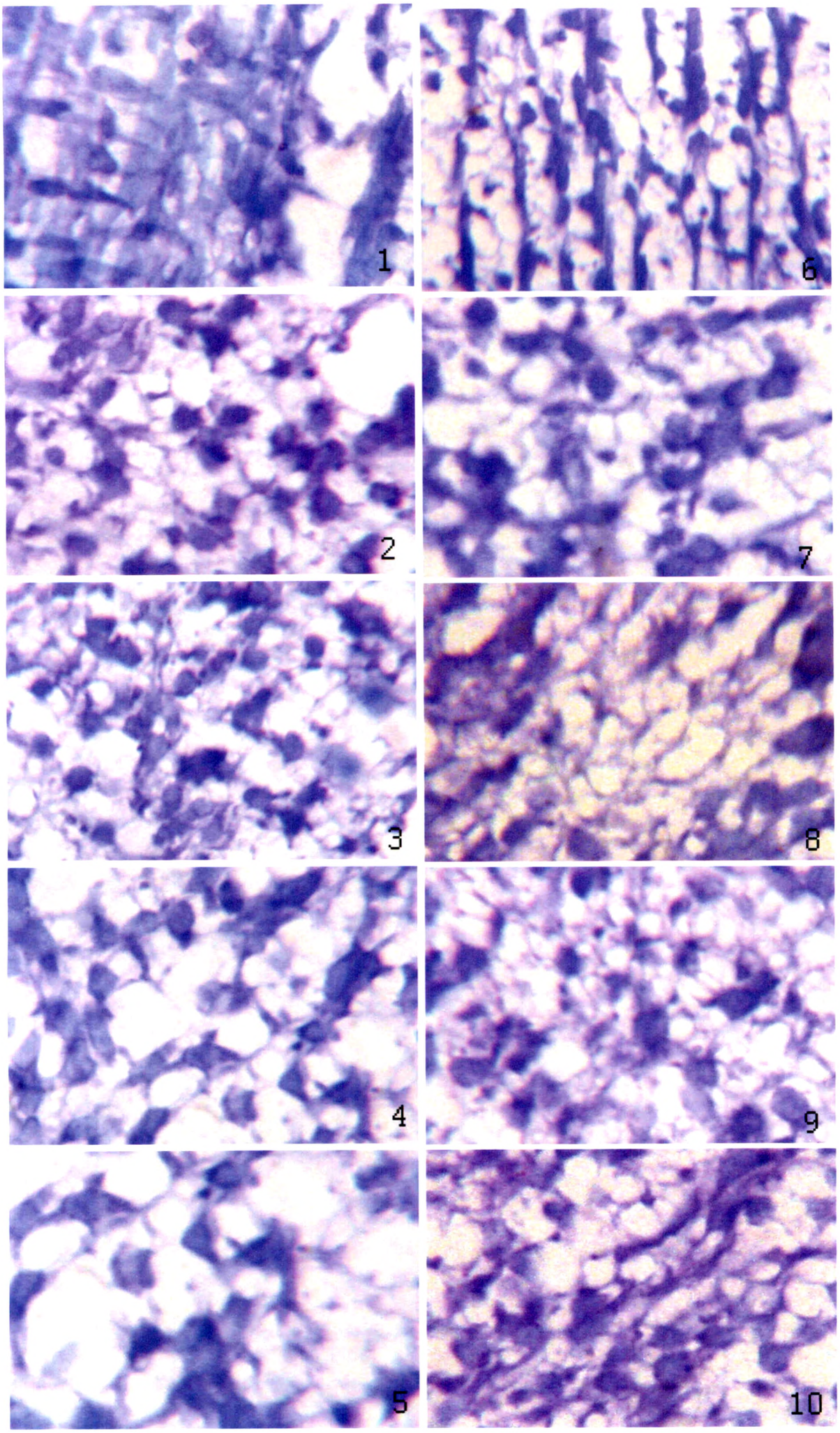
Fig. 7 - Meten. – Stunted axons , aggregated HA at the tips of axonal outgrowths

Fig. 8 - Myelen – Stunted axons , aggregated HA at the tips of axonal outgrowths

Fig. 9 - Dien -. Stunted axons , aggregated HA at the tips of axonal outgrowths

Fig. 10 - Telen. – Stunted axons , aggregated HA at the tips of axonal outgrowths.

PLATE VIII



0.5 mM H_2O_2 treated

Initiation at 24 hrs of development

i) Initial incubation (24 hrs) + Dose exposure incubation (24 hrs)= Final development (48 hrs) –

HA was moderate at the cell surface. No alterations were observed in the cytoplasm of the cells and at intercellular spaces.

ii) Initial incubation (24 hrs) + Dose exposure incubation (48 hrs) = Final development (72 hrs) –

HA was moderate in amount at the cell surfaces. intercellular space, while trace amount was present in the cytoplasm of the cells.

iii) Initial incubation (24 hrs) + Dose exposure incubation (72 hrs) = Final developmental (96 hrs) –

Large amount of HA was noted at the cell surfaces and intercellular spaces, but was observed in the cytoplasm of the neuronal cells.

iv) Initial incubation (24 hrs) + Dose exposure incubation (96 hrs) = Final development (120 hrs) –

HA was moderate at the cell surfaces and intercellular spaces. There was weak content of the HA in the cytoplasm of the neuronal cells of the ependymal and mantle zone of the brain regions.

Initiation at 34 hrs of development

v) Initial incubation (34 hrs) + (24 hrs) dose exposure incubation hrs = Final development (58 hrs) –

Moderate amount of HA was present at the cytoplasm, intercellular spaces. of the neuronal cells.

vi) Initial incubation (34 hrs) + Dose exposure incubation (48 hrs) = Final development (82 hrs) –

Moderate amount of the HA was present at the cell surfaces and intercellular spaces.

vii) Initial incubation (34 hrs) + Dose exposure incubation (72 hrs) = Final development (106 hrs) –

Large amount of the HA was increased at the cell surface, intercellular space and the marginal zone of the brain, and weak content was observed in the cytoplasm of the neuronal cells of the brain regions.

viii) Initial incubation hrs + Dose exposure incubation hrs = Final development hrs (34+ 96 = 130) –

Significant amount of HA was present at the cell surfaces and intercellular spaces.

Initiation at 40 hrs of development

ix) Initial incubation (40 hrs) + Dose exposure incubation (24 hrs) = Final development (64 hrs) –

Moderate amount of HA observed at the cell surfaces and intercellular spaces.

x) Initial incubation (40 hrs) + Dose exposure incubation (48 hrs) = Final development (88 hrs) –

Moderate amount of HA was noted at the cell surface, intercellular spaces.

xi) Initial incubation (40 hrs) + Dose exposure incubation (72 hrs) = Final development (112 hrs) –

Significant amount of the HA was noted at the cell surfaces, intercellular spaces in the mantle layer and marginal layer.

xii) Initial incubation (40 hrs) + Dose exposure incubation (96 hrs) = Final development (136 hrs) –

Significant amount of HA was noted at the cell surfaces, intercellular spaces of the brain regions.

Initiation at 48 hrs of development

xiii) Initial incubation (48 hrs) + Dose exposure incubation (24 hrs) = Final development (72 hrs)-

Moderate amount of HA was observed at the cell surface and intercellular spaces of the neuronal cells in the mantle and marginal layer.

xiv) Initial incubation (48 hrs) + Dose exposure incubation (48 hrs) = Final developmental (96 hrs) –

Moderate amount of HA was noted at the cell surfaces and intercellular spaces of the brain regions.

xv) Initial incubation (48 hrs) + Dose exposure incubation (72 hrs) = Final developmental (120 hrs) –

Significant amount of HA was noted at the cell surfaces, intercellular spaces of mantle and the marginal zone of the brain regions.

Initiation at 72 hrs of development

xvi) Initial incubation (72 hrs) + Dose exposure incubation (24 hrs) = Final development (96 hrs) –

Moderate amount of the HA was noted at cell surfaces and intercellular spaces.

xvii) Initial incubation (72 hrs) + Dose exposure incubation (48 hrs) = Final development (120 hrs) –

Large amount was noted at the cell surfaces, intercellular spaces of the mantle and marginal zone of the brain.

Initiation at 96 hrs of development

xviii) Initial incubation (96 hrs) + Dose exposure incubation (24 hrs) = Final development (120 hrs) –

Moderate content was noted at the cell surfaces and intercellular spaces.

Initiation at 120 hrs of development

xix) Initial incubation (120 hrs) + Dose exposure incubation (24 hrs) = Final development (144 hrs) –

Weak amount of HA was noted at the cell surfaces and intercellular space of the mantle zone and the marginal zone of the brain.

0.5 mM H_2O_2 + 3 mg vitamin C treated

Initiation at 24 hrs of development

i) Initial incubation (24 hrs) + Dose exposure incubation (24 hrs) = Final development (48 hrs) –

Weak amount of HA was noted at the cell surfaces and intercellular space.

ii) Initial incubation (24 hrs) + Dose exposure incubation (48 hrs) = Final development (72 hrs) –

HA was in trace amount was noted at the cell surfaces and intercellular spaces of the brain regions.

iii) Initial incubation (24 hrs) + Dose exposure incubation (72 hrs) = Final development (96 hrs)-

Cell surfaces, intercellular spaces showed trace amount of the HA.

iv) Initial incubation (24 hrs) + Dose exposure incubation (96 hrs) = Final development (120 hrs) –

Trace amount of HA was noted at the cell surfaces and intercellular spaces.

Initiation at 34 hrs of development

v) Initial incubation (34 hrs) + Dose exposure incubation (24 hrs) = Final development (58 hrs) –

Weak amount of HA was noted at the cell surfaces, intercellular spaces.

vi) Initial incubation (34 hrs) + Dose exposure incubation (48 hrs) = Final development (82 hrs) –

Cytoplasm was without the HA content, and it was in trace amount at the cell surfaces and intercellular spaces.

viii) Initial incubation (34 hrs) + Dose exposure incubation (72 hrs) = Final development (106 hrs) –

The result was similar to that was observed for the normal embryo of the same developmental stage. Where the cytoplasm was without the HA and cell surfaces, intercellular spaces was with trace amount of the HA.

vii) Initial incubation (34 hrs) + Dose exposure incubation (96 hrs) = Final development (130 hrs) –

Trace amount of HA was noted at the cell surface, intercellular spaces but it was not observed in the cytoplasm.

Initiation at 40 hrs of development

ix) Initial incubation (40 hrs) + Dose exposure incubation (24 hrs) = Final developmental (64 hrs) –

Weak amount of HA was noted except the cytoplasm of the neuronal cells of the all brain regions.

x) Initial incubation (40 hrs) + Dose exposure incubation (48 hrs) = Final developmental (88 hrs) –

Trace amount of HA at the cell surfaces, intercellular spaces. **xi) Initial incubation (40 hrs) + Dose exposure incubation (72 hrs) = Final development (112 hrs) –**

HA was trace amount at the cell surfaces, intercellular spaces at mantle and marginal layer of the brain.

xii) Initial incubation (40 hrs) + Dose exposure incubation (96 hrs) = Final development (136 hrs) –

The results was similar to that was noted at the normal embryo of the same developmental group. Trace amount was at cell surfaces, intercellular spaces.

Initiation at 48 hrs of development

xiii) Initial incubation (48 hrs) + Dose exposure incubation (24 hrs) = Final development (72 hrs) –

Weak content was noted at the cell surfaces, intercellular spaces of mantle and marginal zone of the five brain regions.

xiv) Initial incubation (48 hrs) + Dose exposure incubation (48 hrs) = Final development (96 hrs) –

Trace amount was noted at the cell surfaces and intercellular spaces.

xv) Initial incubation (48 hrs) + Dose exposure incubation (96 hrs) = Final development (120 hrs) –

HA was in trace amount at the cell surfaces and intercellular spaces.

Initiation at 72 hrs of development

xvi) Initial incubation (72 hrs) + Dose exposure incubation (24 hrs) = Final development (96 hrs) –

Weak amount of HA was noted at the cell surfaces and intercellular spaces.

xvii) Initial incubation (72 hrs) + Dose exposure incubation (48 hrs) = Final development (120 hrs) –

Trace content of the HA at the cell surfaces and intercellular spaces of the mantle and marginal zone of the five brain regions.

Initiation at 96 hrs of development

xviii) Initial incubation (96 hrs) + Dose exposure incubation (24 hrs) = Final development (120 hrs) –

HA was observed with traces at the cell surfaces and intercellular spaces of the five brain regions.

Initiation at 120 hrs of development

xix) Initial incubation (120 hrs) + Dose exposure incubation (24 hrs) = Final development (144 hrs) –

Trace content at the cell surfaces and intercellular spaces.

Discussion:

From the different staining techniques used (Table) it is revealed that hyaluronan is present in weak concentration and restricted to the growing tips of axonal and dendral membrane extensions in the five regions of brain and especially in mantle and marginal layer of the brain of normal embryo.

H_2O_2 treatment had increased the content at the stunted neural membrane growths of axons and dendrites. The increase may be in response to stress of H_2O_2. The localization of hyluronan is not altered by H_2O_2 induced cytotoxicity.

Hydrogen peroxide + Vitamin C (3 mg) treatment had normalized the content and had not influenced the sites o distribution.

The initiation of H_2O_2/ H_2O_2 + Vitamin C (3 mg) treatment at different developmental hrs cumulated with different hrs of exposure to the treatment had not influenced its distribution sites. The amount of content of hyluronan, which had been increased after H2O2 exposure, was also normalized on H_2O_2 + 3 mg vitamin C exposure.

The results indicate that hyaluronan is less in comparison to remaining studied components during brain development.

It is known that during the embryonic development the deposits of hyuronan creates a cell free area by expansion of the extracellular space and facilitates the cell migration (Toole, 1982). It is known to be expressed and regulated through expression and regulation of Hyluronan synthatase (Brinck, 2000). It does not form proteoglyacans but functions through hyluronan binding protein which are present mainly extracellularly but can occurs intracellularly.

The localization of Hyluronan in present results are indicators that it is involved in migration of cells as well as migration of axoneuronal and dendral tips that are present specifically in mantle and marginal layer.

CD 44 is the principle cell surface receptor involved in hyluronan binding (Aruffo *et. al.* 1990) CD 44 distribution seems to be present on the tips of the axons and dendrites. On H_2O_2 exposure since these expanding network is stunted, the interacted CD_{44} and Hyluronan complex seems to stabilized and in response to H_2O_2 generated free radicals increased Hyluronan seems to be synthesized as its concentration at these site was increased. These results not only confirm the earlier observations based on 3H-hyaluronan and receptor of hyaluronan binding indicating involvement of hyaluron receptor in early brain development(Banerjee and Toole, 1991) but also confirm that the leading domain of axons and dendrites are involved in hyaluronan mediated migration activity.

Thus Hyluronan a GAG not in the form of proteoglycans plays role in migration with its localized, specified role. It seems to work through selectively binding to the intercellular space proteins since H_2O_2 + vitamin C (3 mg) had

protected the migratory activities of the cell. Since cell migration and cell locomotion (in present observations axonal and dendrites extensions) involves a series of complex interactions between cytoskeleton, cell surface receptors and matrix (in present observations intercellular space) components (Sheetz 1996; Lauffenburger 1998)

Migratory activities are known to be decreased by increase in hyluronidase activity (Spicer, 1999). In present results, though on exposure of H_2O_2 the migrations are inhibited as revealed through the stunted growths of axons and dendrites. The inhibition of migration of extending membrane processes is not due to Hyluronidase mediated removal of Hyluronan, instead since its intensity/concentration is increased at these sites indicate the stabilization of cytoskeletal, cell surfaces and intercellular involvements with Hyluronan binding.

Simultaneous treatment of vitamin C (3 mg) since had protected the normal migratory activities leading to production of normal embryos on hatching vitamin C through its free radical scavenging/ antioxidant role (direct or through in vivo production of natural antioxidants of cell viz. glutathione) must be protecting the stabilization of these complexes so that migrations are not interfered by the free radical.

Section IV Sialic acid

Observations:

Alterations in SA are depicted in Plate no I- VII and Table no I a-Id

Normal:

48 hrs development-

Trace content of SA was present in the cytoplasm, intercellular space and cell surface contain weak amount of SA. PAS positive O-acyl SA (C_7, C_8 or C_9) was present in weak amount in the cell surface and cytoplasm; the content was trace in at intercellular space.

58 hrs development-

Trace content of SA was present in the cytoplasm and intercellular space while cell surface was with weak amount of SA. O-acyl SA (C_7, C_8 or C_9) was weak in amount at cell surfaces and cytoplasm, trace at intercellular space.

64 hrs development-

SA was trace in content at the cytoplasm and intercellular space. Weak amount was noted at the cell surface. PAS positive O-acyl SA (C_7, C_8 or C_9) was

present in weak content at cell surface and in cytoplasm. Trace content was noted intercellular spaces.

72 hrs development-

Cytoplasm and intercellular space contain trace amount of SA, weak amount was observed visually at cell surface. O-acyl SA (C_7, C_8 or C_9) was observed in trace amount at intercellular space, weak content in cytoplasm and cell surface of the ependymal and mantle zone, marginal zone also contains the weak amount of O-acyl SA (C_7, C_8 or C_9)

82 hrs development-

The content of SA was trace in the cytoplasm and intercellular space while weak at cell surface and of the ependymal and mantle layer, marginal layer content of SA was weak by visual observations. Intercellular space of the neuroepithelial cells contains weak amount of O-acyl SA (C_7, C_8 or C_9) cell surface and cell surface showed trace amount of O-glycosylated SA.

88 hrs development-

Trace amount was observed at the cytoplasm and intercellular space, moderate amount at the cell surface in ependymal and mantle zone of the five brain regions, marginal zone contain moderate amount of SA. O-acyl SA (C_7, C_8 or C_9) was with weak amount at the cell surface and in the cytoplasm, trace in the intercellular space. Marginal zone contains weak amount of O-acyl SA (C_7, C_8 or C_9)

96 hrs development-

Cytoplasm and intercellular space contains trace amount of SA, cell surface contain moderate amount of SA of ependymal and mantle zone. Marginal zone contains moderate amount of SA. O-acyl SA (C_7, C_8 or C_9) was present in trace amount at the intercellular space and moderate at cytoplasm and cell surfaces of all three zones of the five brain regions.

106 hrs development-

Cells of the ependymal and mantle layer showed trace content of SA in the cytoplasm and intercellular space. Moderate content at the cell surfaces, and marginal zone of the brain regions where axonal outgrowths was extended. O-acyl SA (C_7, C_8 or C_9) was with moderate content in the cytoplasm and cell surfaces. Intercellular space showed trace amount.

Sialic acid(SA):
Table no. II. a

Initial incubation (24 hrs) + Dose exposure incubation (24 hrs) = Final incubation (48 hrs).									
Staining techniques	Brain regions	Normal Neuroepithelial layer			Inference of observations	0.5 mM H_2O_2 treated Neuroepithelial layer			Inference of observations
		Cyt	Cs	Ics		Cyt	Cs	Ics	
1)AB pH 2.5	Mesen	±	++	±	Moderate reaction at shistochemically detectable SA was not noted at Cyt and Ics	+	+++++	±	Weak reaction at Cyt and intense at Cs.Histochemically detectable SA was not noted at Ics
	Meten	±	++	±		+	+++++	±	
	Myel	±	++	±		+	+++++	±	
	Dien	±	++	±		+	+++++	±	
	Telen	±	++	±		+	+++++	±	
2)PA*Bh Sap PAS	Mesen	++	++	±	Moderate staining at Cs and Cyt. but histochemically detectable SA was not noted at Ics.	+++++	+++++	±	Increased color intensity at Cyt and Cs but not to the Ics
	Meten	++	++	±		+++++	+++++	±	
	Myel	++	++	±		+++++	+++++	±	
	Dien	++	++	±		+++++	+++++	±	
	Telen	++	++	±		+++++	+++++	±	
3) AZ 3.5	Mesen	±	++	±	Moderate reaction at shistochemically detectable SA was not noted at Cyt and Ics	+	+++++	±	Results observed was similar to AB 2.5 results
	Meten	±	++	±		+	+++++	±	
	Myel	±	++	±		+	+++++	±	
	Dien	±	++	±		+	+++++	±	
	Telen	±	++	±		+	+++++	±	
4) Sap-AB 2.5	Mesen	±	+++	±	Color intensity increased after the saponification	+	++++++	±	Increased color intensity at Cs
	Meten	±	+++	±		+	++++++	±	
	Myel	±	+++	±		+	++++++	±	
	Dien	±	+++	±		+	++++++	±	
	Telen	±	+++	±		+	++++++	±	
5)Mild meth AB2.5	Mesen	±	±	±	Intensity of coloration decreases	±	±	±	Intensity of coloration decreases
	Meten	±	±	±		±	±	±	
	Myel	±	±	±		±	±	±	
	Dien	±	±	±		±	±	±	
	Telen	±	±	±		±	±	±	

Table no. II.a continued

Initial incubation (24 hrs) + Dose exposure incubation (24 hrs) = Final incubation (48 hrs).									
Staininig techniques	Brain Regions	Normal Neuroepithelial layer			Inference Of observations	0.5 mM H_2O_2 Neuroepithelial layer			Inference Of observations
		Cyt	Cs	Ics		Cyt	Cs	Ics	
6)Mild meth Sap AB 2.5	Mesen	±	++	±	Weak reaction at Cs and Ics, trace in the cytoplasm	+	+++++	±	Regained color intensity
	Meten	±	++	±		+	+++++	±	
	Myel	±	++	±		+	+++++	±	
	Dien	±	++	±		+	+++++	±	
	Telen	±	++	±		+	+++++	±	
7)Act meth AB 2.5	Mesen	±	±	±	Moderate color intensity was observed at all Cyt, Cs and Ics	±	±	±	Moderate coloration in Cyt and Ics, intense at Cs
	Meten	±	±	±		±	±	±	
	Myel	±	±	±		±	±	±	
	Dien	±	±	±		±	±	±	
	Telen	±	±	±		±	±	±	
8)Act met Sap AB pH2.5	Mesen	±	++	±	Regained color intensity	+	+++++	±	Regained color intensity
	Meten	±	++	±		+	+++++	±	
	Myel	±	++	±		+	+++++	±	
	Dien	±	++	±		+	+++++	±	
	Telen	±	++	±		+	+++++	±	
9)AB 2.5 PAS	Mesen	+M	++B	±P	Weak magenta color in Cyt, moderate blue at Cs and trace purple at Ics	+	+++++B	±	Intensely stained blue coloration at thc Cs
	Meten	+M	++B	±P		+	+++++B	±	
	Myel	+M	++B	⊥P		+	+++++B	±	
	Dien	+M	++B	±P		+	+++++B	±	
	Telen	+M	++B	±P		+	+++++B	±	
11)Acid hydrolysis	Mesen	-	-	-	Total loss of color intensity was observed	-	-	-	Total loss of color intensity was observed
	Meten	-	-	-		-	-	-	
	Myel	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	

Table no. II.a continued

Initial incubation (24 hrs) + Dose exposure incubation (24 hrs) = Final incubation (48 hrs).									
Stain	Brain Region	0.5mMH_2O_2+ 3mg vitamin C treated			Inference Of observation	Control 3 mg vitamin C treated			Inference Of observation
		Neuroepithelial layer				Neuroepithelial layer			
		Cyt	Cs	Ics		Cyt	Cs	Ics	
1)AB 2.5	Mesen	±	+++	±	Weak reaction in Cyt and Ics, moderate at Cs	±	++	±	Moderate reaction at shistochemically detectable SA was not noted at Cyt and Ics
	Meten	±	+++	±		±	++	±	
	Myel	±	+++	±		±	++	±	
	Dien	±	+++	±		±	++	±	
	Telen	±	+++	±		±	++	±	
2)PA*Bh Sap PAS	Mesen	+++	+++	±	Moderate reaction in Cyt, weak at Cs and Ics	++	++	±	Moderate staining at Cs and Cyt. but histochemically detectable SA was not noted at Ics.
	Meten	+++	+++	±		++	++	±	
	Myel	+++	+++	±		++	++	±	
	Dien	+++	+++	±		++	++	±	
	Telen	+++	+++	±		++	++	±	
3) AZ 3.5	Mesen	±	+++	±	Color intensity was weak in Cyt and Ics, moderate at Cs	±	++	±	Moderate reaction at shistochemically detectable SA was not noted at Cyt and Ics
	Meten	±	+++	±		±	++	±	
	Myel	±	+++	±		±	++	±	
	Dien	±	+++	±		±	++	±	
	Telen	±	+++	±		±	++	±	
4) Sap-AB 2.5	Mesen	±	+++	±	Coloration not altered after saponifacation	±	+++	±	Color intensity increased after the saponification
	Meten	±	+++	±		±	+++	±	
	Myel	±	+++	±		±	+++	±	
	Dien	±	+++	±		±	+++	±	
	Telen	±	+++	±		±	+++	±	
5)Mild meth AB2.5	Mesen	±	±	±	Weak coloration in Cyt, moderate at Cs and trace at Ics	±	±	±	Intensity of coloration decreases
	Meten	±	±	±		±	±	±	
	Myel	±	±	±		±	±	±	
	Dien	±	±	±		±	±	±	
	Telen	±	±	±		±	±	±	

Table no II. a continued

Initial incubation (24 hrs) + Dose exposure incubation (24 hrs) = Final incubation (48 hrs).									
Staining techniques	Brain Region	0.5 mM H_2O_2 +3 mg vitamin C treated			Inference Of observations	Control 3 mg vitamin C treated			Inference Of observations
		Neuroepithelial layer				Neuroepithelial layer			
		Cyt	Cs	Ics		Cyt	Cs	Ics	
6)Mild meth Sap AB 2.5	Mesen	±	+++	±	Regained coloration	±	++	±	Weak reaction at Cs and Ics, trace in the cytoplasm
	Meten	±	+++	±		±	++	±	
	Myel	±	+++	±		±	++	±	
	Dien	±	+++	±		±	++	±	
	Telen	±	+++	±		±	++	±	
7)Act meth AB 2.5	Mesen	±	±	±	Weak coloration in Cyt, moderate at Cs and trace at Ics	±	±	±	Moderate color intensity was observed at all Cyt, Cs and Ics
	Meten	±	±	±		±	±	±	
	Myel	±	±	±		±	±	±	
	Dien	±	±	±		±	±	±	
	Telen	±	±	±		±	±	±	
8)Act met Sap AB pH2.5	Mesen	±	+++	±	Regained coloration	±	++	±	Regained color intensity
	Meten	±	+++	±		±	++	±	
	Myel	±	+++	±		±	++	±	
	Dien	±	+++	±		±	++	±	
	Telen	±	+++	±		±	++	±	
9)AB 2.5 PAS	Mesen	+M	+++B	±P	Weak magenta in Cyt, moderate blue at Cs and weak purple at Ics	+M	++B	±P	Weak magenta color in Cyt, moderate blue at Cs and trace purple at Ics
	Meten	+M	+++B	±P		+M	++B	±P	
	Myel	+M	+++B	±P		+M	++B	±P	
	Dien	+M	+++B	±P		+M	++B	±P	
	Telen	+M	+++B	±P		+M	++B	±P	
10)Acid hydrolysis	Mesen	-	-	-	Loss of coloration	-	-	-	Total loss of color intensity was observed
	Meten	-	-	-		-	-	-	
	Myel	-	-	-		-	-	-	
	Dien	-	-	-		-	-	-	
	Telen	-	-	-		-	-	-	

Table no. II.b Initial incubation (24hrs) + dose exposure incubation (48hrs) = Final incubation (72 hrs)

Initial incubation (24 hrs) + exposure incubation (48 hrs) =Final incubation (72 hrs).																		
Stain Tech.	Brain region	Normal								0.5mM H_2O_2 treated								
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations	
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics			
1)AB 2.5	Mes	±	++	±	±	++	±	++	Moderate coloration at Cs histochemically detectable SA not noted in Ics	+	+4	±	+4	+4	±	+4	Intense coloration in Cyt, at Cs, Ics and ML	
	Met	±	++	±	±	++	±	++		+	+4	±	+4	+4	±	+4		
	Mye	±	++	±	±	++	±	++		+	+4	±	+4	+4	±	+4		
	Die	±	++	±	±	++	±	++		+	+4	±	+4	+4	±	+4		
	Tel	±	++	±	±	++	±	++		+	+4	±	+4	+4	±	+4		
2)PA* BhSap PAS	Mes	++	++	±	++	++	±	++	Moderate coloration at Cs and Cyt histochemically detectable Sa was not at Ics	+4	+4	±	+4	+4	±	+4	Intense coloration in Cyt, moderate at other site	
	Met	++	++	±	++	++	±	++		+4	+4	±	+4	+4	±	+4		
	Mye	++	++	±	++	++	±	++		+4	+4	±	+4	+4	±	+4		
	Die	++	++	±	++	++	±	++		+4	+4	±	+4	+4	±	+4		
	Tel	++	++	±	++	++	±	++		+4	+4	±	+4	+4	±	+4		
3) AZ 3.5	Mes	±	++	±	±	+	±	++	Moderate coloration at Cs histochemically detectable SA not noted in Ics	+	+4	±	+4	+4	±	+4	Intense coloration in Cyt, at Cs, Ics and ML	
	Met	±	++	±	±	+	±	++		+	+4	±	+4	+4	±	+4		
	Mye	±	++	±	±	+	±	++		+	+4	±	+4	+4	±	+4		
	Die	±	++	±	±	+	±	++		+	+4	±	+4	+4	±	+4		
	Tel	±	++	±	±	+	±	++		+	+4	±	+4	+4	±	+4		
4) Sap-AB-2.5	Mes	±	+++	±	±	+++	±	+++	Increased intensity of coloration	+5	+5	±	+5	+5	±	+5	Increased intensity of coloration	
	Met	±	+++	±	±	+++	±	+++		+5	+5	±	+5	+5	±	+5		
	Mye	±	+++	±	±	+++	±	+++		+5	+5	±	+5	+5	±	+5		
	Die	±	+++	±	±	+++	±	+++		+5	+5	±	+5	+5	±	+5		
	Tel	±	+++	±	±	+++	±	+++		+5	+5	±	+5	+5	±	+5		
5)Mild meth AB 2.5	Mes	±	±	±	±	±	±	±	Loss of coloration	±	±	±	±	±	±	±	Loss of coloration	
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±		
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±		
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±		
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±		

Table no. II. b continued

6)Mild meth-Sap AB 2.5	Mes	±	++	±	±	++	±	++	Regained with equal intensity of coloration	+	+4	±	+4	+4	±	+4	Regained with equal intensity of coloration
	Met	±	++	±	±	++	±	++		+	+4	±	+4	+4	±	+4	
	Mye	±	++	±	±	++	±	++		+	+4	±	+4	+4	±	+4	
	Die	±	++	±	±	++	±	++		+	+4	±	+4	+4	±	+4	
	Tel	±	++	±	±	++	±	++		+	+4	±	+4	+4	±	+4	
7)Act meth AB 2.5	Mes	±	±	±	±	±	±	±	Loss of coloration	±	±	±	±	±	±	±	Loss of coloration
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
8)Act met Sap AB 2.5	Mes	±	++	±	±	++	±	++	Regained with equal intensity of coloration	+	+4	±	+4	+4	±	+4	Regained with equal intensity of coloration
	Met	±	++	±	±	++	±	++		+	+4	±	+4	+4	±	+4	
	Mye	±	++	±	±	++	±	++		+	+4	±	+4	+4	±	+4	
	Die	±	++	±	±	++	±	++		+	+4	±	+4	+4	±	+4	
	Tel	±	++	±	±	++	±	++		+	+4	±	+4	+4	±	+4	
9)AB 2.5-PAS	Mes	±M	++B	±P	±M	++B	±P	++B	Weak magenta in Cyt, blue at Cs and ML, purple at Ics	+M	+4B	±P	+4M	+4B	±P	+4B	Moderate content was increased than normal
	Met	±M	++B	±P	±M	++B	±P	++B		+M	+4B	±P	+4M	+4B	±P	+4B	
	Mye	±M	++B	±P	±M	++B	±P	++B		+M	+4B	±P	+4M	+4B	±P	+4B	
	Die	±M	++B	±P	±M	++B	±P	++B		+M	+4B	±P	+4M	+4B	±P	+4B	
	Tel	±M	++B	±P	±M	++B	±P	++B		+M	+4B	±P	+4M	+4B	±P	+4B	
11)Acid hydrol	Mes	-	-	-	-	-	-	-	Loss of coloration	-	-	-	-	-	-	-	Loss of coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. II. b Continued initial incubation 24hrs + dose exposure incubation (48hrs) - Final incubation 72 hrs

Initial incubation (24 hrs) + exposure incubation (48 hrs) =Final incubation (72 hrs).																			
Staining Tech.	Brain zone	0.5 mM H_2O_2 + 3mg vitamin C treated								Control 3mg vitamin C treated									
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations		
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics				
1)AB2.5	Mes	±	++	±	±	++	±	++	Moderate coloration at Cshistochemicall ydetectable SA not noted in Ics	±	++	±	±	++	±	++	Moderate coloration at Cshistochemica llydetectable SA not noted in Ics		
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++			
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++			
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++			
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++			
2)PA* Bh-Sap PAS	Mes	++	++	±	++	++	±	++	Moderate coloration at Cs and Cyt histochemicallyd etectable Sa was not at Ics	++	++	±	++	++	±	++	Moderate coloration at Cs and Cyt histochemically detectable Sa was not at Ics		
	Met	++	++	±	++	++	±	++		++	++	±	++	++	±	++			
	Mye	++	++	±	++	++	±	++		++	++	±	++	++	±	++			
	Die	++	++	±	++	++	±	++		++	++	±	++	++	±	++			
	Tel	++	++	±	++	++	±	++		++	++	±	++	++	±	++			
3) AZ 3.5	Mes	±	++	±	±	+	±	++	Moderate coloration at Cs histochemically detectable SA not noted in Ics	±	++	±	±	+	±	++	Moderate coloration at Cs histochemically detectable SA not noted in Ics		
	Met	±	++	±	±	+	±	++		±	++	±	±	+	±	++			
	Mye	±	++	±	±	+	±	++		±	++	±	±	+	±	++			
	Die	±	++	±	±	+	±	++		±	++	±	±	+	±	++			
	Tel	±	++	±	±	+	±	++		±	++	±	±	+	±	++			
4) Sap-AB 2.5	Mes	±	+++	±	±	+++	±	+++	Increased intensity of coloration	±	+++	±	±	+++	±	+++	Increased intensity of coloration		
	Met	±	+++	±	±	+++	+	+++		±	+++	±	±	+++	±	+++			
	Mye	±	+++	±	±	+++	±	+++		±	+++	±	±	+++	±	+++			
	Die	±	+++	±	±	+++	±	+++		±	+++	±	±	+++	±	+++			
	Tel	±	+++	±	±	+++	±	+++		±	+++	±	±	+++	±	+++			
5) Mild-meth-AB 2.5	Mes	±	±	±	±	±	±	±	Loss of coloration	±	±	±	±	±	±	±	Restored coloration		
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±			
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±			
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±			
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±			

Table no. II. b continued

7)Mild meth Sap AB 2.5	Mes	±	++	±	±	++	±	++	Regained with equal intensity of coloration	±	++	±	±	++	±	++	Regained with equal intensity of coloration
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
8)Act meth AB 2.5	Mes	±	±	±	±	±	±	±	Restored coloration	±	±	±	±	±	±	±	Restored coloration
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9)Act met Sap AB2.5	Mes	±	++	±	±	++	±	++	Regained with equal intensity of coloration	±	++	±	±	++	±	++	Regained with equal intensity of coloration
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
10)AB 2.5-PAS	Mes	±M	++B	±P	±M	++B	±P	++B	Weak magenta in Cyt, blue at Cs and ML, purple at Ics	±M	++B	±P	±M	++B	±P	++B	Weak magenta in Cyt, blue at Cs and ML, purple at Ics
	Met	±M	++B	±P	±M	++B	±P	++B		±M	++B	±P	±M	++B	±P	++B	
	Mye	±M	++B	±P	±M	++B	±P	++B		±M	++B	±P	±M	++B	±P	++B	
	Die	±M	++B	±P	±M	++B	±P	++B		±M	++B	±P	±M	++B	±P	++B	
	Tel	±M	++B	±P	±M	++B	±P	++B		±M	++B	±P	±M	++B	±P	++B	
10)Acid hydro	Mes	-	-	-	-	-	-	-	Loss of coloration	-	-	-	-	-	-	-	Loss of coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. II. c continued

Initial incubation (24 hrs) + exposure incubation (72 hrs) =Final incubation (96 hrs).																		
Staning techn	Brain region	Normal								0.5 mM H_2O_2 treated								
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations	
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics			
1)AB 2.5	Mes	±	++	±	±	++	±	++	Moderate intensity at Cs, ML. trace intensity was noted at Ics	++	+++	±	++	+++	±	+++	Moderate intensity at al sites	
	Met	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++		
	Mye	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++		
	Die	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++		
	Tel	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++		
2)PA* Bh Sap PAS	Mes	++	++	±	++	++	±	++	Moderate intensity at Cs and Cyt trace at Ics	+++	+++	±	+++	+++	±	+++	Moderate intensity at al sites	
	Met	++	++	±	++	++	±	++		+++	+++	±	+++	+++	±	+++		
	Mye	++	++	±	++	++	±	++		+++	+++	±	+++	+++	±	+++		
	Die	++	++	±	++	++	±	++		+++	+++	±	+++	+++	±	+++		
	Tel	++	++	±	++	++	±	++		+++	+++	±	+++	+++	±	+++		
3) AZ 3.5	Mes	±	++	±	±	++	±	++	Moderate intensity at Cs, ML. trace intensity was noted at Ics	++	+++	±	++	+++	±	+++	Moderate intensity at al sites	
	Met	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++		
	Mye	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++		
	Die	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++		
	Tel	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++		
4)Sap-AB2.5	Mes	±	+++	±	±	+++	±	+++	Increased of color intensity	++	+4	±	++	+4	±	+4	Increased of color intensity	
	Met	±	+++	±	±	+++	±	+++		++	+4	±	++	+4	±	+4		
	Mye	±	+++	±	±	+++	±	+++		++	+4	±	++	+4	±	+4		
	Die	±	+++	±	±	+++	±	+++		++	+4	±	++	+4	±	+4		
	Tel	±	+++	±	±	+++	±	+++		++	+4	±	++	+4	±	+4		
5)Mild meth AB 2.5	Mes	±	±	±	±	±	±	±	Loss of coloration	±	±	±	±	±	±	±	Loss of coloration	
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±		
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±		
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±		
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±		

Table no. II. c continued

6)Mild meth-Sap AB 2.5	Mes	±	++	++	±	++	++	++	Regained with	++	+++	±	++	+++	±	+++	Regained with
	Met	±	++	++	±	++	++	++		++	+++	±	++	+++	±	+++	
	Mye	±	++	++	±	++	++	++		++	+++	±	++	+++	±	+++	
	Die	±	++	++	±	++	++	++		++	+++	±	++	+++	±	+++	
	Tel	±	++	++	±	++	++	++		++	+++	±	++	+++	±	+++	
7)Act meth AB 2.5	Mes	±	±	±	±	±	±	±	Restoration of color intensity	±	±	±	±	±	±	±	Restoration of color intensity
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
8)Act met Sap AB 2.5	Mes	±	++	++	±	++	++	++	Regained with	++	+++	±	++	+++	±	+++	Regained with
	Met	±	++	++	±	++	++	++		++	+++	±	++	+++	±	+++	
	Mye	±	++	++	±	++	++	++		++	+++	±	++	+++	±	+++	
	Die	±	++	++	±	++	++	++		++	+++	±	++	+++	±	+++	
	Tel	±	++	++	±	++	++	++		++	+++	±	++	+++	±	+++	
9)AB 2.5-PAS	Mes	±M	++B	++P	±M	++B	++P	++P	Trace magenta in Cyt.moderate at Cs, purple in Ics	++ M	+3B	±P	++ M	+3B	±P	+3B	Increasd intensity at all sites
	Met	±M	++B	++P	±M	++B	++P	++P		++ M	+3B	±P	++ M	+3B	±P	+3B	
	Mye	±M	++B	++P	±M	++B	++P	++P		++ M	+3B	±P	++ M	+3B	±P	+3B	
	Die	±M	++B	++P	±M	++B	++P	++P		++ M	+3B	±P	++ M	+3B	±P	+3B	
	Tel	±M	++B	++P	±M	++B	++P	++P		++ M	+3B	±P	++ M	+3B	±P	+3B	
11)Acid hydrol	Mes	-	-	-	-	-	-	-	Loss of coloration	-	-	-	-	-	-	-	Loss of coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. II. c continued

Initial incubation (24 hrs) + exposure incubation (72 hrs) =Final incubation (96 hrs).																		
Staining Tech.	Brain zones	0.5 mM H_2O_2 + 3 mg vitamin C treated								Control 3 mg vitamin C treated								
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 2.5	Mes	±	++	±	±	++	±	++	Moderate intensity at Cs, ML. trace intensity was noted at Ics	±	++	++	±	++	++	++	Moderate intensity at Cs, ML. trace intensity was noted at Ics
	Met	±	++	±	±	++	±	++		±	++	++	±	++	++	++	
	Mye	±	++	±	±	++	±	++		±	++	++	±	++	++	++	
	Die	±	++	±	±	++	±	++		±	++	++	±	++	++	++	
	Tel	±	++	±	±	++	±	++		±	++	++	±	++	++	++	
2)PA* BhSap PAS	Mes	++	++	±	++	++	±	++	Moderate intensity at Cs and Cyt trace at Ics	++	±	+	++	±	+	++	Moderate intensity at Cs and Cyt trace at Ics
	Met	++	++	±	++	++	±	++		++	±	+	++	±	+	++	
	Mye	++	++	±	++	++	±	++		++	±	+	++	±	+	++	
	Die	++	++	±	++	++	±	++		++	±	+	++	±	+	++	
	Tel	++	++	±	++	++	±	++		++	±	+	++	±	+	++	
3) AZ 3.5	Mcs	±	++	±	±	++	±	++	Moderate intensity at Cs, ML. trace intensity was noted at Ics	±	++	++	±	++	++	++	Moderate intensity at Cs, ML. trace intensity was noted at Ics
	Met	±	++	±	±	++	±	++		±	++	++	±	++	++	++	
	Mye	±	++	±	±	++	±	++		±	++	++	±	++	++	++	
	Die	±	++	±	±	++	±	++		±	++	++	±	++	++	++	
	Tel	±	++	±	±	++	±	++		±	++	++	±	++	++	++	
4) Sap-AB-2.5	Mes	±	+++	±	±	+++	±	+++	Increased of color intensity	±	±	±	±	±	±	±	Increased of color intensity
	Met	±	+++	±	±	+++	±	+++		±	±	±	±	±	±	±	
	Mye	±	+++	±	±	+++	±	+++		±	±	±	±	±	±	±	
	Die	±	+++	±	±	+++	±	+++		±	±	±	±	±	±	±	
	Tel	±	+++	±	±	+++	±	+++		±	±	±	±	±	±	±	
5)Mild meth AB 2.5	Mes	±	±	±	±	±	±	±	Loss of coloration	±	±	±	±	±	±	±	Loss of coloration
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	

Table no. II. c continued

6)Mild meth-Sap AB 2.5	Mes	±	++	++	±	++	++	++	Regained with	±	++	++	±	++	++	++	Regained with
	Met	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Mye	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Die	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Tel	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
7)Act meth AB 2.5	Mes	±	±	±	±	±	±	±	Restoration of color intensity	±	±	±	±	±	±	±	Restoration of color intensity
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
8)Act met Sap AB 2.5	Mes	±	++	++	±	++	++	++	Regained with	±	++	++	±	++	++	++	Regained with
	Met	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Mye	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Die	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Tel	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
9)AB 2.5-PAS	Mes	±M	++B	++P	±M	++B	++P	++P	Trace magenta in Cyt.moderate at Cs, purple in Ics	±M	++B	++P	±M	++B	++P	++P	Trace magenta in Cyt.moderate at Cs, purple in Ics
	Met	±M	++B	++P	±M	++B	++P	++P		±M	++B	++P	±M	++B	++P	++P	
	Mye	±M	++B	++P	±M	++B	++P	++P		±M	++B	++P	±M	++B	++P	++P	
	Die	±M	++B	++P	±M	++B	++P	++P		±M	++B	++P	±M	++B	++P	++P	
	Tel	±M	++B	++P	±M	++B	++P	++P		±M	++B	++P	±M	++B	++P	++P	
10)Acid hydrol	Mes	-	-	-	-	-	-	-	Loss of coloration	-	-	-	-	-	-	-	Loss of coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. II. d

Initial incubation (24 hrs) + exposure incubation (96 hrs) =Final incubation (120 hrs).																		
Staning techn	Brain region	Normal								0.5 mM H_2O_2 treated								
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 2.5	Mes	+	++	±	+	++	±	++	Weak intensity in Cyt, moderate at Cs, ML, trace at Ics	++	+++	±	++	+++	±	+++	Moderate intensity at all sites
	Met	+	++	±	+	++	±	++		++	+++	±	++	+++	±	+++	
	Mye	+	++	±	+	++	±	++		++	+++	±	++	+++	±	+++	
	Die	+	++	±	+	++	±	++		++	+++	±	++	+++	±	+++	
	Tel	+	++	±	+	++	±	++		++	+++	±	++	+++	±	+++	
2)PA* BhSap PAS	Mes	++	++	±	++	++	±	++	Moderate intensity in Cyt, Cs and trace at Ics	++	+++	±	++	+++	±	+++	Moderate intensity at all sites
	Met	++	++	±	++	++	±	++		++	+++	±	++	+++	±	+++	
	Mye	++	++	±	++	++	±	++		++	+++	±	++	+++	±	+++	
	Die	++	++	±	++	++	±	++		++	+++	±	++	+++	±	+++	
	Tel	++	++	±	++	++	±	++		++	+++	±	++	+++	±	+++	
3) AZ 3.5	Mes	+	++	±	++	++	±	++	Weak intensity in Cyt, moderate at Cs, ML, trace at Ics	++	+++	±	++	+++	±	+++	Moderate intensity at all sites
	Met	+	++	±	+	++	±	++		++	+++	±	++	+++	±	+++	
	Mye	+	++	±	+	++	±	++		++	+++	±	++	+++	±	+++	
	Die	+	++	±	+	++	±	++		++	+++	±	++	+++	±	+++	
	Tel	+	++	±	+	++	±	++		++	+++	±	++	+++	±	+++	
4) Sap-AB-2.5	Mes	±	+4	±	±	+4	±	+4	Increased intensity of coloration	±	+4	±	±	+4	±	+4	Increased intensity of coloration
	Met	±	+4	±	±	+4	±	+4		±	+4	±	±	+4	±	+4	
	Mye	±	+4	±	±	+4	±	+4		±	+4	±	±	+4	±	+4	
	Die	±	+4	±	±	+4	±	+4		±	+4	±	±	+4	±	+4	
	Tel	±	+4	±	±	+4	±	+4		±	+4	±	±	+4	±	+4	
5)Mild meth AB 2.5	Mes	±	+	±	±	+	±	+	Loss of coloration	±	±	±	±	±	±	±	Loss of coloration
	Met	±	+	±	±	+	±	+		±	±	±	±	±	±	±	
	Mye	±	+	±	±	+	±	+		±	±	±	±	±	±	±	
	Die	±	+	±	±	+	±	+		±	±	±	±	±	±	±	
	Tel	±	+	±	±	+	±	+		±	±	±	±	±	±	±	

Table no. II. d continued

6)Mild meth-Sap AB 2.5	Mes	+	++	++	+	++	++	++	Regained coloration with equal intensity	++	+++	±	++	+++	±	+++	Regained coloration with equal intensity
	Met	+	++	++	+	++	++	++		++	+++	±	++	+++	±	+++	
	Mye	+	++	++	+	++	++	++		++	+++	±	++	+++	±	+++	
	Die	+	++	++	+	++	++	++		++	+++	±	++	+++	±	+++	
	Tel	+	++	++	+	++	++	++		++	+++	±	++	+++	±	+++	
7)Act meth AB 2.5	Mes	±	+	+	±	+	+	+	Restored intensity of coloration	±	±	±	±	±	±	±	Restored intensity of coloration
	Met	±	+	+	±	+	+	+		±	±	±	±	±	±	±	
	Mye	±	+	+	±	+	+	+		±	±	±	±	±	±	±	
	Die	±	+	+	±	+	+	+		±	±	±	±	±	±	±	
	Tel	±	+	+	±	+	+	+		±	±	±	±	±	±	±	
8)Act met Sap AB 2.5	Mes	+	++	++	+	++	++	++	Regained coloration with equal intensity	++	+++	±	++	+++	±	+++	Moderate intensity at all sites
	Met	+	++	++	+	++	++	++		++	+++	±	++	+++	±	+++	
	Mye	+	++	++	+	++	++	++		++	+++	±	++	+++	±	+++	
	Die	+	++	++	+	++	++	++		++	+++	±	++	+++	±	+++	
	Tel	+	++	++	+	++	++	++		++	+++	±	++	+++	±	+++	
9)AB 2.5-PAS	Mes	+M	++B	++P	+M	++B	++P	++P	Weak magenta in Cyt, moderate blue at Cs, purple at Ics	++M	+3B	++P	++M	+3B	++P	+3B	Moderate increased intensity at respective sites
	Met	+M	++B	++P	+M	++B	++P	++P		++M	+3B	++P	++M	+3B	++P	+3B	
	Mye	+M	++B	++P	+M	++B	++P	++P		++M	+3B	++P	++M	+3B	++P	+3B	
	Die	+M	++B	++P	+M	++B	++P	++P		++M	+3B	++P	++M	+3B	++P	+3B	
	Tel	+M	++B	++P	+M	++B	++P	++P		++M	+3B	++P	++M	+3B	++P	+3B	
10)Acid hydrol-AB 2.5	Mes	-	-	-	-	-	-	-	Loss of coloration	-	-	-	-	-	-	-	Loss of coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. II. d continued

Initial incubation (24 hrs) + exposure incubation (96 hrs) =Final incubation (120 hrs).																		
Staining Tech.	Brain zones	0.5 mM H_2O_2 + 3 mg vitamin C treated								Control 3 mg vitamin C treated								
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations	
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics			
1)AB 2.5	Mes	+	++	±	+	++	±	++	Weak intensity in Cyt, moderate at Cs, ML, trace at Ics	+	++	±	+	++	±	++	Weak intensity in Cyt, moderate at Cs, ML, trace at Ics	
	Met	+	++	±	+	++	±	++		+	++	±	+	++	±	++		
	Mye	+	++	±	+	++	±	++		+	++	±	+	++	±	++		
	Die	+	++	±	+	++	±	++		+	++	±	+	++	±	++		
	Tel	+	++	±	+	++	±	++		+	++	±	+	++	±	++		
2)PA* BhSap PAS	Mes	++	++	±	++	++	±	++	Moderate intensity in Cyt, Cs and trace at Ics	++	++	±	++	++	±	++	Moderate intensity in Cyt, Cs and trace at Ics	
	Met	++	++	±	++	++	±	++		++	++	±	++	++	±	++		
	Mye	++	++	±	++	++	±	++		++	++	±	++	++	±	++		
	Die	++	++	±	++	++	±	++		++	++	±	++	++	±	++		
	Tel	++	++	±	++	++	±	++		++	++	±	++	++	±	++		
3) AZ 3.5	Mes	+	++	±	++	++	±	++	Weak intensity in Cyt, moderate at Cs, ML, trace at Ics	+	++	±	++	++	±	++	Weak intensity in Cyt, moderate at Cs, ML, trace at Ics	
	Met	+	++	±	+	++	±	++		+	++	±	+	++	±	++		
	Mye	+	++	±	+	++	±	++		+	++	±	+	++	±	++		
	Die	+	++	±	+	++	±	++		+	++	±	+	++	±	++		
	Tel	+	++	±	+	++	±	++		+	++	±	+	++	±	++		
4) Sap-AB-2.5	Mes	±	+4	±	±	+4	±	+4	Increased intensity of coloration	±	+4	±	±	+4	±	+4	Increased intensity of coloration	
	Met	±	+4	±	±	+4	±	+4		±	+4	±	±	+4	±	+4		
	Mye	±	+4	±	±	+4	±	+4		±	+4	±	±	+4	±	+4		
	Die	±	+4	±	±	+4	±	+4		±	+4	±	±	+4	±	+4		
	Tel	±	+4	±	±	+4	±	+4		±	+4	±	±	+4	±	+4		
5)Mild meth AB 2.5	Mes	±	+	±	±	+	±	+	Loss of coloration	±	+	±	±	+	±	+	Loss of coloration	
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+		
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+		
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+		
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+		

Table no. II. d continued

6)Mild meth-Sap AB 2.5	Mes	+	++	++	+	++	++	++	Regained coloration with equal intensity	+	++	++	+	++	++	++	Regained coloration with equal intensity
	Met	+	++	++	+	++	++	++		+	++	++	+	++	++	++	
	Mye	+	++	++	+	++	++	++		+	++	++	+	++	++	++	
	Die	+	++	++	+	++	++	++		+	++	++	+	++	++	++	
	Tel	+	++	++	+	++	++	++		+	++	++	+	++	++	++	
7)Act meth AB 2.5	Mes	±	+	+	±	+	+	+	Restored intensity of coloration	±	+	+	±	+	+	+	Restored intensity of coloration
	Met	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Mye	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Die	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Tel	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
8)Act met Sap AB 2.5	Mes	+	++	++	+	++	++	++	Regained coloration with equal intensity	+	++	++	+	++	++	++	Regained coloration with equal intensity
	Met	+	++	++	+	++	++	++		+	++	++	+	++	++	++	
	Mye	+	++	++	+	++	++	++		+	++	++	+	++	++	++	
	Die	+	++	++	+	++	++	++		+	++	++	+	++	++	++	
	Tel	+	++	++	+	++	++	++		+	++	++	+	++	++	++	
9)AB 2.5-PAS	Mes	+M	++B	++P	+M	++B	++P	++P	Weak magenta in Cyt,moderate blue at Cs, purple at Ics	+M	++B	++P	+M	++B	++P	++P	Weak magenta in Cyt, moderate blue at Cs, purple at Ics
	Met	+M	++B	++P	+M	++B	++P	++P		+M	++B	++P	+M	++B	++P	++P	
	Mye	+M	++B	++P	+M	++B	++P	++P		+M	++B	++P	+M	++B	++P	++P	
	Die	+M	++B	++P	+M	++B	++P	++P		+M	++B	++P	+M	++B	++P	++P	
	Tel	+M	++B	++P	+M	++B	++P	++P		+M	++B	++P	+M	++B	++P	++P	
10) acid hydr. AB 2.5	Mes	-	-	-	-	-	-	-	Loss of coloration	-	-	-	-	-	-	-	Loss of coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. II.e Initial incubation (48 hrs) + exposure incubation (24 hrs) =Final incubation (72 hrs).

Stain Tech.	Brain region	Normal								0.5mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 2.5	Mes	±	+	±	±	+	±	+	Trace reaction in Cyt, weak at Cs, Ics and ML	++	+4	±	++	+4	±	+4	Intense coloration in Cyt, at Cs, Ics and ML
	Met	±	+	±	±	+	±	+		++	+4	±	++	+4	±	+4	
	Mye	±	+	±	±	+	±	+		++	+4	±	++	+4	±	+4	
	Die	±	+	±	±	+	±	+		++	+4	±	++	+4	±	+4	
	Tel	±	+	±	±	+	±	+		++	+4	±	++	+4	±	+4	
2)PA* BhSap PAS	Mes	+	±	±	+	±	±	+	Weak content in Cyt and Ics, and trace at Cs	+++	++	±	+++	++	±	++	Intense coloration in Cyt, moderate at other site
	Met	+	±	±	+	±	±	+		+++	++	±	+++	++	±	++	
	Mye	+	±	±	+	±	±	+		+++	++	±	+++	++	±	++	
	Die	+	±	±	+	±	±	+		+++	++	±	+++	++	±	++	
	Tel	+	±	±	+	±	±	+		+++	++	±	+++	++	±	++	
3) AZ 3.5	Mes	±	+	±	±	+	±	+	Trace reaction in Cyt, weak at Cs, Ics and ML	++	+4	±	++	+4	±	+4	Intense coloration in Cyt, at Cs, Ics and ML
	Met	±	+	±	±	+	±	+		++	+4	±	++	+4	±	+4	
	Mye	±	+	±	±	+	±	+		++	+4	±	++	+4	±	+4	
	Die	±	+	±	±	+	±	+		++	+4	±	++	+4	±	+4	
	Tel	±	+	±	±	+	±	+		++	+4	±	++	+4	±	+4	
4) Sap-AB-2.5	Mes	±	±	±	±	±	±	±	Restored intensity of coloration	++	+5	±	++	+5	±	+5	Restored intensity of coloration
	Met	±	±	±	±	±	±	±		++	+5	±	++	+5	±	+5	
	Mye	±	±	±	±	±	±	±		++	+5	±	++	+5	±	+5	
	Die	±	±	±	±	±	±	±		++	+5	±	++	+5	±	+5	
	Tel	±	±	±	±	±	±	±		++	+5	±	++	+5	±	+5	
5)Mild meth AB 2.5	Mes	±	±	±	±	±	±	±	Restored coloration	±	±	±	±	±	±	±	Restored intensity of coloration
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	

Table no. II. b Table no. II.e Initial incubation (48 hrs) + exposure incubation (24 hrs) =Final incubation (72 hrs). continued

6)Mild meth-Sap AB 2.5	Mes	±	+	+	±	+	+	+	Regained with equal intensity of coloration	++	+4	±	++	+4	±	+4	Regained with equal intensity of coloration
	Met	±	+	+	±	+	+	+		++	+4	±	++	+4	±	+4	
	Mye	±	+	+	±	+	+	+		++	+4	±	++	+4	±	+4	
	Die	±	+	+	±	+	+	+		++	+4	±	++	+4	±	+4	
	Tel	±	+	+	±	+	+	+		++	+4	±	++	+4	±	+4	
7)Act meth AB 2.5	Mes	±	±	±	±	±	±	±	Restored coloration	±	±	±	±	±	±	±	Restored coloration
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
8)Act met Sap AB 2.5	Mes	±	+	+	±	+	+	+	Regained with equal intensity of coloration	++	+4	±	++	+4	±	+4	Regained with equal intensity of coloration
	Met	±	+	+	±	+	+	+		++	+4	±	++	+4	±	+4	
	Mye	±	+	+	±	+	+	+		++	+4	±	++	+4	±	+4	
	Die	±	+	+	±	+	+	+		++	+4	±	++	+4	±	+4	
	Tel	±	+	+	±	+	+	+		++	+4	±	++	+4	±	+4	
9)AB 2.5-PAS	Mes	+M	+B	+P	+M	+B	+P	+B	Weak magenta in Cyt, blue at Cs and ML, purple at Ics	++M	+4B	±P	++M	+4B	±P	+4B	Moderate content was increased than normal
	Met	+M	+B	+P	+M	+B	+P	+B		++M	+4B	±P	++M	+4B	±P	+4B	
	Mye	+M	+B	+P	+M	+B	+P	+B		++M	+4B	±P	++M	+4B	±P	+4B	
	Die	+M	+B	+P	+M	+B	+P	+B		++M	+4B	±P	++M	+4B	±P	+4B	
	Tel	+M	+B	+P	+M	+B	+P	+B		++M	+4B	±P	++M	+4B	±P	+4B	
11)Acid hydrol	Mes	-	-	-	-	-	-	-	Loss of coloration	-	-	-	-	-	-	-	Loss of coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. II. b Table no. II.e Initial incubation (48 hrs) + exposure incubation (24 hrs) =Final incubation (72 hrs). continued

Staining Tech.	Brain zone	0.5 mM H_2O_2 + 3mg vitamin C treated								Control 3mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB2.5	Mes	±	++	±	±	++	±	++	Trace reaction in Cyt, Ics moderate at Cs, ML	±	+	±	±	+	±	+	Trace reaction in Cyt, weak at Cs, Ics and ML
	Met	±	++	±	±	++	±	++		±	+	±	±	+	±	+	
	Mye	±	++	±	±	++	±	++		±	+	±	±	+	±	+	
	Die	±	++	±	±	++	±	++		±	+	±	±	+	±	+	
	Tel	±	++	±	±	++	±	++		±	+	±	±	+	±	+	
2)PA* Bh-Sap PAS	Mes	++	++	±	++	++	±	++	Trace content at Ics, and trace at Cs was with moderate intensity	+	±	±	+	±	±	+	Weak content in Cyt and Ics, and trace at Cs
	Met	++	++	±	++	++	±	++		+	±	±	+	±	±	+	
	Mye	++	++	±	++	++	±	++		+	±	±	+	±	±	+	
	Die	++	++	±	++	++	±	++		+	±	±	+	±	±	+	
	Tel	++	++	±	++	++	±	++		+	±	±	+	±	±	+	
3) AZ 3.5	Mes	±	++	±	±	++	±	++	Trace reaction in Cyt, Ics moderate at Cs, ML	±	+	±	±	+	±	+	Trace reaction in Cyt, weak at Cs, Ics and ML
	Met	±	++	±	±	++	±	++		±	+	±	±	+	±	+	
	Mye	±	++	±	±	++	±	++		±	+	±	±	+	±	+	
	Die	±	++	±	±	++	±	++		±	+	±	±	+	±	+	
	Tel	±	++	±	±	++	±	++		±	+	±	±	+	±	+	
4) Sap-AB 2.5	Mes	±	+++	±	±	+++	±	+++	Restored intensity of coloration	±	±	±	±	±	±	±	Restored intensity of coloration
	Met	±	+++	±	±	+++	±	+++		±	±	±	±	±	±	±	
	Mye	±	+++	±	±	+++	±	+++		±	±	±	±	±	±	±	
	Die	±	+++	±	±	+++	±	+++		±	±	±	±	±	±	±	
	Tel	±	+++	±	±	+++	±	+++		±	±	±	±	±	±	±	
5) Mild-meth-AB 2.5	Mes	±	±	±	±	±	±	±	Restored coloration	±	±	±	±	±	±	±	Restored coloration
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	

Table no. II. b Table no. II.e Initial incubation (48 hrs) + exposure incubation (24 hrs) =Final incubation (72 hrs).continued

7)Mild meth Sap AB 2.5	Mes	±	++	±	±	++	±	++	Regained with equal intensity of coloration	±	+	+	±	+	+	+	Regained with equal intensity of coloration
	Met	±	++	±	±	++	±	++		±	+	+	±	+	+	+	
	Mye	±	++	±	±	++	±	++		±	+	+	±	+	+	+	
	Die	±	++	±	±	++	±	++		±	+	+	±	+	+	+	
	Tel	±	++	±	±	++	±	++		±	+	+	±	+	+	+	
8)Act meth AB 2.5	Mes	±	±	±	±	±	±	±	Blocked coloration	±	±	±	±	±	±	±	Blocked coloration
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9)Act met Sap AB2.5	Mes	±	++	±	±	++	±	++	Regained with equal intensity of coloration	±	+	+	±	+	+	+	Regained with equal intensity of coloration
	Met	±	++	±	±	++	±	++		±	+	+	±	+	+	+	
	Mye	±	++	±	±	++	±	++		±	+	+	±	+	+	+	
	Die	±	++	±	±	++	±	++		±	+	+	±	+	+	+	
	Tel	±	++	±	±	++	±	++		±	+	+	±	+	+	+	
10)AB 2.5-PAS	Mes	±M	++B	±P	±M	++B	±P	++B	Weak magenta in Cyt,moderate blue at Cs and ML, purple at Ics	+M	+B	+P	+M	+B	+P	+B	Weak magenta in Cyt, blue at Cs and ML, purple at Ics
	Met	±M	++B	±P	±M	++B	±P	++B		+M	+B	+P	+M	+B	+P	+B	
	Mye	±M	++B	±P	±M	++B	±P	++B		+M	+B	+P	+M	+B	+P	+B	
	Die	±M	++B	±P	±M	++B	±P	++B		+M	+B	+P	+M	+B	+P	+B	
	Tel	±M	++B	±P	±M	++B	±P	++B		+M	+B	+P	+M	+B	+P	+B	
10)Acid hydro	Mes	-	-	-	-	-	-	-	Loss of coloration	-	-	-	-	-	-	-	Loss of coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. II.d Initial incubation (48 hrs) + exposure incubation (48 hrs) =Final incubation (96 hrs).

Stain Tech.	Brain region	Normal								0.5mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 2.5	Mes	±	++	±	±	++	±	++	Trace reaction in Cyt, moderate at Cs, Ics and ML	++	+4	±	++	+4	±	+4	Intense coloration in Cyt, at Cs, Ics and ML
	Met	±	++	±	±	++	±	++		++	+4	±	++	+4	±	+4	
	Mye	±	++	±	±	++	±	++		++	+4	±	++	+4	±	+4	
	Die	±	++	±	±	++	±	++		++	+4	±	++	+4	±	+4	
	Tel	±	++	±	±	++	±	++		++	+4	±	++	+4	±	+4	
2)PA* BhSap PAS	Mes	++	++	±	++	++	±	++	Moderate content in Cyt weak Ics, and trace at Cs	+++	+4	±	+++	+4	±	+4	Intense coloration in Cyt, at Cs, Ics and ML
	Met	++	++	±	++	++	±	++		+++	+4	±	+++	+4	±	+4	
	Mye	++	++	±	++	++	±	++		+++	+4	±	+++	+4	±	+4	
	Die	++	++	±	++	++	±	++		+++	+4	±	+++	+4	±	+4	
	Tel	++	++	±	++	++	±	++		+++	+4	±	+++	+4	±	+4	
3) AZ 3.5	Mes	±	++	±	±	++	±	++	Trace reaction in Cyt, moderate at Cs, Ics and ML	+++	+++	±	+++	+++	±	+++	Intense coloration in Cyt, at Cs, Ics and ML
	Met	±	++	±	±	++	±	++		+++	+++	±	+++	+++	±	+++	
	Mye	±	++	±	±	++	±	++		+++	+++	±	+++	+++	±	+++	
	Die	±	++	±	±	++	±	++		+++	+++	±	+++	+++	±	+++	
	Tel	±	++	±	±	++	±	++		+++	+++	±	+++	+++	±	+++	
4) Sap-AB-2.5	Mes	±	+++	±	±	+++	±	+++	Restored intensity of coloration	±	±	±	±	±	±	±	Restored intensity of coloration
	Met	±	+++	±	±	+++	±	+++		±	±	±	±	±	±	±	
	Mye	±	+++	±	±	+++	±	+++		±	±	±	±	±	±	±	
	Die	±	+++	±	±	+++	±	+++		±	±	±	±	±	±	±	
	Tel	±	+++	±	±	+++	±	+++		±	±	±	±	±	±	±	
5)Mild meth AB 2.5	Mes	±	±	±	±	±	±	±	Restored intensity of coloration	±	±	±	±	±	±	±	Restored intensity of coloration
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	

Table no. II. d Initial incubation (48 hrs) + exposure incubation (48 hrs) =Final incubation (96 hrs). continued

6)Mild meth-Sap AB 2.5	Mes	±	++	±	±	++	±	++	Regained with equal intensity of coloration	+++	+++	+++	+++	+++	+++	+++	Regained with equal intensity of coloration
	Met	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
7)Act meth AB 2.5	Mes	±	±	±	±	±	±	±	Restored coloration	±	±	±	±	±	±	±	Restored coloration
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
8)Act met Sap AB 2.5	Mes	±	++	±	±	++	±	++	Regained with equal intensity of coloration	+++	+++	+++	+++	+++	+++	+++	Regained with equal intensity of coloration
	Met	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
9)AB 2.5-PAS	Mes	±M	++B	±P	±M	++B	±P	++B	Weak magenta in Cyt, blue at Cs and ML, purple at Ics	++M	++P	++B	++M	++P	++B	++M	Moderate content was increased than normal
	Met	±M	++B	±P	±M	++B	±P	++B		++M	++P	++B	++M	++P	++B	++M	
	Mye	±M	++B	±P	±M	++B	±P	++B		++M	++P	++B	++M	++P	++B	++M	
	Die	±M	++B	±P	±M	++B	±P	++B		++M	++P	++B	++M	++P	++B	++M	
	Tcl	±M	++B	±P	±M	++B	±P	++B		++M	++P	++B	++M	++P	++B	++M	
11)Acid hydrol	Mes	-	-	-	-	-	-	-	Loss of coloration	-	-	-	-	-	-	-	Loss of coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. II. d Initial incubation (48 hrs) + exposure incubation (48 hrs) =Final incubation (96 hrs).continued

Initial incubation (48 hrs) + exposure incubation (48 hrs) =Final incubation (96 hrs).																			
Staining Tech.	Brain region	0.5 mM H_2O_2 + 3mg vitamin C treated								Control 3mg vitamin C treated									
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations		
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics				
1)AB 2.5	Mes	+	++	++	+	++	++	++	Weak reaction in Cyt, moderate at Cs, Ics and ML	±	++	±	±	++	±	++	Trace reaction in Cyt, moderate at Cs, Ics and ML		
	Met	+	++	++	+	++	++	++		±	++	±	±	++	±	++			
	Mye	+	++	++	+	++	++	++		±	++	±	±	++	±	++			
	Die	+	++	++	+	++	++	++		±	++	±	±	++	±	++			
	Tel	+	++	++	+	++	++	++		±	++	±	±	++	±	++			
2)PA* Bh-Sap PAS	Mes	++	±	+	++	±	+	+	Moderate content in Cyt weak Ics, and trace at Cs	++	++	±	++	++	±	++	Moderate content in Cyt weak Ics, and trace at Cs		
	Met	++	±	+	++	±	+	+		++	++	±	++	++	±	++			
	Mye	++	±	+	++	±	+	+		++	++	±	++	++	±	++			
	Die	++	±	+	++	±	+	+		++	++	±	++	++	±	++			
	Tel	++	±	+	++	±	+	+		++	++	±	++	++	±	++			
3) AZ 3.5	Mes	±	++	++	±	++	++	++	Trace reaction in Cyt, moderate at Cs, Ics and ML	±	++	±	±	++	±	++	Trace reaction in Cyt, moderate at Cs, Ics and ML		
	Met	±	++	++	±	++	++	++		±	++	±	±	++	±	++			
	Mye	±	++	++	±	++	++	++		±	++	±	±	++	±	++			
	Die	±	++	++	±	++	++	++		±	++	±	±	++	±	++			
	Tel	±	++	++	±	++	++	++		±	++	±	±	++	±	++			
4) Sap-AB 2.5	Mes	±	±	±	±	±	±	±	Restored intensity of coloration	±	+++	±	±	+++	±	+++	Restored intensity of coloration		
	Met	±	±	±	±	±	±	±		±	+++	±	±	+++	±	+++			
	Mye	±	±	±	±	±	±	±		±	+++	±	±	+++	±	+++			
	Die	±	±	±	±	±	±	±		±	+++	±	±	+++	±	+++			
	Tel	±	±	±	±	±	±	±		±	+++	±	±	+++	±	+++			
5) Mild-meth-AB 2.5	Mes	±	±	±	±	±	±	±	Restored intensity of coloration	±	±	±	±	±	±	±	Restored intensity of coloration		
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±			
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±			
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±			
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±			

Table no. II. b continued

7)Mild meth Sap AB 2.5	Mes	±	++	++	±	++	++	++	Regained with equal intensity of coloration	±	++	±	±	++	±	++	Regained with equal intensity of coloration
	Met	±	++	++	±	++	++	++		±	++	±	±	++	±	++	
	Mye	±	++	++	±	++	++	++		±	++	±	±	++	±	++	
	Die	±	++	++	±	++	++	++		±	++	±	±	++	±	++	
	Tel	±	++	++	±	++	++	++		±	++	±	±	++	±	++	
8)Act meth AB 2.5	Mes	±	±	±	±	±	±	±	Restored coloration	±	±	±	±	±	±	±	Restored coloration
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9)Act met Sap AB2.5	Mes	±	++	++	±	++	++	++	Regained with equal intensity of coloration	±	++	±	±	++	±	++	Regained with equal intensity of coloration
	Met	±	++	++	±	++	++	++		±	++	±	±	++	±	++	
	Mye	±	++	++	±	++	++	++		±	++	±	±	++	±	++	
	Die	±	++	++	±	++	++	++		±	++	±	±	++	±	++	
	Tel	±	++	++	±	++	++	++		±	++	±	±	++	±	++	
10)AB 2.5-PAS	Mes	±M	++B	++P	±M	++B	++P	++P	Weak magenta in Cyt, blue at Cs and ML, purple at Ics	±M	++B	±P	±M	++B	±P	++B	Weak magenta in Cyt, blue at Cs and ML, purple at Ics
	Met	±M	++B	++P	±M	++B	++P	++P		±M	++B	±P	±M	++B	±P	++B	
	Mye	±M	++B	++P	±M	++B	++P	++P		±M	++B	±P	±M	++B	±P	++B	
	Die	±M	++B	++P	±M	++B	++P	++P		±M	++B	±P	±M	++B	±P	++B	
	Tel	±M	++B	++P	±M	++B	++P	++P		±M	++B	±P	±M	++B	±P	++B	
10)Acid hydro	Mes	-	-	-	-	-	-	-	Loss of coloration	-	-	-	-	-	-	-	Loss of coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. II. d Initial incubation (48 hrs) + exposure incubation (72 hrs) =Final incubation (120 hrs).

Staning techn	Brain region	Normal								0.5 mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 2.5	Mes	±	++	±	±	++	±	++	Moderate intensity at Cs, Ics and ML. weak in Cyt	++	+++	±	++	+++	±	+++	Moderate intensity at al sites
	Met	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++	
	Mye	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++	
	Die	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++	
	Tel	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++	
2)PA* BhSap PAS	Mes	++	++	±	++	++	±	++	Weak intensity at Cs, Ics moderate at Cyt, weak at ML	+++	+++	±	+++	+++	±	+++	Intense intensity in Cyt, Moderate intensity at other sites
	Met	++	++	±	++	++	±	++		+++	+++	±	+++	+++	±	+++	
	Mye	++	++	±	++	++	±	++		+++	+++	±	+++	+++	±	+++	
	Die	++	++	±	++	++	±	++		+++	+++	±	+++	+++	±	+++	
	Tel	++	++	±	++	++	±	++		+++	+++	±	+++	+++	±	+++	
3) AZ 3.5	Mes	±	++	±	±	++	±	++	Moderate intensity at Cs, Ics and ML. weak in Cyt	++	+++	±	++	+++	±	+++	Moderate intensity at al sites
	Met	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++	
	Mye	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++	
	Die	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++	
	Tel	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++	
4) Sap-AB-2.5	Mes	++	++	++	++	++	++	++	Restoration of color intensity	+++	++++	±	+++	++++	±	++++	Restoration of color intensity
	Met	++	++	++	++	++	++	++		+++	++++	±	+++	++++	±	++++	
	Mye	++	++	++	++	++	++	++		+++	++++	±	+++	++++	±	++++	
	Die	++	++	++	++	++	++	++		+++	++++	±	+++	++++	±	++++	
	Tel	++	++	++	++	++	++	++		+++	++++	±	+++	++++	±	++++	
5)Mild meth AB 2.5	Mes	±	±	±	±	±	±	±	Restoration of color intensity	±	±	±	±	±	±	±	Restoration of color intensity
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	

Table no. II.d Initial incubation (48 hrs) + exposure incubation (72 hrs) =Final incubation (120 hrs).

6)Mild meth-Sap AB 2.5	Mes	±	++	±	±	++	±	++	Regained with	++	+++	±	++	+++	±	+++	Regained with
	Met	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++	
	Mye	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++	
	Die	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++	
	Tel	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++	
7)Act meth AB 2.5	Mes	±	±	±	±	±	±	±	Restoration of color intensity	±	±	±	±	±	±	±	Restoration of color intensity
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
8)Act met Sap AB 2.5	Mes	±	++	±	±	++	±	++	Regained with	++	+++	±	++	+++	±	+++	Regained with
	Met	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++	
	Mye	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++	
	Die	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++	
	Tel	±	++	±	±	++	±	++		++	+++	±	++	+++	±	+++	
9)AB 2.5-PAS	Mes	±M	++B	±P	±M	++B	±P	++B	Weak magenta in Cyt.moderate blue at Cs, purple in Ics	++M	+3B	++P	++M	+3B	++P	++P	Increasd intensity at all sites
	Met	±M	++B	±P	±M	++B	±P	++B		++M	+3B	++P	++M	+3B	++P	++P	
	Mye	±M	++B	±P	±M	++B	±P	++B		++M	+3B	++P	++M	+3B	++P	++P	
	Die	±M	++B	±P	±M	++B	±P	++B		++M	+3B	++P	++M	+3B	++P	++P	
	Tel	±M	++B	±P	±M	++B	±P	++B		++M	+3B	++P	++M	+3B	++P	++P	
11)Acid hydrol	Mes	-	-	-	-	-	-	-	Loss of coloration	-	-	-	-	-	-	-	Loss of coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Dic	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. II. d Initial incubation (48 hrs) + exposure incubation (72 hrs) =Final incubation (120 hrs).

Staining Tech.	Brain zones	0.5 mM H_2O_2 + 3 mg vitamin C treated								Control 3 mg vitamin C treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 2.5	Mes	±	++	±	±	++	±	++	Moderate intensity at Cs, Ics and ML. weak in Cyt	±	++	±	±	++	±	++	Moderate intensity at Cs, Ics and ML. weak in Cyt
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
2)PA* BhSap PAS	Mes	++	++	±	++	++	±	++	Weak intensity at Cs, Ics moderate at Cyt, weak at ML	++	++	±	++	++	±	++	Weak intensity at Cs, Ics moderate at Cyt, weak at ML
	Met	++	++	±	++	++	±	++		++	++	±	++	++	±	++	
	Mye	++	++	±	++	++	±	++		++	++	±	++	++	±	++	
	Die	++	++	±	++	++	±	++		++	++	±	++	++	±	++	
	Tel	++	++	±	++	++	±	++		++	++	±	++	++	±	++	
3) AZ 3.5	Mes	±	++	±	±	++	±	++	Moderate intensity at Cs, Ics and ML. weak in Cyt	±	++	±	±	++	±	++	Moderate intensity at Cs, Ics and ML. weak in Cyt
	Met	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Mye	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Die	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
	Tel	±	++	±	±	++	±	++		±	++	±	±	++	±	++	
4) Sap-AB-2.5	Mes	++	++	++	++	++	++	++	Restoration of color intensity	++	++	++	++	++	++	++	Restoration of color intensity
	Met	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Mye	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Die	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
	Tel	++	++	++	++	++	++	++		++	++	++	++	++	++	++	
5)Mild meth AB 2.5	Mes	±	±	±	±	±	±	±	Restoration of color intensity	±	±	±	±	±	±	±	Restoration of color intensity
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	

Table no. II.d Initial incubation (48 hrs) + exposure incubation (72 hrs) =Final incubation (120 hrs).

6)Mild meth-Sap AB 2.5	Mes	+	++	++	+	++	++	++	Regained with	+	++	++	+	++	++	++	Regained with
	Met	+	++	++	+	++	++	++		+	++	++	+	++	++	++	
	Mye	+	++	++	+	++	++	++		+	++	++	+	++	++	++	
	Die	+	++	++	+	++	++	++		+	++	++	+	++	++	++	
	Tel	+	++	++	+	++	++	++		+	++	++	+	++	++	++	
7)Act meth AB 2.5	Mes	±	±	±	±	±	±	±	Restoration of color intensity	±	±	±	±	±	±	±	Restoration of color intensity
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
8)Act met Sap AB 2.5	Mes	+	++	++	+	++	++	++	Regained with	+	++	++	+	++	++	++	Regained with
	Met	+	++	++	+	++	++	++		+	++	++	+	++	++	++	
	Mye	+	++	++	+	++	++	++		+	++	++	+	++	++	++	
	Die	+	++	++	+	++	++	++		+	++	++	+	++	++	++	
	Tel	+	++	++	+	++	++	++		+	++	++	+	++	++	++	
9)AB 2.5-PAS	Mes	+M	++B	++P	+M	++B	++P	++P	Weak magenta in Cyt.moderate blue at Cs, purple in Ics	+M	++B	++P	+M	++B	++P	++P	Weak magenta in Cyt.moderate blue at Cs, purple in Ics
	Met	+M	++B	++P	+M	++B	++P	++P		+M	++B	++P	+M	++B	++P	++P	
	Mye	+M	++B	++P	+M	++B	++P	++P		+M	++B	++P	+M	++B	++P	++P	
	Die	+M	++B	++P	+M	++B	++P	++P		+M	++B	++P	+M	++B	++P	++P	
	Tel	+M	++B	++P	+M	++B	++P	++P		+M	++B	++P	+M	++B	++P	++P	
10)Acid hydrol	Mes	-	-	-	-	-	-	-	Loss of coloration	-	-	-	-	-	-	-	Loss of coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. II. e Initial incubation (72hrs) + exposure incubation (24hrs) =Final incubation (96 hrs).

Stain Tech.	Brain region	Normal								0.5mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 2.5	Mesn	±	++	±	±	++	±	++	Trace reaction in Cyt, moderate at Cs, Ics and ML	+++	+++	+++	+++	+++	+++	+++	Intense coloration at all sites
	Meten	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Myel	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Dien	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Teln	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
2)PA* BhSap PAS	Mesn	++	++	±	++	++	±	++	Moderate content in Cyt weak Ics, and trace at Cs	+++	++	++	+++	++	++	++	Moderate intesity at Ics,Cs and ML, intense in Cyt
	Meten	++	++	±	++	++	±	++		+++	++	++	+++	++	++	++	
	Myel	++	++	±	++	++	±	++		+++	++	++	+++	++	++	++	
	Dien	++	++	±	++	++	±	++		+++	++	++	+++	++	++	++	
	Teln	++	++	±	++	++	±	++		+++	++	++	+++	++	++	++	
3) AZ 3.5	Mesn	±	++	±	±	++	±	++	Trace reaction in Cyt, moderate at Cs, Ics and ML	+++	+++	+++	+++	+++	+++	+++	Intense coloration at all sites
	Meten	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Myel	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Dien	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Teln	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
4) Sap-AB-2.5	Mesn	±	+++	±	±	+++	±	+++	Restored intensity of coloration	+++	+++	+++	+++	+++	+++	+++	Intense coloration at all sites
	Meten	±	+++	±	±	+++	±	+++		+++	+++	+++	+++	+++	+++	+++	
	Myel	±	+++	±	±	+++	±	+++		+++	+++	+++	+++	+++	+++	+++	
	Dien	±	+++	±	±	+++	±	+++		+++	+++	+++	+++	+++	+++	+++	
	Teln	±	+++	±	±	+++	±	+++		+++	+++	+++	+++	+++	+++	+++	
5)Mild meth AB 2.5	Mesn	±	±	±	±	±	±	±	Restored intensity of coloration	±	±	±	±	±	±	±	Restored coloration
	Meten	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mycl	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Dien	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Teln	±	±	±	±	±	±	±		±	±	±	±	±	±	±	

Table no. II. e Initial incubation (72hrs) + exposure incubation (24hrs) =Final incubation (96 hrs). continued

6)Mild meth-Sap AB 2.5	Mesn	±	++	±	±	++	±	++	Regained with equal intensity of coloration	+++	+++	+++	+++	+++	+++	+++	Regained with equal intensity of coloration
	Meten	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Myel	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Dien	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Teln	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
7)Act meth AB 2.5	Mesn	±	±	±	±	±	±	±	Restored coloration	±	±	±	±	±	±	±	Restored coloration
	Meten	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Myel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Dien	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Teln	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
8)Act met Sap AB 2.5	Mesn	±	++	±	±	++	±	++	Regained with equal intensity of coloration	+++	+++	+++	+++	+++	+++	+++	Regained with equal intensity of coloration
	Meten	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Myel	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Dien	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Teln	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
9)AB 2.5-PAS	Mesn	±M	++B	±P	±M	++B	±P	++B	Weak magenta in Cyt, blue at Cs and ML, purple at Ics	++M	++P	++B	++M	++P	++B	++M	Moderate coloration at all sites
	Meten	±M	++B	±P	±M	++B	±P	++B		++M	++P	++B	++M	++P	++B	++M	
	Myel	±M	++B	±P	±M	++B	±P	++B		++M	++P	++B	++M	++P	++B	++M	
	Dien	±M	++B	±P	±M	++B	±P	++B		++M	++P	++B	++M	++P	++B	++M	
	Teln	±M	++B	±P	±M	++B	±P	++B		++M	++P	++B	++M	++P	++B	++M	
11)Acid hydrol	Mesn	-	-	-	-	-	-	-	Loss of coloration	-	-	-	-	-	-	-	Loss of coloration
	Meten	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Myel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Dien	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Teln	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
12) Sialid dig. AB 2.5	Mesn	±	++	±	±	++	±	++	Loss of coloration	-	-	-	-	-	-	-	Loss of coloration
	Meten	±	++	±	±	++	±	++		-	-	-	-	-	-	-	
	Myel	±	++	±	±	++	±	++		-	-	-	-	-	-	-	
	Dien	±	++	±	±	++	±	++		-	-	-	-	-	-	-	
	Teln	±	++	±	±	++	±	++		-	-	-	-	-	-	-	

Table no. II. e Initial incubation (72 hrs) + exposure incubation (24 hrs) =Final incubation (96 hrs). continued

Initial incubation (72 hrs) + exposure incubation (48hrs) =Final incubation (120 hrs).																		
Staining Tech.	Brain zone	0.5 mM H_2O_2 + 3mg vitamin C treated								Control 3mg vitamin C treated								
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB2.5	Mesn	±	+	+	±	+	+	+	Trace reaction in Cyt, weak at Cs, Ics and ML	±	+	+	±	+	+	+	Trace reaction in Cyt, weak at Cs, Ics and ML
	Meten	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Myel	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Dien	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Teln	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
2)PA* Bh-Sap PAS	Mesn	+	±	+	+	±	+	+	Weak content in Cyt and Ics, and trace at Cs	+	±	+	+	±	+	+	Weak content in Cyt and Ics, and trace at Cs
	Meten	+	±	+	+	±	+	+		+	±	+	+	±	+	+	
	Myel	+	±	+	+	±	+	+		+	±	+	+	±	+	+	
	Dien	+	±	+	+	±	+	+		+	±	+	+	±	+	+	
	Teln	+	±	+	+	±	+	+		+	±	+	+	±	+	+	
3) AZ 3.5	Mesn	±	+	+	±	+	+	+	Trace reaction in Cyt, weak at Cs, Ics and ML	±	+	+	±	+	+	+	Trace reaction in Cyt, weak at Cs, Ics and ML
	Meten	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Myel	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Dien	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Teln	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
4) Sap-AB 2.5	Mesn	±	+	+	±	+	+	+	Restored intensity of coloration	±	+	+	±	+	+	+	Restored intensity of coloration
	Meten	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Myel	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Dien	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Teln	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
5) Mild-meth-AB 2.5	Mesn	±	+	+	±	+	+	+	Restored coloration	±	+	+	±	+	+	+	Restored coloration
	Mctcn	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Myel	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Dien	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Teln	±	+	+	±	+	+	+		±	+	+	±	+	+	+	

Table no. II. e Initial incubation (72 hrs) + exposure incubation (24 hrs) =Final incubation (96 hrs). continued

6)Mild meth Sap AB 2.5	Mesn	±	±	±	±	±	±	±	Regained with equal intensity of coloration	±	±	±	±	±	±	±	Regained with equal intensity of coloration
	Meten	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Myel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Dien	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Teln	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
7)Act meth AB 2.5	Mesn	±	+	+	±	+	+	+	Restored coloration	±	+	+	±	+	+	+	Restored coloration
	Meten	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Myel	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Dien	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Teln	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
8)Act met Sap AB2.5	Mesn	±	±	±	±	±	±	±	Regained with equal intensity of coloration	±	+	+	±	+	+	+	Regained with equal intensity of coloration
	Meten	±	±	±	±	±	±	±		±	+	+	±	+	+	+	
	Myel	±	±	±	±	±	±	±		±	+	+	±	+	+	+	
	Dien	±	±	±	±	±	±	±		±	+	+	±	+	+	+	
	Teln	±	±	±	±	±	±	±		±	+	+	±	+	+	+	
9)AB 2.5-PAS	Mesn	+M	+P	+B	+M	+P	+B	+M	Weak magenta in Cyt, blue at Cs and ML, purple at Ics	+M	+P	+B	+M	+P	+B	+M	Weak magenta in Cyt, blue at Cs and ML, purple at Ics
	Meten	+M	+P	+B	+M	+P	+B	+M		+M	+P	+B	+M	+P	+B	+M	
	Myel	+M	+P	+B	+M	+P	+B	+M		+M	+P	+B	+M	+P	+B	+M	
	Dien	+M	+P	+B	+M	+P	+B	+M		+M	+P	+B	+M	+P	+B	+M	
	Teln	+M	+P	+B	+M	+P	+B	+M		+M	+P	+B	+M	+P	+B	+M	
10)Acid hydro	Mesn	-	-	-	-	-	-	-	Loss of coloration	-	-	-	-	-	-	-	Loss of coloration
	Meten	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Myel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Dien	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Teln	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. II. e Initial incubation (72 hrs) + exposure incubation (48 hrs) =Final incubation (120 hrs).

Stain Tech.	Brain region	Normal								0.5mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 2.5	Mes	±	++	±	±	++	±	++	Moderate intensity at Cs, Ics and ML. weak in Cyt	+++	+++	+++	+++	+++	+++	+++	Intense reaction at all sites
	Met	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
2)PA* BhSap PAS	Mes	++	++	±	++	++	±	++	Weak intensity at Cs, Ics moderate at Cyt, weak at ML	+++	++	++	+++	++	++	++	Increased color intensity at all sites
	Met	++	++	±	++	++	±	++		+++	++	++	+++	++	++	++	
	Mye	++	++	±	++	++	±	++		+++	++	++	+++	++	++	++	
	Die	++	++	±	++	++	±	++		+++	++	++	+++	++	++	++	
	Tel	++	++	±	++	++	±	++		+++	++	++	+++	++	++	++	
3) AZ 3.5	Mes	±	++	±	±	++	±	++	Moderate intensity at Cs, Ics and ML. weak in Cyt	+++	+++	+++	+++	+++	+++	+++	Intense reaction
	Met	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
4) Sap-AB-2.5	Mes	++	++	++	++	++	++	++	Restoration of color intensity	+++	+++	+++	+++	+++	+++	+++	Intense reaction
	Met	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Die	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	++	++	++	++	++	++	++		+++	+++	+++	+++	+++	+++	+++	
5)Mild meth AB 2.5	Mes	±	±	±	±	±	±	±	Restoration of color intensity	±	±	±	±	±	±	±	Decreased color intensity
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	

Table no. II. e Initial incubation (72 hrs) + exposure incubation (48 hrs) =Final incubation (120 hrs).

6)Mild meth-Sap AB 2.5	Mes	±	++	±	±	++	±	++	Regained with	+++	+++	+++	+++	+++	+++	+++	Intense reaction
	Met	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
7)Act meth AB 2.5	Mes	±	±	±	±	±	±	±	Restoration of color intensity	±	±	±	±	±	±	±	Restoration of color intensity
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
8)Act met Sap AB 2.5	Mes	±	++	±	±	++	±	++	Regained with	+++	+++	+++	+++	+++	+++	+++	Intense reaction
	Met	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Mye	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Die	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
	Tel	±	++	±	±	++	±	++		+++	+++	+++	+++	+++	+++	+++	
9)AB 2.5-PAS	Mes	±M	++B	±P	±M	++B	±P	++B	Weak magenta in Cyt.moderate blue at Cs, purple in Ics	++M	++P	++B	++M	++P	++B	++M	Moderate coloration at all corresponding sites
	Met	±M	++B	±P	±M	++B	±P	++B		++M	++P	++B	++M	++P	++B	++M	
	Mye	±M	++B	±P	±M	++B	±P	++B		++M	++P	++B	++M	++P	++B	++M	
	Die	±M	++B	±P	±M	++B	±P	++B		++M	++P	++B	++M	++P	++B	++M	
	Tel	±M	++B	±P	±M	++B	±P	++B		++M	++P	++B	++M	++P	++B	++M	
11)Acid hydrol	Mes	-	-	-	-	-	-	-	Loss of coloration	-	-	-	-	-	-	-	loss of coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. II. e Initial incubation (72 hrs) + exposure incubation (48 hrs) =Final incubation (120 hrs).continued

Initial incubation (48hrs) + exposure incubation (48 hrs) =Final incubation (98 hrs).																			
Staining Tech.	Brain zone	0.5 mM H_2O_2 + 3mg vitamin C treated								Control 3mg vitamin C treated									
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations		
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics				
1)AB2.5	Mes	±	+	+	±	+	+	+	Trace reacl at cyt. weak at Cs, Ics & ML	±	+	+	±	+	+	+	Trace reacl at cyt weak at Cs, Ics & ML		
	Met	±	+	+	±	+	+	+		±	+	+	±	+	+	+			
	Mye	±	+	+	±	+	+	+		±	+	+	±	+	+	+			
	Die	±	+	+	±	+	+	+		±	+	+	±	+	+	+			
	Tel	±	+	+	±	+	+	+		±	+	+	±	+	+	+			
2)PA* Bh-Sap PAS	Mes	+	±	+	+	±	+	+	Weak colora tion in Cyt & Ics, ML; Trace at other	+	±	+	+	±	+	+	Weak colora tion in cyt. & Ics, ML Trace in Cyt		
	Met	+	±	+	+	±	+	+		+	±	+	+	±	+	+			
	Mye	+	±	+	+	±	+	+		+	±	+	+	±	+	+			
	Die	+	±	+	+	±	+	+		+	±	+	+	±	+	+			
	Tel	+	±	+	+	±	+	+		+	±	+	+	±	+	+			
3) AZ 3.5	Mes	±	+	+	±	+	+	+	Trace reaction at cyt. weak at Cs, Ics & ML	±	+	+	±	+	+	+	Trace reaction at cyt, weak at Cs, Ics & ML		
	Met	±	+	+	±	+	+	+		±	+	+	±	+	+	+			
	Mye	±	+	+	±	+	+	+		±	+	+	±	+	+	+			
	Die	±	+	+	±	+	+	+		±	+	+	±	+	+	+			
	Tel	±	+	+	±	+	+-	+		±	+	+	±	+	+	+			
4) Sap-AB 2.5	Mes	±	++	++	±	++	++	++	No change in colorad' at Cyt.	±	++	++	±	++	++	++	Increased colorat'n		
	Met	±	++	++	±	++	++	++		±	++	++	±	++	++	++			
	Mye	±	++	++	±	++	++	++		±	++	++	±	++	++	++			
	Die	±	++	++	±	++	++	++		±	++	++	±	++	++	++			
	Tel	±	++	++	±	++	++	++		±	++	++	±	++	++	++			
5) Mild-meth-AB 2.5	Mes	±	±	±	±	±	±	+	Restortion of color intensity	±	±	±	±	±	±	±	Restortion of color intensity		
	Met	±	±	±	±	±	±	+		±	±	±	±	±	±	±			
	Mye	±	±	±	±	±	±	+		±	±	±	±	±	±	±			
	Die	±	±	±	±	±	±	+		±	±	±	±	±	±	±			
	Tel	±	±	±	±	±	±	+		±	±	±	±	±	±	±			

Table no. II. e Initial incubation (72 hrs) + exposure incubation (48 hrs) =Final incubation (120 hrs). continued

7)Mild meth Sap AB 2.5	Mes	±	++	++	±	++	++	++	Regained reaction	±	++	++	±	++	++	++	Regained reaction
	Met	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Mye	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Die	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
	Tel	±	++	++	±	++	++	++		±	++	++	±	++	++	++	
8)Act meth AB 2.5	Mes	±	+	+	±	+	+	+	Restortion of reaction	±	+	+	±	+	+	+	Restortion of reaction
	Met	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Mye	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Die	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
	Tel	±	+	+	±	+	+	+		±	+	+	±	+	+	+	
9)Act met Sap AB2.5	Mes	±	++	++	±	++	++	++	Regained reaction	±	+	+	±	+	+	+	Regained reaction
	Met	±	++	++	±	++	++	++		±	+	+	±	+	+	+	
	Mye	±	++	++	±	++	++	++		±	+	+	±	+	+	+	
	Die	±	++	++	±	++	++	++		±	+	+	±	+	+	+	
	Tel	±	++	++	±	++	++	++		±	+	+	±	+	+	+	
10)AB 2.5-PAS	Mes	+M	+P	+B	+M	+P	+B	+M	Regained reaction	+M	+P	+B	+M	+P	+B	+M	~~Moderate~~ weak reaction at all sites
	Met	+M	+P	+B	+M	+P	+B	+M		+M	+P	+B	+M	+P	+B	+M	
	Mye	+M	+P	+B	+M	+P	+B	+M		+M	+P	+B	+M	+P	+B	+M	
	Die	+M	+P	+B	+M	+P	+B	+M		+M	+P	+B	+M	+P	+B	+M	
	Tel	+M	+P	+B	+M	+P	+B	+M		+M	+P	+B	+M	+P	+B	+M	
10)Acid hydro	Mes	-	-	-	-	-	-	-	Loss of coloration	-	-	-	-	-	-	-	Loss of coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. II.f Initial incubation (96hrs) + exposure incubation (24 hrs) =Final incubation (120 hrs).

Stain Tech.	Brain region	Normal								0.5mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 2.5	Mes	+	++	±	+	++	±	++	Weak intensity in Cyt and moderate at Cs, Ics and ML	+++	+++	±	+++	+++	±	+++	Intense coloration in Cyt and Cs
	Met	+	++	±	+	++	±	++		+++	+++	±	+++	+++	±	+++	
	Mye	+	++	±	+	++	±	++		+++	+++	±	+++	+++	±	+++	
	Die	+	++	±	+	++	±	++		+++	+++	±	+++	+++	±	+++	
	Tel	+	++	±	+	++	±	++		+++	+++	±	+++	+++	±	+++	
2)PA* BhSap PAS	Mes	++	+	±	+	++	±	++	Moderate intensity in Cyt, Ics and weak at Cs	+++	++	±	+++	++	±	++	Intense reaction at Cyt and Vs
	Met	++	+	±	+	++	±	++		+++	++	±	+++	++	±	++	
	Mye	++	+	±	+	++	±	++		+++	++	±	+++	++	±	++	
	Die	++	+	±	+	++	±	++		+++	++	±	+++	++	±	++	
	Tel	++	+	±	+	++	±	++		+++	++	±	+++	++	±	++	
3) AZ 3.5	Mes	+	++	±	+	++	±	++	Weak intensity in Cyt and moderate at Cs, Ics and ML	+++	+++	±	+++	+++	±	+++	Intense coloration in Cyt and Cs
	Met	+	++	±	+	++	±	++		+++	+++	±	+++	+++	±	+++	
	Mye	+	++	±	+	++	±	++		+++	+++	±	+++	+++	±	+++	
	Die	+	++	±	+	++	±	++		+++	+++	±	+++	+++	±	+++	
	Tel	+	++	±	+	++	±	++		+++	+++	±	+++	+++	±	+++	
4) Sap-AB-2.5	Mes	±	+++	±	±	+++	±	+++	Increased intensity of coloration	+++	+++	±	+++	+++	±	+++	Increased intensity of coloration
	Met	±	+++	±	±	+++	±	+++		+++	+++	±	+++	+++	±	+++	
	Mye	±	+++	±	±	+++	±	+++		+++	+++	±	+++	+++	±	+++	
	Die	±	+++	±	±	+++	±	+++		+++	+++	±	+++	+++	±	+++	
	Tel	±	+++	±	±	+++	±	+++		+++	+++	±	+++	+++	±	+++	
5)Mild meth AB 2.5	Mes	±	+	±	±	+	±	+	Loss of coloration	±	±	±	±	±	±	±	Loss of coloration
	Met	±	+	±	±	+	±	+		±	±	±	±	±	±	±	
	Mye	±	+	±	±	+	±	+		±	±	±	±	±	±	±	
	Die	±	+	±	±	+	±	+		±	±	±	±	±	±	±	
	Tel	±	+	±	±	+	±	+		±	±	±	±	±	±	±	

Table no. II.f Initial incubation (96hrs) + exposure incubation (24 hrs) =Final incubation (120 hrs).

6)Mild meth-Sap AB 2.5	Mes	+	++	±	+	++	±	++	Regained coloration intensity	+++	+++	±	+++	+++	±	+++	Regained coloration intensity
	Met	+	++	±	+	++	±	++		+++	+++	±	+++	+++	±	+++	
	Mye	+	++	±	+	++	±	++		+++	+++	±	+++	+++	±	+++	
	Die	+	++	±	+	++	±	++		+++	+++	±	+++	+++	±	+++	
	Tel	+	++	±	+	++	±	++		+++	+++	±	+++	+++	±	+++	
7)Act meth AB 2.5	Mes	±	±	±	±	±	±	±	Decreased intensity of coloration	±	±	±	±	±	±	±	Decreased intensity of coloration
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
8)Act met Sap AB 2.5	Mes	+	++	±	+	++	±	++	Regained coloration	+++	+++	±	+++	+++	±	+++	Regained coloration
	Met	+	++	±	+	++	±	++		+++	+++	±	+++	+++	±	+++	
	Mye	+	++	±	+	++	±	++		+++	+++	±	+++	+++	±	+++	
	Die	+	++	±	+	++	±	++		+++	+++	±	+++	+++	±	+++	
	Tel	+	++	±	+	++	±	++		+++	+++	±	+++	+++	±	+++	
9)AB 2.5-PAS	Mes	+M	++B	+P	+M	+P	++B	+M	Weak coloration at appropriate site	+3M	+3B	±P	+3M	+3B	±P	+3M	Intense coloration at all sites
	Met	+M	++B	+P	+M	+P	++B	+M		+3M	+3B	±P	+3M	+3B	±P	+3M	
	Mye	+M	++B	+P	+M	+P	++B	+M		+3M	+3B	±P	+3M	+3B	±P	+3M	
	Die	+M	++B	+P	+M	+P	++B	+M		+3M	+3B	±P	+3M	+3B	±P	+3M	
	Tel	+M	++B	+P	+M	+P	++B	+M		+3M	+3B	±P	+3M	+3B	±P	+3M	
11)Acid hydrol	Mes	-	-	-	-	-	-	-	Loss of coloration	-	-	-	-	-	-	-	Loss of coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. II.f Initial incubation (96hrs) + exposure incubation (24 hrs) =Final incubation (120 hrs).

Initial incubation (96hrs) + exposure incubation (24 hrs) =Final incubation (120 hrs).																		
Staining Tech.	Brain zone	0.5 mM H_2O_2 + 3mg vitamin C treated								Control 3mg vitamin C treated								
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations	
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics			
1)AB 2.5	Mes	+	++	±	+	++	±	++	Weak intensity in Cyt, moderate at all other sites	+	++	±	+	++	±	++	Weak intensity in Cyt and moderate at Cs, Ics and ML	
	Met	+	++	±	+	++	±	++		+	++	±	+	++	±	++		
	Mye	+	++	±	+	++	±	++		+	++	±	+	++	±	++		
	Die	+	++	±	+	++	±	++		+	++	±	+	++	±	++		
	Tel	+	++	±	+	++	±	++		+	++	±	+	++	±	++		
2)PA* Bh-Sap PAS	Mes	++	+	±	+	++	±	++	Moderate intensity in Cyt, weak in all other sites	++	+	±	+	++	±	++	Moderate intensity in Cyt, Ics and weak at Cs	
	Met	++	+	±	+	++	±	++		++	+	±	+	++	±	++		
	Mye	++	+	±	+	++	±	++		++	+	±	+	++	±	++		
	Die	++	+	±	+	++	±	++		++	+	±	+	++	±	++		
	Tel	++	+	±	+	++	±	++		++	+	±	+	++	±	++		
3) AZ 3.5	Mes	+	++	±	+	++	±	++	Weak intensity in Cyt, moderate at all other sites	+	++	±	+	++	±	++	Weak intensity in Cyt and moderate at Cs, Ics and ML	
	Met	+	++	±	+	++	±	++		+	++	±	+	++	±	++		
	Mye	+	++	±	+	++	±	++		+	++	±	+	++	±	++		
	Die	+	++	±	+	++	±	++		+	++	±	+	++	±	++		
	Tel	+	++	±	+	++	±	++		+	++	±	+	++	±	++		
4) Sap-AB 2.5	Mes	±	+++	±	±	+++	±	+++	Restored intensity of coloration	±	+++	±	±	+++	±	+++	Restored intensity of coloration	
	Met	±	+++	±	±	+++	±	+++		±	+++	±	±	+++	±	+++		
	Mye	±	+++	±	±	+++	±	+++		±	+++	±	±	+++	±	+++		
	Die	±	+++	±	±	+++	±	+++		±	+++	±	±	+++	±	+++		
	Tel	±	+++	±	±	+++	±	+++		±	+++	±	±	+++	±	+++		
5) Mild-meth-AB 2.5	Mes	±	+	±	±	+	±	+	Restored intensity of coloration	±	+	±	±	+	±	+	Restored intensity of coloration	
	Met	±	+	±	±	+	±	+		±	+	±	±	+	±	+		
	Mye	±	+	±	±	+	±	+		±	+	±	±	+	±	+		
	Die	±	+	±	±	+	±	+		±	+	±	±	+	±	+		
	Tel	±	+	±	±	+	±	+		±	+	±	±	+	±	+		

Table no. II.f Initial incubation (96hrs) + exposure incubation (24 hrs) =Final incubation (120 hrs).

7)Mild meth Sap AB 2.5	Mes	+	++	±	+	++	±	++	Regained coloration intensity	+	++	±	+	++	±	++	Regained coloration intensity
	Met	+	++	±	+	++	±	++		+	++	±	+	++	±	++	
	Mye	+	++	±	+	++	±	++		+	++	±	+	++	±	++	
	Die	+	++	±	+	++	±	++		+	++	±	+	++	±	++	
	Tel	+	++	±	+	++	±	++		+	++	±	+	++	±	++	
8)Act meth AB 2.5	Mes	±	±	±	±	±	±	±	Decreased intensity of coloration	±	±	±	±	±	±	±	Decreased intensity of coloration
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
9)Act met Sap AB2.5	Mes	+	++	±	+	++	±	++	Regained coloration	+	++	±	+	++	±	++	Regained coloration
	Met	+	++	±	+	++	±	++		+	++	±	+	++	±	++	
	Mye	+	++	±	+	++	±	++		+	++	±	+	++	±	++	
	Die	+	++	±	+	++	±	++		+	++	±	+	++	±	++	
	Tel	+	++	±	+	++	±	++		+	++	±	+	++	±	++	
10)AB 2.5-PAS	Mes	+M	++B	+P	+M	+P	++B	+M	Weak coloration at appropriate site	+M	++B	+P	+M	+P	++B	+M	Weak coloration at appropriate site
	Met	+M	++B	+P	+M	+P	++B	+M		+M	++B	+P	+M	+P	++B	+M	
	Mye	+M	++B	+P	+M	+P	++B	+M		+M	++B	+P	+M	+P	++B	+M	
	Die	+M	++B	+P	+M	+P	++B	+M		+M	++B	+P	+M	+P	++B	+M	
	Tel	+M	++B	+P	+M	+P	++B	+M		+M	++B	+P	+M	+P	++B	+M	
10)Acid hydro	Mes	-	-	-	-	-	-	-	Loss of coloration	-	-	-	-	-	-	-	Loss of coloration
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. II.g Initial incubation (120hrs) + exposure incubation (24 hrs) =Final incubation (144 hrs).

Stain Tech.	Brain region	Normal								0.5mM H_2O_2 treated							
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics		
1)AB 2.5	Mes	+	++	++	+	++	++	++	Weak intensity in Cyt, Ics moderate at all other sites	++	++	++	++	++	++	++	Moderate intensity at all other sites
	Met	+	++	++	+	++	++	++		++	++	++	++	++	++	++	
	Mye	+	++	++	+	++	++	++		++	++	++	++	++	++	++	
	Die	+	++	++	+	++	++	++		++	++	++	++	++	++	++	
	Tel	+	++	++	+	++	++	++		++	++	++	++	++	++	++	
2)PA* BhSap PAS	Mes	++	+	+	++	+	+	++	Moderate intensity in Cyt and Ics, weak intensity at all other sites	++	++	++	++	++	++	++	Moderate intensity at all other sites
	Met	++	+	+	++	+	+	++		++	++	++	++	++	++	++	
	Mye	++	+	+	++	+	+	++		++	++	++	++	++	++	++	
	Die	++	+	+	++	+	+	++		++	++	++	++	++	++	++	
	Tel	++	+	+	++	+	+	++		++	++	++	++	++	++	++	
3) AZ 3.5	Mes	+	++	++	+	++	++	++	Weak intensity in Cyt, Ics moderate at all other sites	++	++	++	++	++	++	++	Moderate intensity at all other sites
	Met	+	++	++	+	++	++	++		++	++	++	++	++	++	++	
	Mye	+	++	++	+	++	++	++		++	++	++	++	++	++	++	
	Die	+	++	++	+	++	++	++		++	++	++	++	++	++	++	
	Tel	+	++	++	+	++	++	++		++	++	++	++	++	++	++	
4) Sap-AB-2.5	Mes	±	+	+	±	+	+	+	Restored intensity of coloration	±	±	±	±	±	±	±	Restored intensity of coloration
	Met	±	+	+	±	+	+	+		±	±	±	±	±	±	±	
	Mye	±	+	+	±	+	+	+		±	±	±	±	±	±	±	
	Die	±	+	+	±	+	+	+		±	±	±	±	±	±	±	
	Tel	±	+	+	±	+	+	+		±	±	±	±	±	±	±	
5)Mild meth AB 2.5	Mes	±	±	±	±	±	±	±	Restored intensity of coloration	±	±	±	±	±	±	±	Restored intensity of coloration
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Myc	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	

Table no. II. e Initial incubation (120hrs) + exposure incubation (24 hrs) =Final incubation (144 hrs). continued

6)Mild meth-Sap AB 2.5	Mes	+	++	++	+	++	++	++	Regained coloration with equal intensity	++	++	++	++	++	++	++	Regained coloration with equal intensity
	Met	+	++	++	+	++	++	++		++	++	++	++	++	++	++	
	Mye	+	++	++	+	++	++	++		++	++	++	++	++	++	++	
	Die	+	++	++	+	++	++	++		++	++	++	++	++	++	++	
	Tel	+	++	++	+	++	++	++		++	++	++	++	++	++	++	
7)Act meth AB 2.5	Mes	±	±	±	±	±	±	±	Restored intensity of coloration	±	±	±	±	±	±	±	Restored intensity of coloration
	Met	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Mye	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Die	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
	Tel	±	±	±	±	±	±	±		±	±	±	±	±	±	±	
8)Act met Sap AB 2.5	Mes	+	++	++	+	++	++	++	Regained coloration with equal intensity	++	++	++	++	++	++	++	Regained coloration with equal intensity
	Met	+	++	++	+	++	++	++		++	++	++	++	++	++	++	
	Mye	+	++	++	+	++	++	++		++	++	++	++	++	++	++	
	Die	+	++	++	+	++	++	++		++	++	++	++	++	++	++	
	Tel	+	++	++	+	++	++	++		++	++	++	++	++	++	++	
9)AB 2.5-PAS	Mes	+M	++B	++P	+M	++B	++P	++P	Weak magentain Cyt. moderate blueandpurple at other sites	++M	++P	++B	++M	++P	++B	++M	Moderate increase in intensity at all sites
	Met	+M	++B	++P	+M	++B	++P	++P		++M	++P	++B	++M	++P	++B	++M	
	Mye	+M	++B	++P	+M	++B	++P	++P		++M	++P	++B	++M	++P	++B	++M	
	Die	+M	++B	++P	+M	++B	++P	++P		++M	++P	++B	++M	++P	++B	++M	
	Tel	+M	++B	++P	+M	++B	++P	++P		++M	++P	++B	++M	++P	++B	++M	
11)Acid hydrol	Mes	-	-	-	-	-	-	-	Loss of color intensity	-	-	-	-	-	-	-	Loss of color intensity
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mes	-	-	-	-	-	-	-	Loss of color intensity	-	-	-	-	-	-	-	Loss of color intensity
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

Table no. II. e Initial incubation (120hrs) + exposure incubation (24 hrs) =Final incubation (144 hrs). continued

Initial incubation (120hrs) + exposure incubation (24 hrs) =Final incubation (144 hrs).																		
Staining Tech.	Brain zone	0.5 mM H_2O_2 + 3mg vitamin C treated								Control 3mg vitamin C treated								
		Ependymal layer			Mantle layer			ML	Inference to observations	Ependymal layer			Mantle layer			ML	Inference to observations	
		Cyt	Cs	Ics	Cyt	Cs	Ics			Cyt	Cs	Ics	Cyt	Cs	Ics			
1)AB2.5	Mes	+	++	++	+	++	++	++	Weak in Cyt. moderate Cs, Isc, ML	+	++	++	+	++	++	++	Weak intensity in Cyt, Ics moderate at all other sites	
	Met	+	++	++	+	++	++	++		+	++	++	+	++	++	++		
	Mye	+	++	++	+	++	++	++		+	++	++	+	++	++	++		
	Die	+	++	++	+	++	++	++		+	++	++	+	++	++	++		
	Tel	+	++	++	+	++	++	++		+	++	++	+	++	++	++		
2)PA* Bh-Sap PAS	Mes	++	+	+	++	+	+	+	Moderate in Cyt. weak at other sites	++	+	+	++	+	+	++	Moderate intensity in Cyt and Ics, weak intensity at all other sites	
	Met	++	+	+	++	+	+	+		++	+	+	++	+	+	++		
	Mye	++	+	+	++	+	+	+		++	+	+	++	+	+	++		
	Die	++	+	+	++	+	+	+		++	+	+	++	+	+	++		
	Tel	++	+	+	++	+	+	+		++	+	+	++	+	+	++		
3) AZ 3.5	Mes	+	++	++	+	++	++	++	Weak in Cyt. moderate Cs, Isc, ML	+	++	++	+	++	++	++	Weak intensity in Cyt, Ics moderate at all other sites	
	Met	+	++	++	+	++	++	++		+	++	++	+	++	++	++		
	Mye	+	++	++	+	++	++	++		+	++	++	+	++	++	++		
	Die	+	++	++	+	++	++	++		+	++	++	+	++	++	++		
	Tel	+	++	++	+	++	++	++		+	++	++	+	++	++	++		
4) Sap-AB 2.5	Mes	±	+	+	±	+	+	+	Restored intensity of coloration	±	+	+	±	+	+	+	Restored intensity of coloration	
	Met	±	+	+	±	+	+	+		±	+	+	±	+	+	+		
	Mye	±	+	+	±	+	+	+		±	+	+	±	+	+	+		
	Die	±	+	+	±	+	+	+		±	+	+	±	+	+	+		
	Tel	±	+	+	±	+	+	+		±	+	+	±	+	+	+		
5) Mild-meth-AB 2.5	Mes	±	+	+	±	+	+	+	Restored intensity of coloration	±	±	±	±	±	±	±	Restored intensity of coloration	
	Met	±	+	+	±	+	+	+		±	±	±	±	±	±	±		
	Mye	±	+	+	±	+	+	+		±	±	±	±	±	±	±		
	Die	±	+	+	±	+	+	+		±	±	±	±	±	±	±		
	Tel	±	+	+	±	+	+	+		±	±	±	±	±	±	±		

Table no. II. e Initial incubation (120hrs) + exposure incubation (24 hrs) =Final incubation (144 hrs). continued

7)Mild meth Sap AB 2.5	Mes	+	++	++	+	++	++	++	Regained coloration with equal intensity	±	±	±	±	±	±	±	Regained coloration with equal intensity
	Met	+	++	++	+	++	++	++		±	±	±	±	±	±	±	
	Mye	+	++	++	+	++	++	++		±	±	±	±	±	±	±	
	Die	+	++	++	+	++	++	++		±	±	±	±	±	±	±	
	Tel	+	++	++	+	++	++	++		±	±	±	±	±	±	±	
8)Act meth AB 2.5	Mes	±	±	±	±	±	±	±	Restored intensity of coloration	±	+	+	±	+	+	+	Restored intensity of coloration
	Met	±	±	±	±	±	±	±		±	+	+	±	+	+	+	
	Mye	±	±	±	±	±	±	±		±	+	+	±	+	+	+	
	Die	±	±	±	±	±	±	±		±	+	+	±	+	+	+	
	Tel	±	±	±	±	±	±	±		±	+	+	±	+	+	+	
9)Act met Sap AB2.5	Mes	+	++	++	+	++	++	++	Regained coloration with equal intensity	±	+	+	±	+	+	+	Regained coloration with equal intensity
	Met	+	++	++	+	++	++	++		±	+	+	±	+	+	+	
	Mye	+	++	++	+	++	++	++		±	+	+	±	+	+	+	
	Die	+	++	++	+	++	++	++		±	+	+	±	+	+	+	
	Tel	+	++	++	+	++	++	++		±	+	+	±	+	+	+	
10)AB 2.5-PAS	Mes	+M	++B	++P	+M	++B	++P	++P		+M	+P	+B	+M	+P	+B	+M	Weak magentain Cyt.moderate blueandpurple at other sites
	Met	+M	++B	++P	+M	++B	++P	++P		+M	+P	+B	+M	+P	+B	+M	
	Mye	+M	++B	++P	+M	++B	++P	++P		+M	+P	+B	+M	+P	+B	+M	
	Die	+M	++B	++P	+M	++B	++P	++P		+M	+P	+B	+M	+P	+B	+M	
	Tel	+M	++B	++P	+M	++B	++P	++P		+M	+P	+B	+M	+P	+B	+M	
10)Acid hydro	Mes	-	-	-	-	-	-	-	Loss of color intensity	-	-	-	-	-	-	-	Loss of color intensity
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
[illegible]	Mes	-	-	-	-	-	-	-	Loss of color intensity	-	-	-	-	-	-	-	Loss of color intensity
	Met	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Mye	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Die	-	-	-	-	-	-	-		-	-	-	-	-	-	-	
	Tel	-	-	-	-	-	-	-		-	-	-	-	-	-	-	

PLATE I

PLATE I- Initial incubation (24 hrs) + Dose exposure incubation (24 hrs) = Final development (48 hrs). AB pH 2.5

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen .- Weak blue coloration was observed at the cellular zones.

Fig. 2 - Meten. - Weak blue coloration was observed at the cellular zones.

Fig. 3 - Myelen - Weak blue coloration was observed at the cellular zones.

Fig. 4 - Dien - Weak blue coloration was observed at the cellular zones.

Fig. 5 - Telen. - Weak blue coloration was observed at the cellular zones.

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000.

Fig. 6 - Mesen. - Intensely increased , diffused coloration was noted from the neuroepithelial cells.

Fig. 7 - Meten. - Intensely increased , diffused coloration was noted from the neuroepithelial cells..

Fig. 8 - Myelen - Intensely increased , diffused coloration was noted from the neuroepithelial cells.

Fig. 9 - Dien - Intensely increased , diffused coloration was noted from the neuroepithelial cells.

Fig. 10 - Telen- Intensely increased , diffused coloration was noted from the neuroepithelial cells.

Fig. 11-15- 0.5 mM H_2O_2 + 3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen - Weak blue coloration was observed at the cellular zones.

Fig. 12 - Meten. - Weak blue coloration was observed at the cellular zones..

Fig. 13 - Myelen - Weak blue coloration was observed at the cellular zones..

Fig. 14 - Dien - Weak blue coloration was observed at the cellular zones.

Fig. 15 - Telen. - Weak blue coloration was observed at the cellular zones.

PLATE I

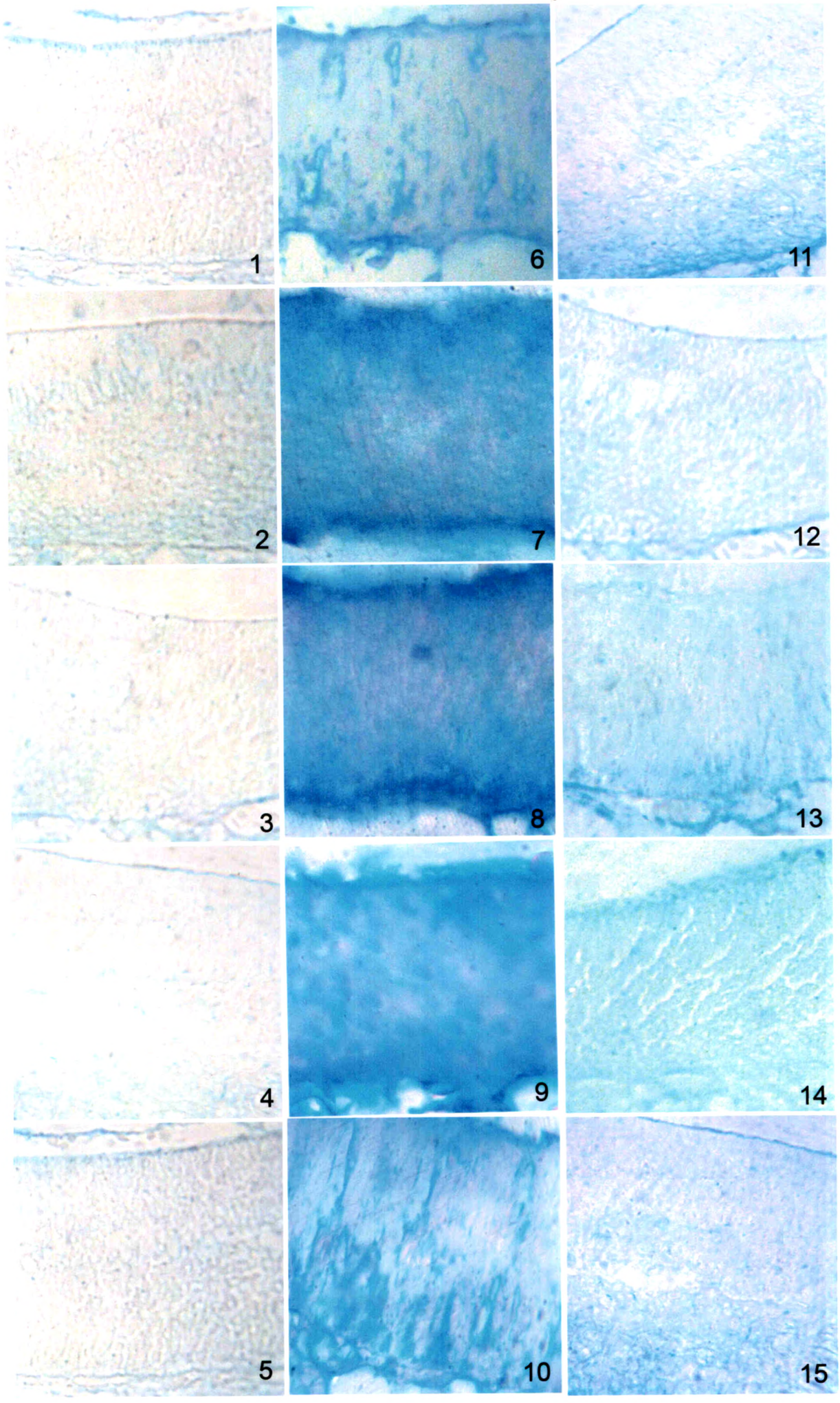


PLATE II

PLATE II- Initial incubation (24 hrs) + Dose exposure incubation (96 hrs) = Final development (120 hrs).

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen .- Cell surfaces was with blue coloration

Fig. 2 - Meten. - Cell compactly arranged without intercellular spaces, basophilic nuclei. Cell surfaces blue colored

Fig. 3 - Myelen - Cells compactly arranged without intercellular spaces intensely tained basophilic nuclei. cell surfaces blue colored

Fig. 4 - Dien - Compactly arranged cells without intercellular spaces. cell surfaces blue

Fig. 5 - Telen. - Compactly arranged cell without intercellular spaces. cell surfaces blue

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000.

Fig. 6 - Mesen. – Cytoplasm stained blue intercellular spaces was without coloration

Fig. 7 - Meten. - Cytoplasm stained with the blue intercellular spaces was not observed.

Fig. 8 - Myelen - Cytoplasm stained with the blue intercellular spaces was not observed.

Fig. 9 - Dien - Cytoplasm stained with the blue intercellular spaces was not observed.

Fig. 10 - Telen- Cytoplasm stained with the blue intercellular spaces was not observed.

Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen – Weakly stained cell surfaces, cytoplasm and intercellular space not stained with Alcian blue.

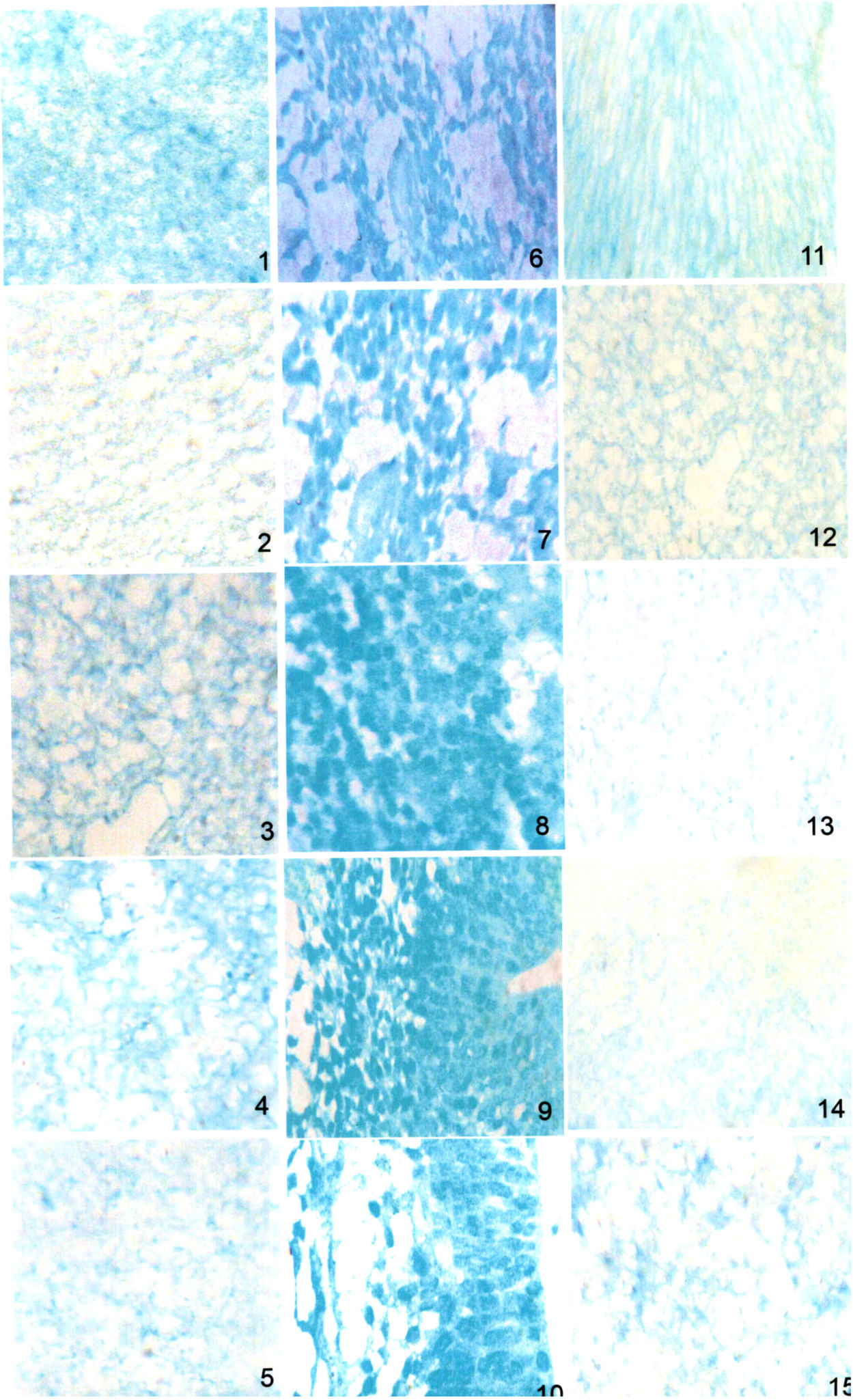
Fig. 12 - Meten. - Weakly stained cell surfaces, cytoplasm and intercellular space not stained with Alcian blue.

Fig. 13 - Myelen - Weakly stained cell surfaces, cytoplasm and intercellular space not stained with Alcian blue..

Fig. 14 - Dien - Weakly stained cell surfaces, cytoplasm and intercellular space not stained with Alcian blue.

Fig. 15 - Telen. - Weakly stained cell surfaces, cytoplasm and intercellular space not stained with Alcian blue.

PLATE II



112 hrs development-

Moderate amount of SA was observed at cell surfaces and marginal zone.Trace amount in cytoplasm and intercellular space. O-acyl form was observed with moderate amount in the cytoplasm and cell surfaces, trace in amount at the intercellular space.

120 hrs development-

Trace content of SA was in the cytoplasm and intercellular space, moderate at cytoplasm and marginal layer. O-acyl SA (C_7, C_8 or C_9) was in moderate concentration at cytoplasm and cell surface, trace amount was noted at the intercellular space.

130 hrs development-

SA was observed in trace amount in the cytoplasm and intercellular space, moderate amount on the cell surfaces and marginal zone. O-acyl SA (C_7, C_8 or C_9) was observed with moderate amount in the cytoplasm and cell surfaces, trace amount was noted at the intercellular space.

136 hrs development-

Weak content of SA was observed in the cytoplasm, moderate at the cell surface. Trace amount was noted at the intercellular space of the five brain regions.

144 hrs development-

Cytoplasm and cell surface showed moderate amount of SA. Intercellular spaces showed trace amount of SA. O-acyl SA (C_7, C_8 or C_9) was observed in trace amount at the intercellular space and moderate amount was noted in the cell surface and cytoplasm.

Control: HBSS-

Content of SA and O-acyl SA (C_7, C_8 or C_9) was observed in same amount as observed in the normal development of the embryos at various developmental hours

Control: 3 mg Vitamin C-

Treatment of 3 mg vitamin C showed similar amount of SA and O-acyl SA (C_7, C_8 or C_9) as observed in the normal and HBSS control embryonic hours.

0.5 mM H_2O_2 treated

Initiation at 24 hrs of development

i) Initial incubation (24 hrs) + Dose exposure incubation (24 hrs)= Final development (48 hrs) –

Intense/ significant amount of SA was increased in the at all the cellular structures of the five brain regions. O-acyl SA (C_7, C_8 or C_9) was increased significantly in the cytoplasm of the cells and cell surfaces. Trace amount at the intercellular spaces.

ii) Initial incubation (24 hrs) + Dose exposure incubation (48 hrs) = Final development (72 hrs) –

Intense or significant amount of SA was increased at the cell surface. Weak content was observed in the cytoplasm and intercellular spaces of the five brain regions. O-acyl SA (C_7, C_8 or C_9) was increased in significant amount in the cytoplasm, moderately increased at the cell surface and the intercellular space same results are observed for the marginal layer of the five brain regions.

iii) Initial incubation (24 hrs) + Dose exposure incubation (72 hrs) = Final developmental (96 hrs) –

Content of SA was decreased from the significant to the moderate as compared to early exposure of this group, but it was moderately increased than the normal amount of SA in all considered cellular structures. O-acyl SA (C_7, C_8 or C_9) was moderately as compared to the normal hrs of same group.

Initial incubation (24 hrs) + Dose exposure incubation (96 hrs) = Final development (120 hrs) –

SA was observed with moderately increased at cellular structures of the five brain regions. O-acyl SA (C_7, C_8 or C_9) was observed with moderately increased amount at the cytoplasm, weak content at the cell surface and intercellular space along with marginal layer.

Initiation at 34 hrs of development

v) Initial incubation (34 hrs) + (24 hrs) dose exposure incubation hrs = Final development (58 hrs) –

Significantly increased at all the cellular structures studied at five brain regions. O-glycosylated SA was increased significantly in the cytoplasm of the cell, and moderately increased amount at the cell surface, intercellular space and marginal layer.

PLATE III

PLATE I- Initial incubation (48 hrs) + Dose exposure incubation (24 hrs) = Final development (72 hrs).

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen .- Neuroepithelial layer present. Compactly arranged cells without cell surfaces, intensely stained basophilic nuclei. Blue color faint at cell surfaces

Fig. 2 - Meten. - Cell compactly arranged without intercellular spaces, basophilic nuclei.

Fig. 3 - Myelen - Cells compactly arranged without intercellular spaces intensely stained basophilic nuclei.

Fig. 4 - Dien - Compactly arranged cells without intercellular spaces.

Fig. 5 - Telen. - Compactly arranged cell without intercellular spaces.

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000. increased blue colored

Fig. 6 - Mesen. - Cells compactly arranged with little intercellular spaces. Foggy cells towards the luminal side.

Fig. 7 - Meten. - Cells compactly arranged, foggy cell towards the luminal side.

Fig. 8 - Myelen - Cells compactly arranged, foggy cells towards the luminal side

Fig. 9 - Dien - Cells compactly arranged without intercellular spaces, foggy cell towards the luminal side.

Fig. 10 - Telen- Cells compactly arranged with foggy appearance.

Fig. 11-15- 0.5 mM H_2O_2+3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen - Cells compactly arranged without intensely stained nuclei.

Fig. 12 - Meten. - Cells compactly arranged not well marked cell boundaries.

Fig. 13 - Myelen - Cells compactly arranged without cell boundaries.

Fig. 14 - Dien - Cells compactly arranged without well stained cells.

Fig. 15 - Telen. - Cells compactly arranged without intercellular spaces. No well marked cell boundaries.

PLATE III

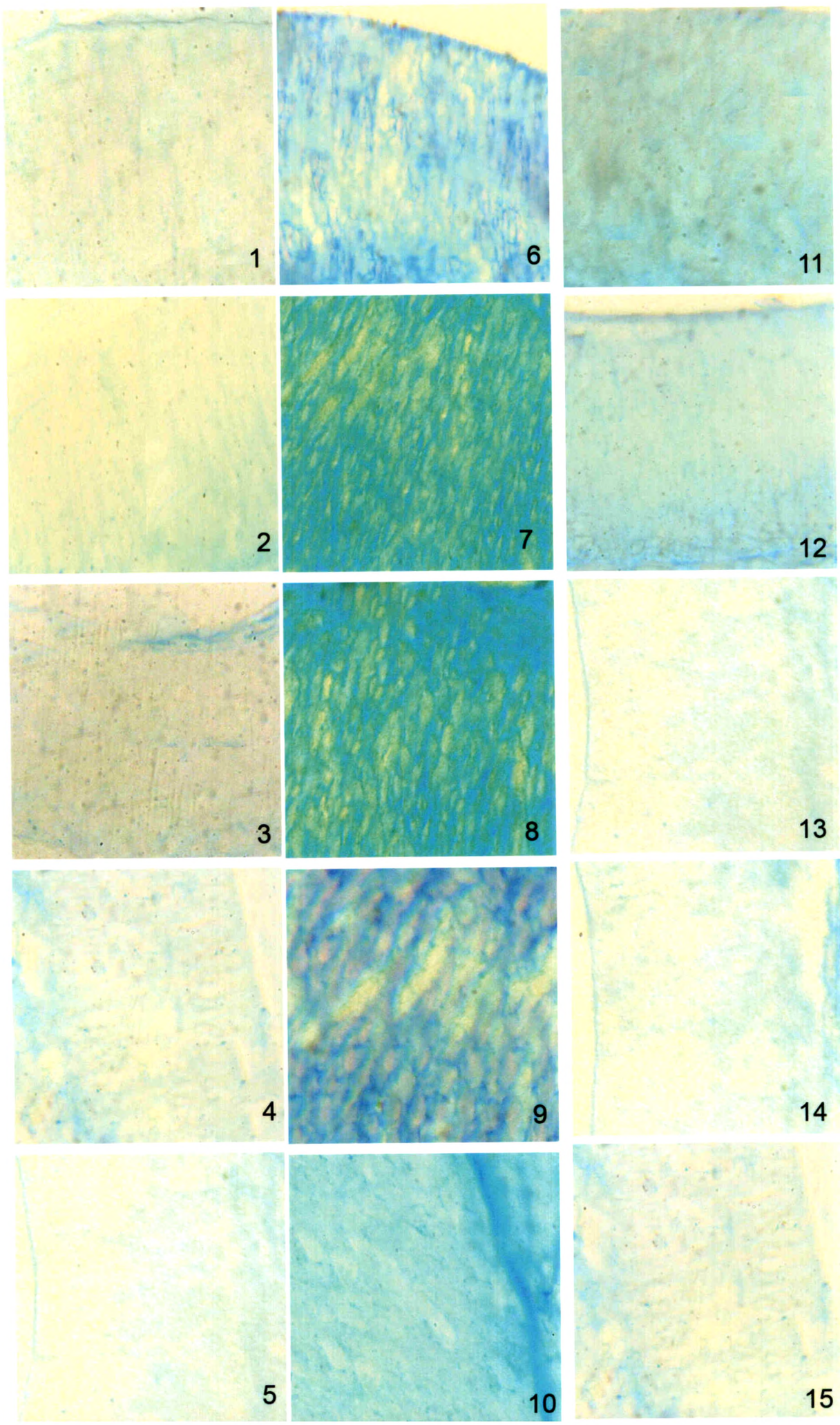


PLATE IV

PLATE I- Initial incubation (72 hrs) + Dose exposure incubation (48 hrs) = Final development (120 hrs).

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen .- Neuroepithelial layer present. Compactly arranged cells without cell surfaces, intensely stained basophilic nuclei. Faint blue coloration

Fig. 2 - Meten. - Cell compactly arranged without intercellular spaces, ~~basophilic nuclei.~~ Faint Blue coloration

Fig. 3 - Myelen - Cells compactly arranged without intercellular spaces intensely stained ~~basophilic nuclei.~~ cell surface with blue coloration

Fig. 4 - Dien - Compactly arranged cells without intercellular spaces. Cell surfaces with blue coloration

Fig. 5 - Telen. - Compactly arranged cell without intercellular spaces. cell surfaces with blue coloration

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000.

Fig. 6 - Mesen. - Cells compactly arranged with little intercellular spaces. Foggy cells towards the luminal side. Intense blue coloration

Fig. 7 - Meten. - Cells compactly arranged, foggy cell towards the luminal side. Intense color

Fig. 8 - Myelen - Cells compactly arranged, foggy cells towards the luminal side. Intense blue color

Fig. 9 - Dien - Cells compactly arranged without intercellular spaces, foggy cell towards the luminal side. Intense blue coloration.

Fig. 10 - Telen- Cells compactly arranged with foggy appearance. Intense blue coloration

Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen - Cells compactly arranged without intensely stained ~~nuclei~~. cell surface

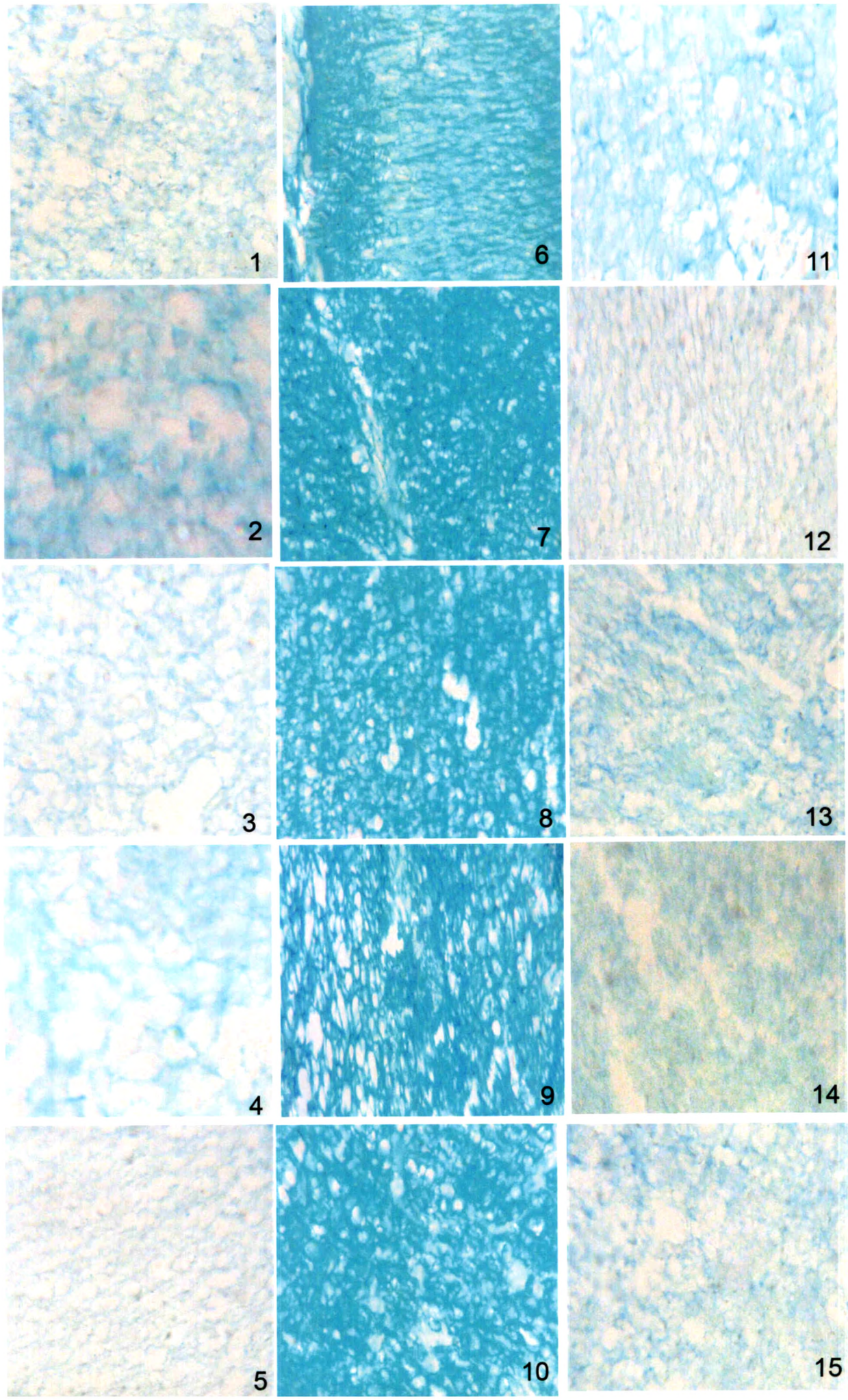
Fig. 12 - Meten. - Cells compactly arranged not well marked cell boundaries. cell surface faint blue

Fig. 13 - Myelen - Cells compactly arranged without cell boundaries. cell surface faint blue

Fig. 14 - Dien - Cells compactly arranged without well stained cells. cell surface faint blue

Fig. 15 - Telen. - Cells compactly arranged without intercellular spaces. No well marked cell boundaries. cell surface faint blue

PLATE IV



vi) Initial incubation (34 hrs) + Dose exposure incubation (48 hrs) = Final development (82 hrs) –

Significantly increased amount of SA was noted visually at all the cellular structures considered in five brain regions. O-acyl SA (C_7, C_8 or C_9) was increased significantly in the cytoplasm while moderately at the cell surface, intercellular space and the marginal zone of the five brain regions.

vii) Initial incubation (34 hrs) + Dose exposure incubation (72 hrs) = Final development (106 hrs) –

Moderate increase of SA was observed in the cytoplasm, cell surface and intercellular space of the ependyma, mantle and marginal zone of the five brain regions.

viii) Initial incubation hrs + Dose exposure incubation hrs = Final development hrs (34+ 96 = 130) –

Moderately increased SA was noted all cellular structures. O-acyl SA (C_7, C_8 or C_9) was increased moderately increased amount in all cellular structures. O-acyl SA (C_7, C_8 or C_9) was noted in moderate content at the all-cellular layers of the five brain regions.

Initiation at 40 hrs of development

ix) Initial incubation (40 hrs) + Dose exposure incubation (24 hrs) = Final development (64 hrs) –

Significantly increased amount of SA was observed in all cellular structures. O-acyl SA (C_7, C_8 or C_9) showed significant increase in the cytoplasm, weak at the cell surface, intercellular space and the marginal layer.

x) Initial incubation (40 hrs) + Dose exposure incubation (48 hrs) = Final development (88 hrs) –

Significantly increase of SA was noted in all cellular structures. O-acyl SA (C_7, C_8 or C_9) was increased significantly in the cytoplasm, weakly at the cell surface, intercellular space and the marginal layer of the brain.

xi) Initial incubation (40 hrs) + Dose exposure incubation (72 hrs) = Final development (112 hrs) –

Significant increase of SA at all cellular structures. O-acyl SA (C_7, C_8 or C_9) was increased significant in the cytoplasm, while weak amount of O-acyl SA (C_7, C_8 or C_9) was noted at the cell surface, intercellular space and the marginal layer of the five brain regions.

xii) Initial incubation (40 hrs) + Dose exposure incubation (96 hrs) = Final development (136 hrs) –

Moderate amount of SA was noted all cellular structures. O-acyl SA (C_7, C_8 or C_9) was increased moderately in the cytoplasm and weak in amount at the cell surface, intercellular space and marginal zone.

Initiation at 48 hrs of development

xiii) Initial incubation (48 hrs) + Dose exposure incubation (24 hrs) = Final development (72 hrs)-

Intensely or significantly amount of SA was observed at all the cellular structure studied. O-acyl SA (C_7, C_8 or C_9) significantly increased in amount in the cytoplasm, and weak in amount at the cell surface, intercellular space and marginal zone of the five brain vesicles.

xiv) Initial incubation (48 hrs) + Dose exposure incubation (48 hrs) = Final developmental (96 hrs) –

Significantly, increased amount of SA was noted in all brain regions, O-acyl SA (C_7, C_8 or C_9) was increased significantly in the cytoplasm and not in the other cellular structures.

xv) Initial incubation (48 hrs) + Dose exposure incubation (72 hrs) = Final developmental (120 hrs) –

Moderate increased amount of SA was noted visually at all cellular structures. O-acyl SA (C_7, C_8 or C_9) was increased was noted significantly at the cytoplasm only.

Initiation at 72 hrs of development

xvi) Initial incubation (72 hrs) + Dose exposure incubation (24 hrs) = Final development (96 hrs) –

Significantly increase in the amount of SA was noted at all the cellular structures studied. O-acyl SA (C_7, C_8 or C_9) was increased significantly at the cytoplasm and weakly at the cell surface, intercellular space and marginal layer of the five brain vesicles.

xvii) Initial incubation (72 hrs) + Dose exposure incubation (48 hrs) = Final development (120 hrs) –

Moderate amount of SA was noted in cytoplasm, at cell surface, intercellular space and the marginal zone.

PLATE V

PLATE I- Initial incubation (72 hrs) + Dose exposure incubation (24 hrs) = Final development (96 hrs).

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen .- Neuroepithelial layer present. Compactly arranged cells without cell surfaces, intensely stained basophilic nuclei.

Fig. 2 - Meten. - Cell compactly arranged without intercellular spaces, basophilic nuclei.

Fig. 3 - Myelen - Cells compactly arranged without intercellular spaces intensely stained basophilic nuclei.

Fig. 4 - Dien - Compactly arranged cells without intercellular spaces.

Fig. 5 - Telen. - Compactly arranged cell without intercellular spaces.

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000. Intense blue coloration

Fig. 6 - Mesen. - Cells compactly arranged with little intercellular spaces. Foggy cells towards the luminal side.

Fig. 7 - Meten. - Cells compactly arranged, foggy cell towards the luminal side.

Fig. 8 - Myelen - Cells compactly arranged, foggy cells towards the luminal side

Fig. 9 - Dien - Cells compactly arranged without intercellular spaces, foggy cell towards the luminal side.

Fig. 10 - Telen- Cells compactly arranged with foggy appearance.

Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen - Cells compactly arranged without intensely stained nuclei.

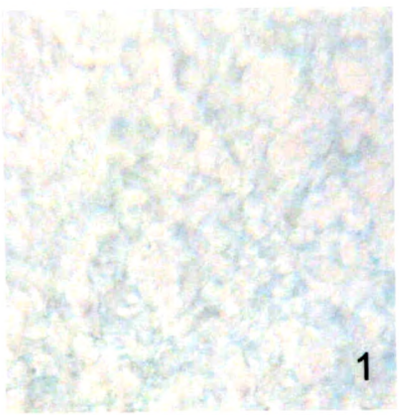
Fig. 12 - Meten. - Cells compactly arranged not well marked cell boundaries.

Fig. 13 - Myelen - Cells compactly arranged without cell boundaries.

Fig. 14 - Dien - Cells compactly arranged without well stained cells.

Fig. 15 - Telen. - Cells compactly arranged without intercellular spaces. No well marked cell boundaries.

PLATE V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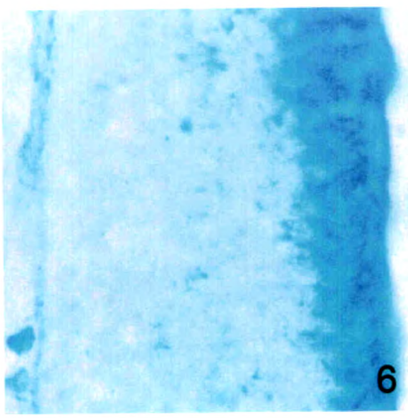
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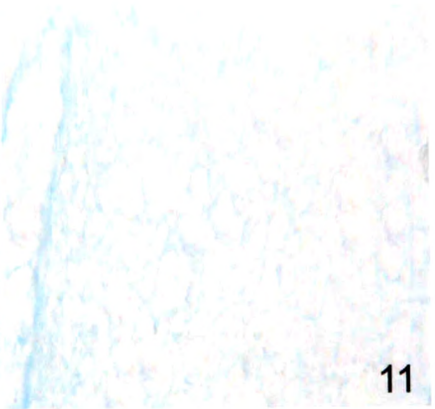
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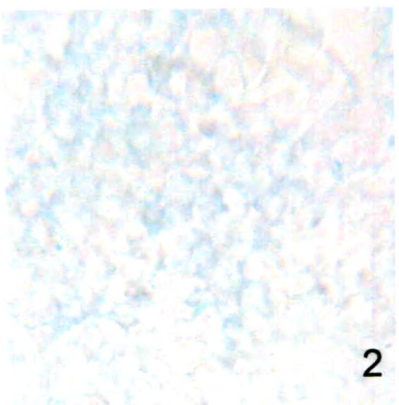
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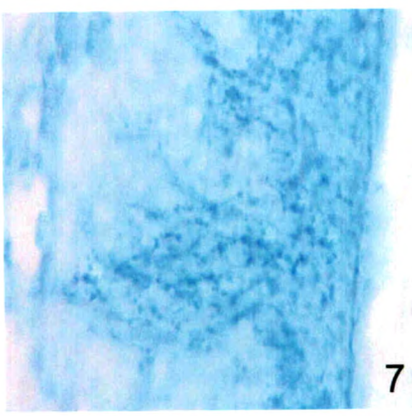
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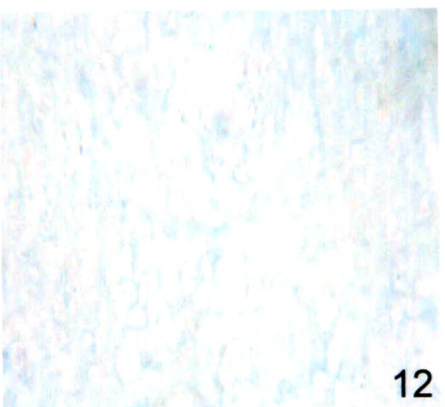
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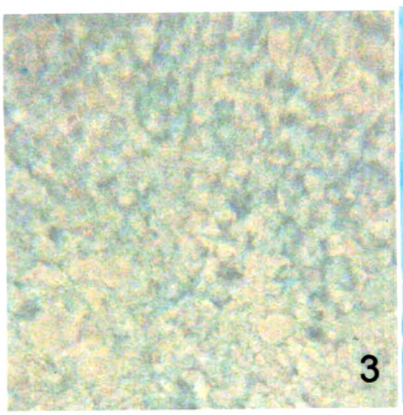
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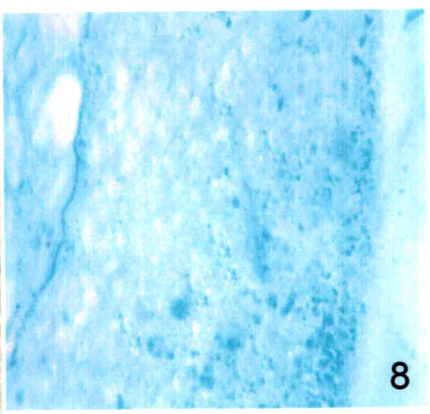
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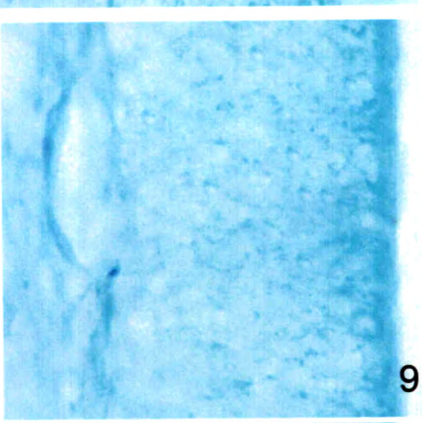
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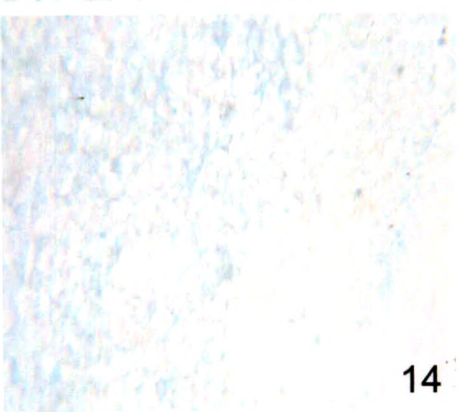
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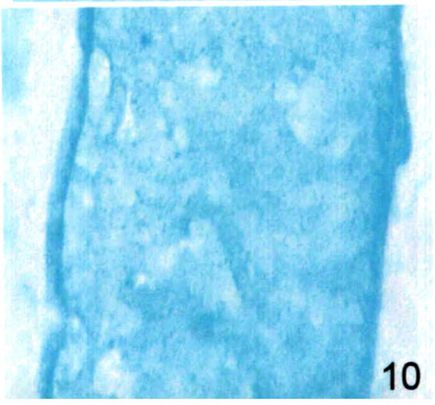
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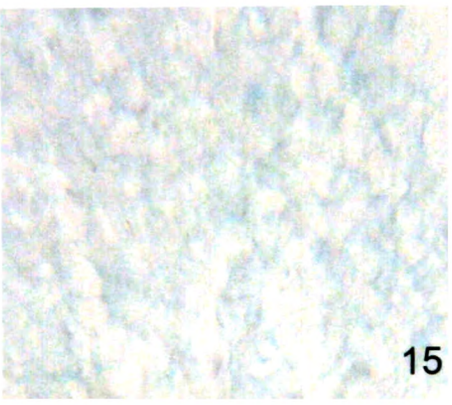
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Initiation at 96 hrs of development

xviii) Initial incubation (96 hrs) + Dose exposure incubation (24 hrs) = Final development (120 hrs) –

Significant increase of SA was noted at the cell surface and in cytoplasm. Weak increased amount was noted in the cytoplasm. O-acyl SA (C_7, C_8 or C_9) was increased significantly in the cytoplasm and weak amount was noted at the cell surface, intercellular space and the marginal zone.

Initiation at 120 hrs of development

xix) Initial incubation (120 hrs) + Dose exposure incubation (24 hrs) = Final development (144 hrs) –

Moderate increase of SA was noted at all the cellular structure. O-acyl SA (C_7, C_8 or C_9) was noted with moderate increase in the cytoplasm and weak at the cell surface, intercellular space and marginal layer.

0.5 mM H_2O_2 + 3 mg vitamin C treated

Initiation at 24 hrs of development

i) Initial incubation (24 hrs) + Dose exposure incubation (24 hrs) = Final development (48 hrs) –

SA content was moderate as compared to normal, and decreased moderately as compared to 0.5 mM H_2O_2 the cell surfaces. Weak amount of SA was noted in the cell surfaces. O-acyl SA (C_7, C_8 or C_9) was moderate in the cytoplasm and at the cell surfaces, weak amount was noted and intercellular spaces.

ii) Initial incubation (24 hrs) + Dose exposure incubation (48 hrs) = Final development (72 hrs) –

Trace amount of SA was noted at the cytoplasm and weak content at the cell surfaces. While trace content was noted at the intercellular spaces of the neuronal cells. O-acyl SA (C_7, C_8 or C_9) content was observed in weak amount in the cytoplasm and trace amount was noted at the cell surfaces and intercellular spaces.

iii) Initial incubation (24 hrs) + Dose exposure incubation (72 hrs) = Final development (96 hrs)-

SA was noted in trace amount in the cytoplasm and weak content on the cell surface. Trace amount at the intercellular spaces of the neuronal cells. O-acyl SA (C_7, C_8 or C_9) content was observed in weak amount in the cytoplasm and at the cell surfaces, trace amount was noted at intercellular spaces.

iv) Initial incubation (24 hrs) + Dose exposure incubation (96 hrs) = Final development (120 hrs) –

The results obtained for the amount of SA and O-acyl SA (C_7, C_8 or C_9) were similar to that was observed in the normal 120 hrs of development where weak amount of SA was present in the cytoplasm, intercellular spaces and while the trace amount were noted in the cytoplasm. O-acyl SA (C_7, C_8 or C_9) was weak in the content in the cytoplasm and trace amount was observed at other cellular site.

Initiation at 34 hrs of development

v) Initial incubation (34 hrs) + Dose exposure incubation (24 hrs) = Final development (58 hrs) –

Moderate SA was noted at the cell surfaces. Trace amount at the intercellular spaces while O-acyl SA (C_7, C_8 or C_9) was moderate in the cytoplasm and cell surfaces.

vi) Initial incubation (34 hrs) + Dose exposure incubation (48 hrs) = Final development (82 hrs) –

SA results observed were similar to that was observed for the normal embryos of 82 hrs development. SA was present in weak amount at the cell surfaces, traces in cytoplasm and intercellular spaces. While O-acyl SA (C_7, C_8 or C_9) was weak in cytoplasm and traces in amount at other cellular structures.

viii) Initial incubation (34 hrs) + Dose exposure incubation (72 hrs) = Final development (106 hrs) –

Weak amount of SA was present in the cell surfaces and trace content were observed in cytoplasm and intercellular spaces. O-acyl SA (C_7, C_8 or C_9) was present in the weak amount in the cytoplasm, trace amount at the cell surfaces and intercellular spaces were observed.

vii) Initial incubation (34 hrs) + Dose exposure incubation (96 hrs) = Final development (130 hrs) –

SA trace amount was in cytoplasm and intercellular spaces, weak in content at cell surface, O-acyl SA (C_7, C_8 or C_9) was weak content in the cytoplasm and trace at the cell surfaces and intercellular spaces.

PLATE VI

PLATE I- Initial incubation (72 hrs) + Dose exposure incubation (48 hrs) = Final development (120 hrs).

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen .- Neuroepithelial layer present. Compactly arranged cells without cell surfaces, intensely stained basophilic nuclei.

Fig. 2 - Meten. - Cell compactly arranged without intercellular spaces, basophilic nuclei.

Fig. 3 - Myelen - Cells compactly arranged without intercellular spaces intensely stained basophilic nuclei.

Fig. 4 - Dien - Compactly arranged cells without intercellular spaces.

Fig. 5 - Telen. - Compactly arranged cell without intercellular spaces.

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000.

Fig. 6 - Mesen. - Cells compactly arranged with little intercellular spaces. Foggy cells towards the luminal side.

Fig. 7 - Meten. - Cells compactly arranged, foggy cell towards the luminal side.

Fig. 8 - Myelen - Cells compactly arranged, foggy cells towards the luminal side

Fig. 9 - Dien - Cells compactly arranged without intercellular spaces, foggy cell towards the luminal side.

Fig. 10 - Telen- Cells compactly arranged with foggy appearance.

Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen - Cells compactly arranged without intensely stained nuclei.

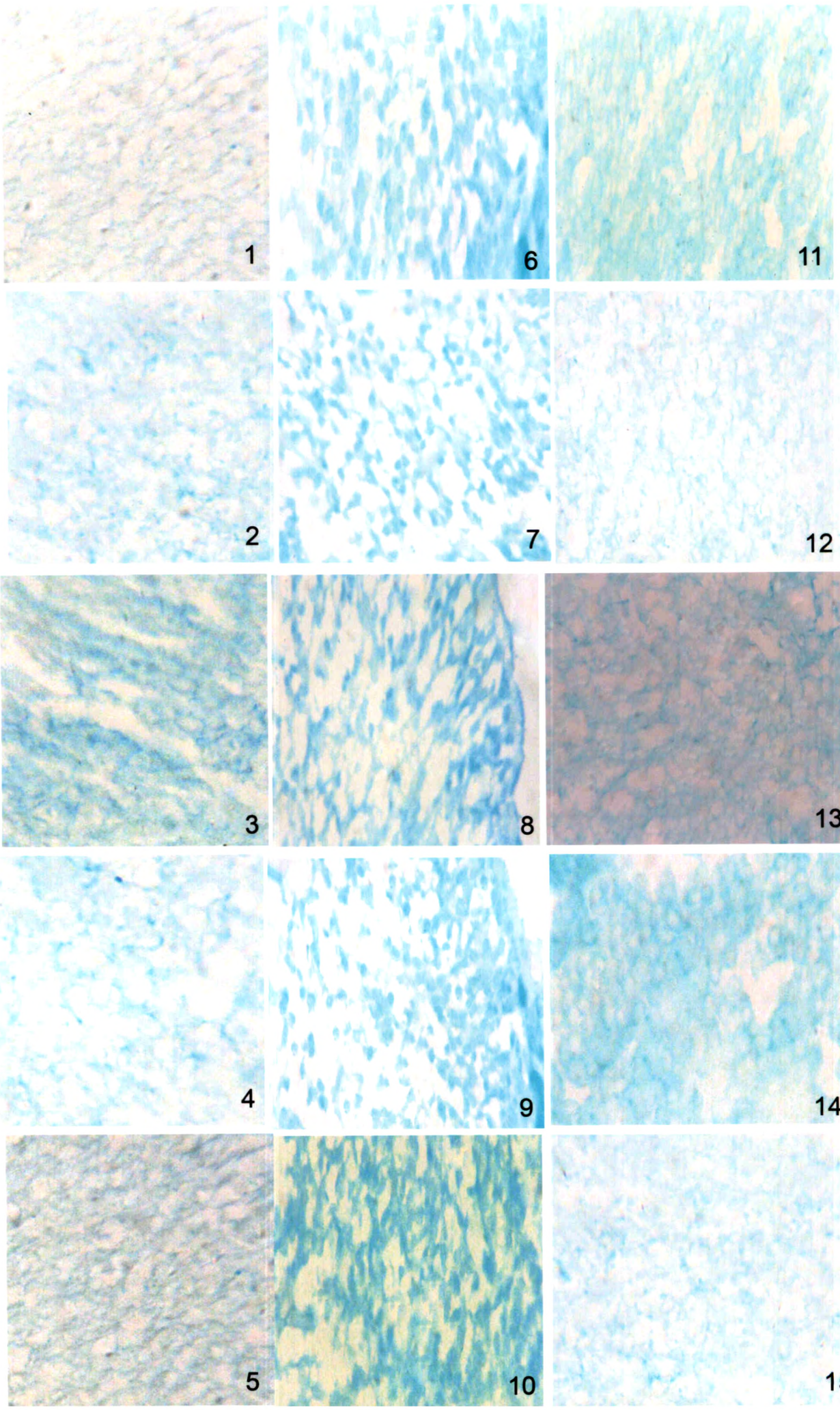
Fig. 12 - Meten. - Cells compactly arranged not well marked cell boundaries.

Fig. 13 - Myelen - Cells compactly arranged without cell boundaries.

Fig. 14 - Dien - Cells compactly arranged without well stained cells.

Fig. 15 - Telen. - Cells compactly arranged without intercellular spaces. No well marked cell boundaries.

PLATE VI



Initiation at 40 hrs of development

ix) Initial incubation (40 hrs) + Dose exposure incubation (24 hrs) = Final developmental (64 hrs) –

Moderate amount of SA at cell surfaces and in traces at intercellular spaces and in cytoplasm was observed along with O-acyl SA (C_7, C_8 or C_9) moderate amount in the cytoplasm.

x) Initial incubation (40 hrs) + Dose exposure incubation (48 hrs) = Final developmental (88 hrs) –

Weak amount of SA was at cell surfaces. Trace amount was observed in cytoplasm and at intercellular spaces of five brain regions. Trace content was in the cytoplasm. O-acyl SA (C_7, C_8 or C_9) amount was moderate in the cytoplasm and in traces at the cell surfaces and intercellular space.

xi) Initial incubation (40 hrs) + Dose exposure incubation (72 hrs) = Final development (112 hrs) –

Results obtained were similar to those observed in normal embryos at 112 hrs of development. Cytoplasm and intercellular spaces was with trace amount of SA while at cell surface weak amount of SA was found. O-acyl SA (C_7, C_8 or C_9) moderate content in the cytoplasm and cell surfaces.

xii) Initial incubation (40 hrs) + Dose exposure incubation (96 hrs) = Final development (136 hrs) –

Trace amount of SA in cytoplasm and intercellular spaces, while weak amount at cell surfaces. O-acyl SA (C_7, C_8 or C_9) was moderate in content in the cytoplasm and cell surfaces.

Initiation at 48 hrs of development

xiii) Initial incubation (48 hrs) + Dose exposure incubation (24 hrs = Final development (72 hrs) –

Moderate SA at cell surfaces.. In cytoplasm and at intercellular spaces trace content of SA was noted. O-acyl SA (C_7, C_8 or C_9) moderate content in the cytoplasm and trace at the cell surface, intercellular space and marginal zone.

xiv) Initial incubation (48 hrs) + Dose exposure incubation (48 hrs) = Final development (96 hrs) –

SA with weak amount at the cell surface and marginal zone, while O-acyl SA (C_7, C_8 or C_9) weak content in the cytoplasm and cell surfaces, trace in amount at the intercellular spaces of the five brain vesicles.

xv) Initial incubation (48 hrs) + Dose exposure incubation (96 hrs) = Final development (120 hrs) –

Weak content at the cell surface. Trace amount at the intercellular space and in the cytoplasm was noted visually for SA. O-acyl SA (C_7, C_8 or C_9) weak content in the cytoplasm, trace at cell surface, intercellular space and marginal zone.

Initiation at 72 hrs of development

xvi) Initial incubation (72 hrs) + Dose exposure incubation (24 hrs) = Final development (96 hrs) –

Moderate content of SA at the cell surface. Intercellular spaces, cytoplasm of cells was observed with trace amount of SA. O-acyl SA (C_7, C_8 or C_9) moderate content in the cytoplasm and trace at other cellular structures.

xvii) Initial incubation (72 hrs) + Dose exposure incubation (48 hrs) = Final development (120 hrs) –

Trace content SA was noted at the cytoplasm, weak content were noted at the cell surface and other cellular structures considered. O-acyl SA (C_7, C_8 or C_9) weak content in the cytoplasm and trace at the other cellular structures studied.

Initiation at 96 hrs of development

xviii) Initial incubation (96 hrs) + Dose exposure incubation (24 hrs) = Final development (120 hrs) –

Moderate amount at cell surface, intercellular space and marginal zone of SA was noted. O-acyl SA (C_7, C_8 or C_9) moderate content in the cytoplasm and weak at other cellular structures was noted.

Initiation at 120 hrs of development

xix) Initial incubation (120 hrs) + Dose exposure incubation (24 hrs) = Final development (144 hrs) –

Weak amount in cytoplasm, moderate amount at cell surface, intercellular space and marginal zone of SA was noted. O-acyl SA (C_7, C_8 or C_9) moderate content in the cytoplasm and weak at other cellular structures was noted

Discussion:

During the morphological development of brain vesicles followed by further growth, all the five vesicles retained the three-layered structure. In ependymal layer neuroepithelial cells are in proliferation and at the transition of mantle layer, the neurons are being migrated while marginal layer mainly concentrates neuroaxonal networking.

PLATE VII

PLATE I- Initial incubation (96 hrs) + Dose exposure incubation (24 hrs) = Final development (120 hrs).

Fig 1-5- Normal embryonic brain X1000.

Fig. 1 - Mesen .- Neuroepithelial layer present. Compactly arranged cells without cell surfaces, intensely stained basophilic nuclei.

Fig. 2 - Meten. - Cell compactly arranged without intercellular spaces, basophilic nuclei.

Fig. 3 - Myelen - Cells compactly arranged without intercellular spaces intensely stained basophilic nuclei.

Fig. 4 - Dien - Compactly arranged cells without intercellular spaces.

Fig. 5 - Telen. - Compactly arranged cell without intercellular spaces.

Fig. 6 -10- 0.5 mM H_2O_2 treated embryonic brain X1000. Intense blue coloration

Fig. 6 - Mesen. - Cells compactly arranged with little intercellular spaces. Foggy cells towards the luminal side.

Fig. 7 - Meten. - Cells compactly arranged, foggy cell towards the luminal side.

Fig. 8 - Myelen - Cells compactly arranged, foggy cells towards the luminal side

Fig. 9 - Dien - Cells compactly arranged without intercellular spaces, foggy cell towards the luminal side.

Fig. 10 - Telen- Cells compactly arranged with foggy appearance.

Fig. 11-15- 0.5 mM H_2O_2 +3 mg vitamin C treated embryonic brain X1000.

Fig. 11 - Mesen - Cells compactly arranged without intensely stained nuclei.

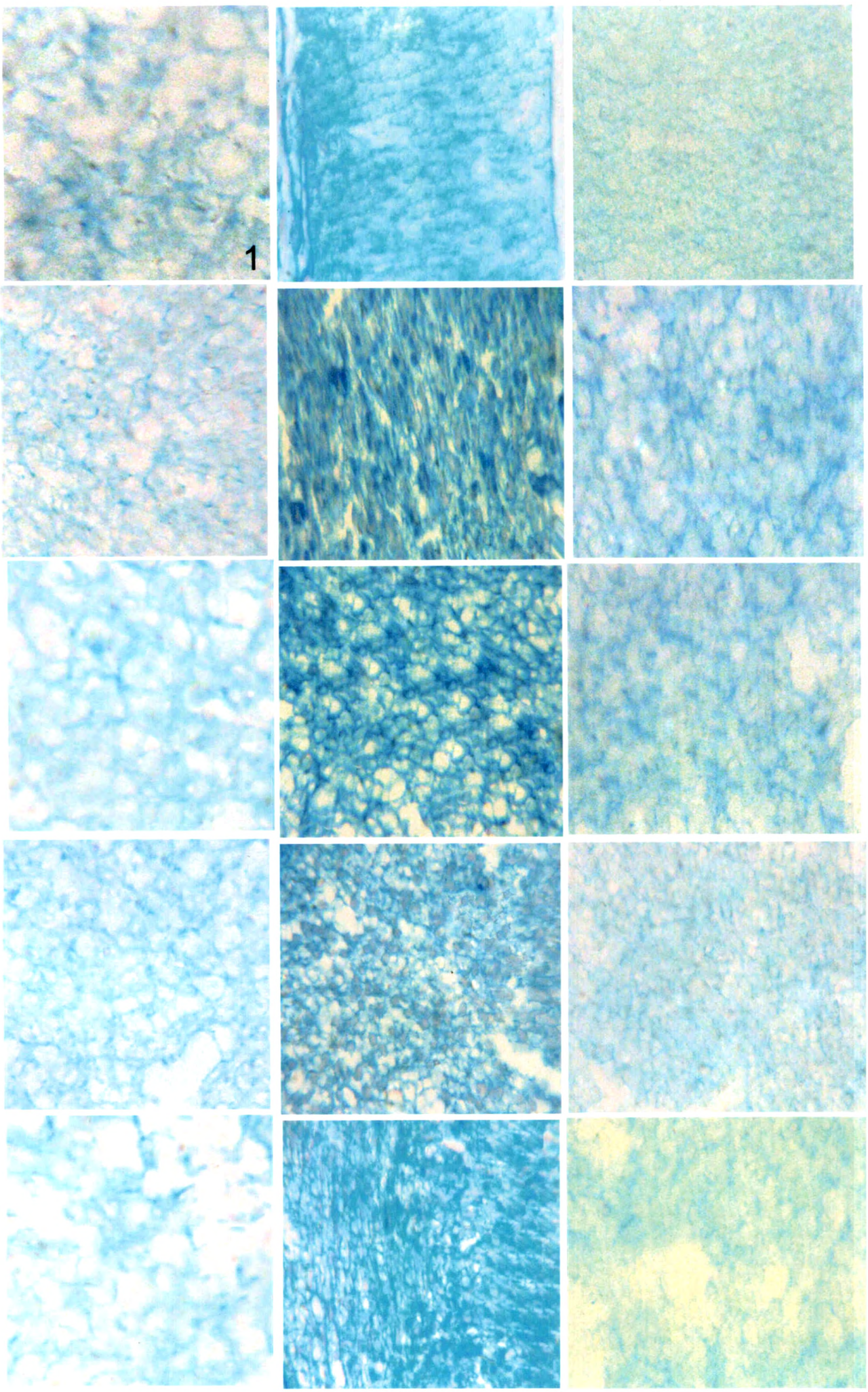
Fig. 12 - Meten. - Cells compactly arranged not well marked cell boundaries.

Fig. 13 - Myelen - Cells compactly arranged without cell boundaries.

Fig. 14 - Dien - Cells compactly arranged without well stained cells.

Fig. 15 - Telen. - Cells compactly arranged without intercellular spaces. No well marked cell boundaries.

PLATE VII



Distribution of SA is mainly in three sites neuronal cytoplasm at cell surfaces in growing number of neurons, axons and the dendrites and the intercellular spaces in all the three regions in which neurons and neuroaxonal network are extended.

SA distribution in brains of normal embryos of different developmental controls can be segregated into two categories as per the intensity of stain in various techniques used to detect the SA. After 48 hrs to 120 hrs SA was weakly distributed at neuronal cell surfaces and in cytoplasm and in intercellular spaces it was in traces. While in the other group i. e. in 120-144 hrs, when brain vesicular development completed the cell surface SA staining intensities of various techniques indicated its weak staining resulting in visibly increased SA at cell surfaces but it was above the marginal increase. This was also true in case of O-acyl SA. It was weakly stained in cytoplasm and intercellular spaces (48-96 hrs groups), and was weakly stained at cell surfaces, it was increased to get over marginal intensity of the staining.

But 0.5 mM H_2O_2 introduced to embryos significant increase was observed in SA distribution of both the types. The increase was prominently observed at cell surfaces and intercellular spaces where they were intensely observed. The results indicated that H_2O_2 induced free radical influence the quantity of SA but not the sites of SA. The cell surfaces and intercellular spaces were the sites where mainly intensity was increased. This was further increased at 120 to 144 hrs. With reference to the exposure time the increase in SA at cell surfaces and intercellular spaces with early hours exposure and that also of short terms, indicated more intense distributions but with late hours exposures and initiation, distributional intensity of SA was comparatively low. These distributions were normalized in quantity on simultaneous treatment of 3 mg vitamin C. Exposure initiation and exposure time together indicated smooth slow decrease at the cell surfaces and intercellular spaces indicating it's normalization. Therefore, that intensity of SA seems to be monitored through vitamin C.

From the present results, it seems that in normal embryo different zones of brain viz. ependymal, mantle and marginal layers of mesencephalon, metencephalon, myelencephalon, diencephalons and telencephalon SA with staining was observed mainly on cell surfaces. This was more evident in the mantle and marginal layer as the neuroaxonal networking increased where alcinophilia at pH 2.5 and pH 1 was evident. Though gangliosides contain SA and is distributed all over the membrane surfaces (Abraham, Rosenberg , 1991) its SA content appears to be quantitatively limited. But

the neuronal cell adhesion molecule (N-CAM) in there number are distributed on neuronal cell surfaces and also on extending axonal and dendral membrane surfaces (Cunningham et. al. 1991) as revealed from molecular topographic studies. Similarly the studies of SA on these molecules indicated α-2-8 linked polysialic acid containing glycan chains linked to core proteins of N-CAM (Finne 1992) which are partially sulfated also and polysialic acid seems to show distinctive changes during embryonic development especially studied from day 5-21 of development revealed through polysialyl tranferase activities in neuronal cells and the enzymatic involvement in N-CAM polysialytion (Inoue et. al 2000).

On the bases of above *in vitro* observations and chemical studies of these molecules, present observations of distribution of SA noted after various staining procedures used indicated that the histochemically demosratable SA is mainly present at surfaces and seems to be contributed by heavily polysialyted N-CAM membrane molecules which seems to increase with the migratory activities of neural cells and hence concentrated on the extending membranes of neuroaxonal networking regions, which are more anastomized in marginal layer of all the five regions of brain. Being sulfated the staining specificities are equally observed by alcinophilia at pH 1 and 2.5 and with moderate combined staining of AB 2.5+ PAS, AB 1 +PAS and AF +AB 2.5. Thus indicating PAS positive SA (O-acyl SA C7, C8 or C9) and sulfated forms of polysialic acid and free SA which was stained only by alcian blue pH 2.5 in both of these combined techniques and also the other techniques used for confirmation of presence of SA.

H_2O_2 treatment had increased SA content at all the sites especially at cell surfaces where maximum staining were reported. Cytosol also showed presence of SA. The intercellular spaces showed the staining but it seems that the staining may be due to extended chains of glycans that are associated with N-CAM surface molecules N-terminus of which anchors SA containing glycan chains (Galuska, 2008) and are known to play role in cell adhesion and migratory activities of neural cells (Chuong *et. al.* 1997; Thiery *et. al.* 1985).

Increase in SA on H_2O_2 treatment may be due to increased stress on brain of OH^- and O^- radicals generated from H_2O_2. SA had shown to have antioxidant activity against H_2O_2 by direct chemical action which is capable to be acting at various pH to produce modified SA residue (Coden, 2004; Iijima, 2004 and 2007; Takahashi *et. al.* 2007; Ogasawara *et. al.* 2007;).

Thus, increase in SA seems to be against the free radicals generated. Since vitamin C when was given simultaneous with H_2O_2 there is normalization of SA content at cell surfaces occurred where they appeared intensely associated with cytoplasmic moderate concentration (for biosynthetic transfer) in only H_2O_2 treated embryos. Since in simultaneous studies from this group of workers (Toraskar, 2008) it has been shown that glutathione metabolism was also significantly influenced, vitamin C seem to play antioxidant/free radical scavenger role partially, directly and also through influencing the cellular free radical scavenger like glutathione. Since in present experiment all the embryos were protected and normal on hatching, the H_2O_2 influenced cytotoxic effects seems to have been monitored by 3 mg vitamin C dose through controlling the SA synthesis stress of neuronal cells.