CHAPTER - III

OBSERVATIONS AND RESULTS :

OBSERVATIONS AND RESULTS

The total protein concentration analysed with Lowry's method is given in table -1. The concentration is highest in the control and it went on decreasing after treatment with UN, which also dependent on the duration of treatment. The normal rat plasma contains a complex mixture of proteins.

The analytical procedures and methods of separation are based on the physical properties of rat plasma. Ultracentrifugation and electrophoresis have emerged recently as the analytical method. These methods have contributed a system of standard nomenclature far all protein solutions.

Presently SDS-Polyacrylamide gel electrophoresis technique has been followed. This technique separates the plasma proteins into a minimum of 29 fractions (Plate-1). The fastest identified band is of Thyroglobulin and it is followed by albumin and the slowest moving band was that of α_2-macroglobulin (reduced). α-macroglobulin splits into α_1 and α_2 macroglobulins, the reduced form of α_2 macroglobulin of molecular weight 190,000 separates at the beginning.

These fractions were classified into three groups according to their concentrations -

Table-1

Alteration in total plasma protein content.

Group	Experimental animal	Total protein content
I	Control	3400mg/100ml of plasma
II	UN 24	2900mg/100ml of plasma
III	UN 48	2700mg/100ml of plasma
IV	UN 72	1900mg/100ml of plasma

UN 24 - Uranyl nitrate treatment for 24 hours.

UN 48 - Uranyl nitrate treatment for 48 hours.

UN 72 - Uranyl nitrate treatment for 72 hours.

CAPTIONS

PLATE - 1

O - Plasma protein profile of control rats

UN24 - Plasma protein profile after 24 h. of UN treatment

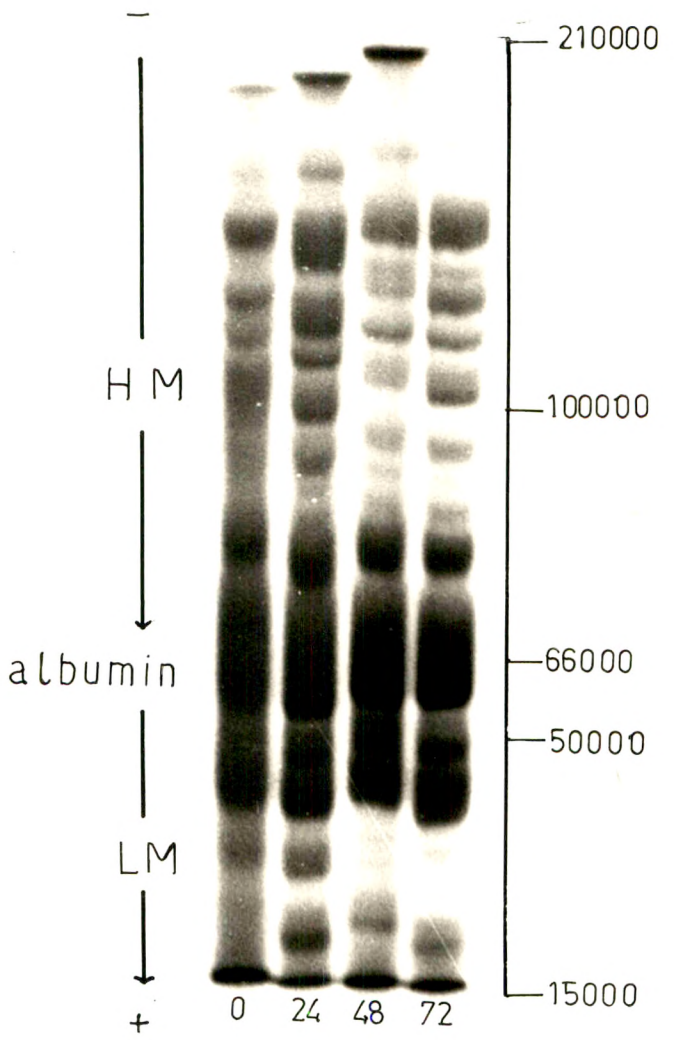
UN48 - Plasma protein profile after 48 h. of UN treatment

UN72 - Plasma protein profile after 72h. of UN treatment

HM - Heavy molecular weight plasma proteins.

LM - Low molecular weight plasma proteins.

PLATE — 1



SDS—PAGE of plasma proteins

I) MAJOR COMPONENTS :

Having concentration more than 150 mg/100ml were LM_1 (190.08), LM_6 (155.52), LM_8 (174.52), LM_9 (171.64), LM_{11} (229.24) and LM_{12}(181.2). All component were of low molecular weight. The components included in this group have low molecular weight.

II) INTERMEDIATE COMPONENTS :

Having a concentration between 50mg/100ml to 150mg/100ml contains the majority of the plasma proteins were LM_2(93.31), LM_3(58.75), LM_4(77.18), LM_5(93.6), LM_7(98.49), LM_{10}(107.13), HM_1(74.30), HM_2(61.05), HM_3(82.94), HM_5 (50.68), HM_6 (66.24), HM_9 (67.96), HM_{10}(72.57), HM_{11}(88.12), HM_{12}(72.30) and HM_{13}(130.75). This group contain proteins of low as well as high molecular weight.

III) THE MINOR COMPONENTS :

Having a concentration below 50mg/100ml were HM_4(38.01), HM_7(28.8), HM_8(47.80), HM_{14}(10.94), HM_{15}(28.80), HM_{16}(4.60) and HM_{17}(8.64). The components included in this group are having very high molecular weight.

Table -2 illustrates the relative mobilities and estimated molecular weights of the plasma proteins of low molecular weight

Table-2

Relative mobilities and estimated molecular weights of low molecular weight (LM) of plasma proteins.

Observations	Protein	Mobilities	Molecular weights (Daltons)
1	LM_1	1.00	15000
2	LM_2	0.95	16000
3	LM_3	0.87	21000
4	LM_4	0.78	27000
5	LM_5 (Thyroglobulin)	0.72	32000
6	LM_6	0.66	37000
7	LM_7	0.59	42000
8	LM_8	0.58	46000
9	LM_9	0.54	52000
10	LM_{10}	0.52	55000
11	LM_{11}	0.47	63000
12	LM_{12} (Albumin)	0.44	66000

LM - Low molecular weight proteins.

Table_3

Relative mobilities and estimated molecular weights of high molecular weight (HM) of Plasma proteins.

Observations	Proteins	Mobilities	Molecular Weights (Daltons)
1	HM_1 (Transferrin)	0.41	73000
2	HM_2 (Plasminogen)	0.38	80000
3	HM_3	0.37	84000
4	HM_4	0.35	88000
5	HM_5	0.33	92000
6	HM_6	0.32	94000
7	HM_7	0.29	103000
8	HM_8	0.25	115000
9	HM_9	0.23	120000
10	HM_{10}	0.18	135000
11	HM_{11}	0.16	145000
12	HM_{12} (IgG)	0.14	150000
13	HM_{13} (IgA)	0.12	160000
14	HM_{14}	0.10	170000
15	HM_{15}	0.06	135000
16	HM_{16} (α_2-macroglobulin red)	0.03	190000
17	HM_{17}	0.01	210000

HM = High Molecular Weight Proteins.

(LM). The molecular weight ranges from 15000 to 66000 D. i.e. from LM_1 to LM_{12}. The molecular weights were determined by using mobilities of each protein fraction.

Table-3 shows the relative mobilities and estimated molecular weight of high molecular weight (HM) proteins.

The above two tables, (Table-2 and Table-3) show some important plasma proteins such as Thyroglobulin (LM_5-32000), Albumin (LM_{12}-66000). Transferrin (HM_1-73000), ceruloplasmin (HM_9-120,000), I_9G (HM_{12}-150,000), I_gA(HM_{13}-160000) and finally a_2-macroglobulin (reduced) (HM_{16}-190000). The remaining plasma protein components/fractions are not confirmed yet. For their confirmation further research is needed.

The plasma albumin (LM_{12}-66000) - It possess no enzymatic or any hormonal activities. The research on plasma albumin has nonetheless, brought out new complexities in the behaviour of globular proteins which without question have contributed much to an ultimate understanding of the less prosaic proteins.

An attempt has been made to sumerize the more important features of the behaviour of plasma albumin as reflected in the new voluminous literature on this protein.

It has been suggested that on isomerization reaction, the N-F transformation, plays a central role in many of the properties which this protein displays. Among them the low pH titration anomaly, co-operative detergent binding, heat and urea denaturation behaviour, and the altered solubility properties in acid solution. While the scheme proposed appears to be internally consistant, it can not be regarded as established and certainly it leaves many unanswered questions.

Two stricking properties stand out upon examination of the literature on the proteins : (a) The unusual affinity for ions, particularly anions in which regard plasma albumin is almost in a class by it self, and (b) The remarkable degree to which the molecule is capable of undergoing extensive conformational alterations apparently in fully reversible manner. That these two properties are inter-related and seem unquestionably true as was first pointed out by Karush (1950). He has attributed the powerful binding affinity to "configuration adaptability". It is difficult to escape the conclusion that these properties are the key to the physiological role of plasma albumin. Thus, it is readily visualized that albumin may serve to reduce the concentration of a variety of low molecular weight ionic substances and even nonionic materials by combining with them and thus buffering their activity. Secondly albumin doubtless serve a transport function, carrying both undersirable and desirable ions to the site of elimination or need.

Other than the buffer and transport functions, and the obvious fact that albumin provids one of the major mobile reserve store of amino acids, there is one further established physiological functions of albumin, namely it's role in the regulation of plasma volume and tissue fluid balance. Scatchard et al. (1944) have found experimentally that 1.0 g albumin yields on osmotic pressure equivalent to 1.2 g of total plasma protein under physiological conditions. In other words, the albumin component is responsible for approximately 75% of the total colloidal osmotic pressure of the plasma. The high efficiency of albumin in this regard is attributed in part to the fact that, its molecular weight is appreciably lower than the average value for the plasma protein (66000 to 70000 D), but more important is the relatively high average net charge of albumin under physiological conditions. This higher than average net charge, is due in part to the low isoelectric point of albumin, and in part to its powerful binding affinity for the anions. From these results Scatchard et al. (1944) calculated that 1 gm of albumin would increase the plasma volume by 18 ml at 25 mmHg pressure, in stricking agreement with the observed value of 17 ml.

The plasma transferrin (HM_1 73000) - The variations of the soluble, protein bound iron in plasma, the "Serum iron", in health and in diseases has been studied for fifty years and the significance of this small iron fraction in the intermediary iron metabolism was recognized long before. It was established that

this iron circulated with the plasma firmly chelated to a specific metal combining B-globulin, usually is named transferrin (Tr) or the Siderophilin. The highest plasma iron value obtained after iron tolerence test in the healthy subjects were about 300 µg/100 ml. Similar values were noted when a tolerable dose of simple iron salt was injected intravenously. On further intravenous injection of iron this limit had been reached without any further increase in the plasma iron level (Waldenstrom, 1944). Crystalline transferrin has been prepared from plasma in the free state (Ksechlin, 1952) or as an iron complex (Laurell, 1953 and Schultz et al., 1957). The later is easier to work with because the molecule is stabilized by the bound iron. Various investigators have utilized different, more or less conventional types of fractionation steps and have succeeded obtaining the final products satisfying the conventional criteria for purity.

The most complete report on physicochemical properties of transferrin is given by Shultz et al. (1957). Its solubility resembles that of albumin and its molecular weight as per SDS-PAGE is about 73000 to 76000 D. The composition of amino acid has been charted by Schultz et al. (1957). The only N-terminal amino acid found was valin. Sugar represent 3.9 % of the molecule with approximately an even proportion of hexose, hexose-amine and acetyl neuraminic acid (Schultz, 1958). On treatment of Tr with neuraminidase, it's electrophoretic mobility decreases considerably. Every transferrin molecule can firmly bind to two atoms of ferric

ions (Laurel and Ingelmn, 1947) or loosely bind to cupric, copper or zinc. Iron can readily replace copper from the Cu-transferrin complex (Holmberg and Laurel, 1947). The iron in the complex is trivalent and bound with essentially ionic bonds (Enrenberg and Laurel, 1955). One bi-carbonate ion per metal iron is bound in farming the complex (Fiala, 1949; and Shade et al., 1949). The specific configuration of the molecule at the binding site has not been cleared up.

Ceruloplasmin (HM_9-120000) - As early as 1927, the Abderhalden and Moller (1928) showed that the copper in serum is non-dialyzable. On acidification of the serum the copper is released (Warburg, 1927). A comprehensive study of the variations of serum-copper in physiological and pathological conditions in man has been presented in 1941 by Heilmeyer et al. in 1948 Holmberg and Laurel showed that more than 90% of the serum copper is firmly bound to an α_2-globulin - Ceruloplasmin - with oxidase properties. The oxidase activity of plasma varies with serum copper level indicating the variation, previously observed in serum copper, is dependent on the variations of the ceruloplasmin content of the plasma (Holmberg and Laurel, 1951; Hornykiewtez and Niebauer, 1953). Ceruloplasmin has been isolated from human plasma and pig plasma (Holmberg and Laurel, 1948). The main preparative step includes ammonium sulphate precipitation, fractionation with ethanol at low ionic strength, denaturation of contaminating proteins by a mixture of chloroform methanol at O°C,

as used earlier by Mann and Keilin (1938) for the isolation of other copper proteins. The ceruloplasmin is precipitated quantitatively at -5°C by 50% aceton at pH 5.7 or below (Bjorling, 1958), even if the solution contains several percent ammonium per sulphate or other simple salts, it can be eluted from precipitate by 38% acetone. Crystalization of ceruloplasmin can be affected by ammonium sulfate as well as by ethanol by low ionic strength.

Despite the regular variation in the ceruloplasmin level in various diseases, available data provide no clue what so ever to it's biological functions. It's capacity to oxidise naturally occurring substances such as ascorbic acid, adrenalin, nor-adrenalin and serotenin is low . It is still vary uncertain whether these substances are normally oxidised at all ceruloplasmin in vivo(Weil-Malherbe and Bone, 1958). The oxidation products hitherto obtained in vitro (Porter et al., 1957) differ considerably from the known main metabolites of these three biogenic amines in vivo.

The biological function of ceruloplasmin need not, however, be linked it to its potential activity as an oxidase. In view of the above mensioned affinity of the ceruloplasmin for the different ions, it might serve as a carrier of some charged substances.

Immunoglobulins (IgG-HM_{12}-150000 and IgA-HM_{13}-160000). One of the puzzling problems in immunology has been how to explain the Switch in biosynthesis from one class of immunoglobulin

to another having the same antigen binding specificity. Historically the switch was first observed and studied after immunization. The first injection of immunogen evokes the primary response in which IgM constitute the predominant class of antibodies detectable by conventional methods. Subsequent injections induced the secondary response characterized by a large increase in antibody of the same specificity but predominantly of the IgG class.

α_2-macroglobulin (reduced) (HM_{16}-190,000)

The considerable evidences have been accumulated that the pathological macroglobulins as well as the normal γ-macroglobulin are immunologically related to ordinary 7S γ-globulin. This relationship has the properties of a cross reaction indicating some antigenic grouping in common. Franklin and Kunkel (1957) demonstrated this relationship for the normal macroglobulin. It was most evident when quantitative precipitin curves were utilized, and was brought out readily when antisera were made against pure 7S material, isolated in the ultracentrifuge from fraction IInd γ-globulin. Such antisera reacted strongly with normal as well as pathological macroglobulin, and all of this reactivity would be removed by absorption with the pure 7S fraction. Evidence for this cross reaction was not obtained by Burtin et al. (1957) by the immuno-electrophoresis but, since their preparation of macroglobulin contain 7S γ-globulin which diffuse rapidly, all cross reacting antibodies were absorbed by the 7S material leaving only specific

antibodies to react with the macroglobulin and thus giving the impression of complete specificity.

Table-4 illustrate the percent concentration of low molecular weight protein after Uranyl nitrate treatment. The protein band from LM_1 to LM_{12} compaired with control along with UN treated 24h. UN 48 h and UN 72 h. Similarly Table-5 exhibit the percent concentration of high molecular weight proteins after Uranyl nitrate treatment. In this table the high molecular weight protein from HM_1 to HM_{17} are compaired that of control and UN treated at 24 h. 48 h and 72 h.

Table -6, illustrates the quantitative alteration in low molecular weight proteins of 24 h, Uranyl nitrate treated rats. The table exhibits comparison of low molecular weight proteins in control and UN treated rats after 24 h. along with percent difference. Where as Table -7 shows the quantitative alterations in high molecular weight proteins of 24 h. UN treated rats. The table ilustrate the comparison of between control rats and UN treated rats after 24 h. along with percent difference. Fig.1 shows the densitometer scans of gels of plasma proteins. (I) control rat and (II) UN treated rats for 24 h.

Table - 8 exhibits the quantitative alteration in low molecular weight of protein after 48 hr. UN treated rats. The Table exhibits the protein comparison between control and UN

Table – 4

Percent concentration of Low Molecular Weight Protein after Uranyl Nitrate Treatment.

Protein	Control mg/100ml	UN24 mg/100ml	UN48 mg/100ml	UN72 mg/100ml
LM_1	190.08±3.924	124.416±6.216	158.4±3.791	96.768±5.012
LM_2	93.312±4.50	39.418±5.75	39.744±3.452	-
LM_3	58.752±5.921	-	-	-
LM_4	77.184±4.703	152.64±5.189	101.954±5.913	105.408±3.478
LM_5	93.6±6.92	32.256±4.215	29.376±5.332	27.768±4.752
LM_6	155.52±3.821	66.24±3.975	39.744±6.710	19.584±5.315
LM_7	98.492±3.494	89.976±3.125	54.72±4.375	32.256±3.928
LM_8	174.528±4.312	205.632±4.513	217.728±3.973	177.984±4.932
LM_9	171.648±5.521	113.472±4.358	55.296±3.755	134.208±6.935
LM_{10}	107.138±4.171	106.56±5.972	136.512±5.126	64.088±4.352
LM_{11}	229.248±5.93	232.704±3.715	359.424±5.931	305.28±5.931
LM_{12}	181.2±4.32	197.568±3.793	139.968±4.319	93.312±5.133

UN24 = Uranyl nitrate treatment for 24 hours.

UN48 = Uranyl nitrate treatment for 48 hours.

UN72 = Uranyl nitrate treatment for 72 hours.

LM = Low molecular weight proteins.

Table-5

Percent Concentration of High Molecular Weight Proteins after Uranyl nitrate treatment.

Protein	Control mg/100ml	UN24 mg/100ml	UN48 mg/100ml	UN72 mg/100ml
HM_1	74.304±3.133	24.192±3.478	12.096±3.921	45.504±5.277
HM_2	61.056±5.321	59.04±4.257	40.32±5.449	33.984±4.315
HM_3	82.944±6.69	45.504±5.738	24.192±6.331	11.54±3.931
HM_4	38.016±6.251	83.52±6.137	65.664±4.002	194.92±5.703
HM_5	50.688±5.57	161.28±6.993	-	13.248±5.100
HM_6	66.24±5.112	150.336±4.552	13.824±4.321	55.296±3.903
HM_7	28.8±5.32	185.472±4.313	78.912±3.733	91.008±4.762
HM_8	47.808±3.16	74.304±4.77	127.872±4.913	-
HM_9	67.968±4.162	48.96±5.891	114.048±5.132	39.168±3.698
HM_{10}	72.576±3.937	214.272±5.335	33.984±3.329	134.784±5.702
HM_{11}	88.128±4.185	43.776±6.697	-	171.072±6.391
HM_{12}	74.30±5.003	14.976±3.798	-	48.656±3.051
HM_{13}	130.752±5.932	66.816±3.119	187.776±3.113	211.968±4.712
HM_{14}	10.944±5.75	9.216±4.813	54.144±4.173	72.576±3.863
HM_{15}	28.800±4.32	13.824±4.710	12.672±5.318	29.952±4.701
HM_{16}	4.608±4.151	16.704±5.155	-	10.368±5.337
HM_{17}	8.64±6.717	26.496±6.225	-	17.28±6.512

HM = High Molecular Weight Proteins.

UN24 = Uranyl nitrate treatment for 24 hours.

UN48 = Uranyl nitrate treatment for 48 hours.

UN72 = Uranyl nitrate treatment for 72 hours.

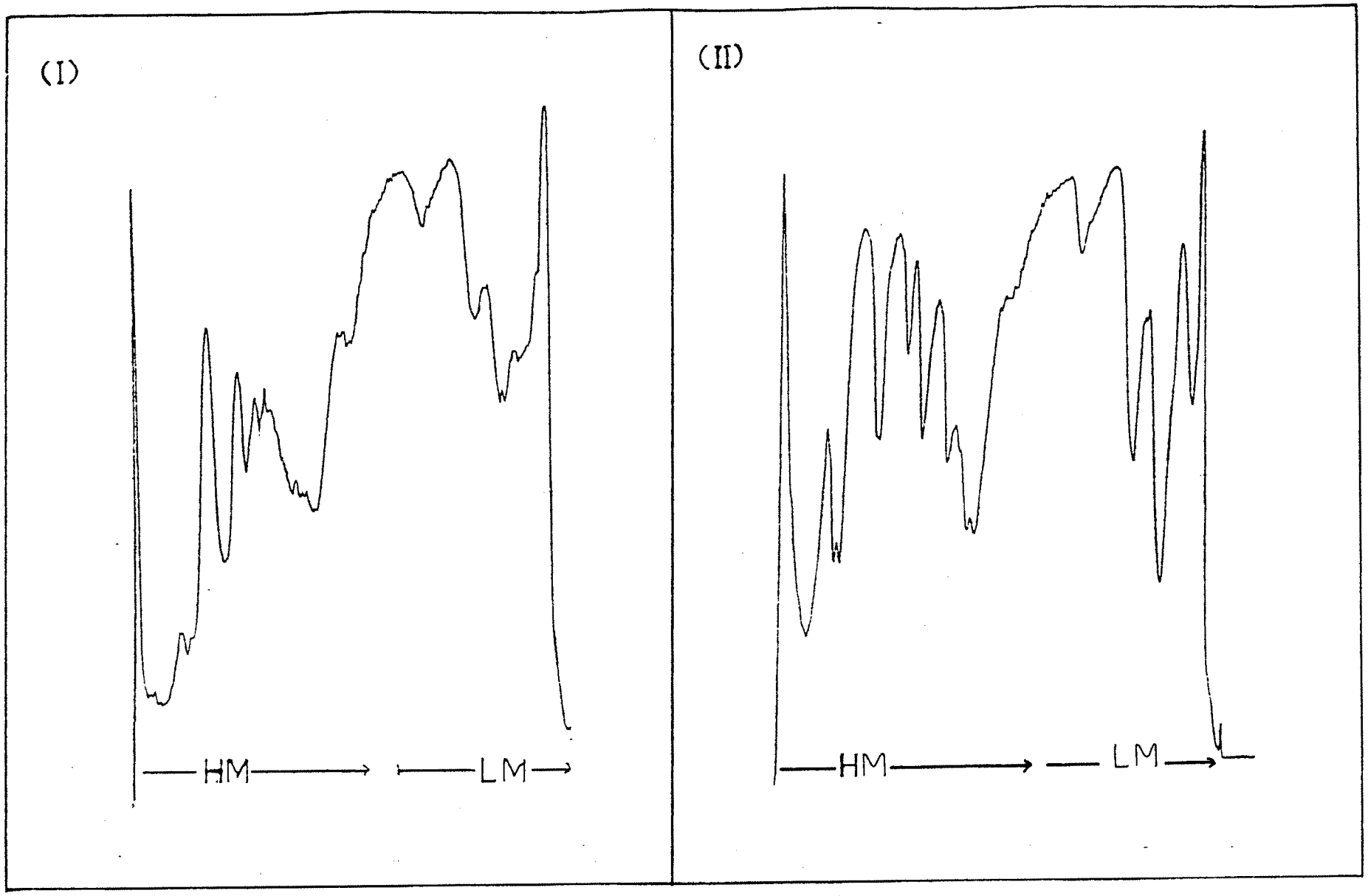


Figure 1 – Densitometer scans of gels of plasma proteins : (I) control and (II) UN24

Table 6

Quantitative alterations in low molecular weight proteins of 24 h. Uranyl nitrate treated rats.

Observations	Proteins	Control mg/100ml	Un24 mg/100ml	% Difference mg/100ml
1	LM_1	190.06	124.42	-34.55
2	LM_2	93.31	39.42	-57.76
3	LM_3	53.75	-	-
4	LM_4	77.18	152.64	+97.76
5	LM_5 (Thyroglobulin)	93.60	32.26	-65.54
6	LM_6	155.52	66.24	-57.41
7	LM_7	98.49	89.98	-8.65
8	LM_8	174.53	205.63	+17.82
9	LM_9	171.65	113.47	-33.89
10	LM_{10}	107.14	106.56	-05.54
11	LM_{11}	229.25	232.70	+01.51
12	LM_{12} (Albumin)	181.20	197.57	+09.03

-ve percent difference = Decrease in % concentration compaired to control.

+ve percent difference = Increase in % concentration compaired to control.

UN24 = Uranyl nitrate treatment for 24 hours.

LM = Low molecular weight plasma proteins.

Table 7

Quantitative alterations in high molecular weight protein of 24 h. Uranyl nitrate treated rats.

Proteins	Control mg/100ml	UN24 mg/100ml	% Difference mg/100ml
HM_1	74.30	24.19	-67.44
HM_2	61.06	59.04	-3.30
HM_3	82.94	45.50	-45.14
HM_4	38.02	83.52	+119.70
HM_5	50.69	161.28	+218.18
HM_6	66.24	150.34	+126.96
HM_7	28.80	185.47	+544.00
HM_8	47.81	74.30	+55.42
HM_9	67.97	48.96	-27.97
HM_{10}	72.58	214.27	+195.24
HM_{11}	88.13	43.78	-50.33
HM_{12}	74.30	14.98	-79.84
HM_{13}	130.75	66.82	-48.90
HM_{14}	10.94	9.22	-15.79
HM_{15}	28.80	13.82	-52.00
HM_{16}	4.61	16.70	+262.50
HM_{17}	8.64	26.50	+206.67

negative (-) percent difference = Decrease in % concentration than control.

+ve % difference = Increase in % concentration than control.

HM = High molecular weight plasma proteins.

UN 24 = Uranyl nitrate treatment for 24 hours.

treated rats with percent variations. Similarly Table -9 shows the quantitative alterations in high molecular weight protein after 48 hours UN treated rats. This table compair and contrast between the control rats and UN treated rats after 48 h. Table also exhibit percent variations after comparing the UN treated rat with control. Fig. 2 shows densitometer scans of gels of plasma proteins (I) control and (II) UN treated rats for 48 h.

Table - 10 illustrate the quantitative alterations in low molecular weight proteins after 72 h. UN treated rats. The table exhibits comparison of protein between control and UN treated rats along with percent variations. Similarly Table-11 shows quantitative alterations in high molecular weight protein after 72 h. UN treated rats. The table compaire and contrast of high molecular weight protein fraction between control and UN treated rats. The table also express the percent variations among them. Fig.3 shows densitometer scans of gels of plasma proteins (I) control rat and (II) UN treated rats for 72 h.

Table_12 illustrate the patterns of low molecular weight protein changes after uranyl nitrate treatment.

Protein fractions with various molecular weight exhibits interesting changes after UN treatment as compared with control. Some protein fractions are decreased in UN treatment after 24 h, but enhance after 48 h, and once again deplited after 72 h. UN

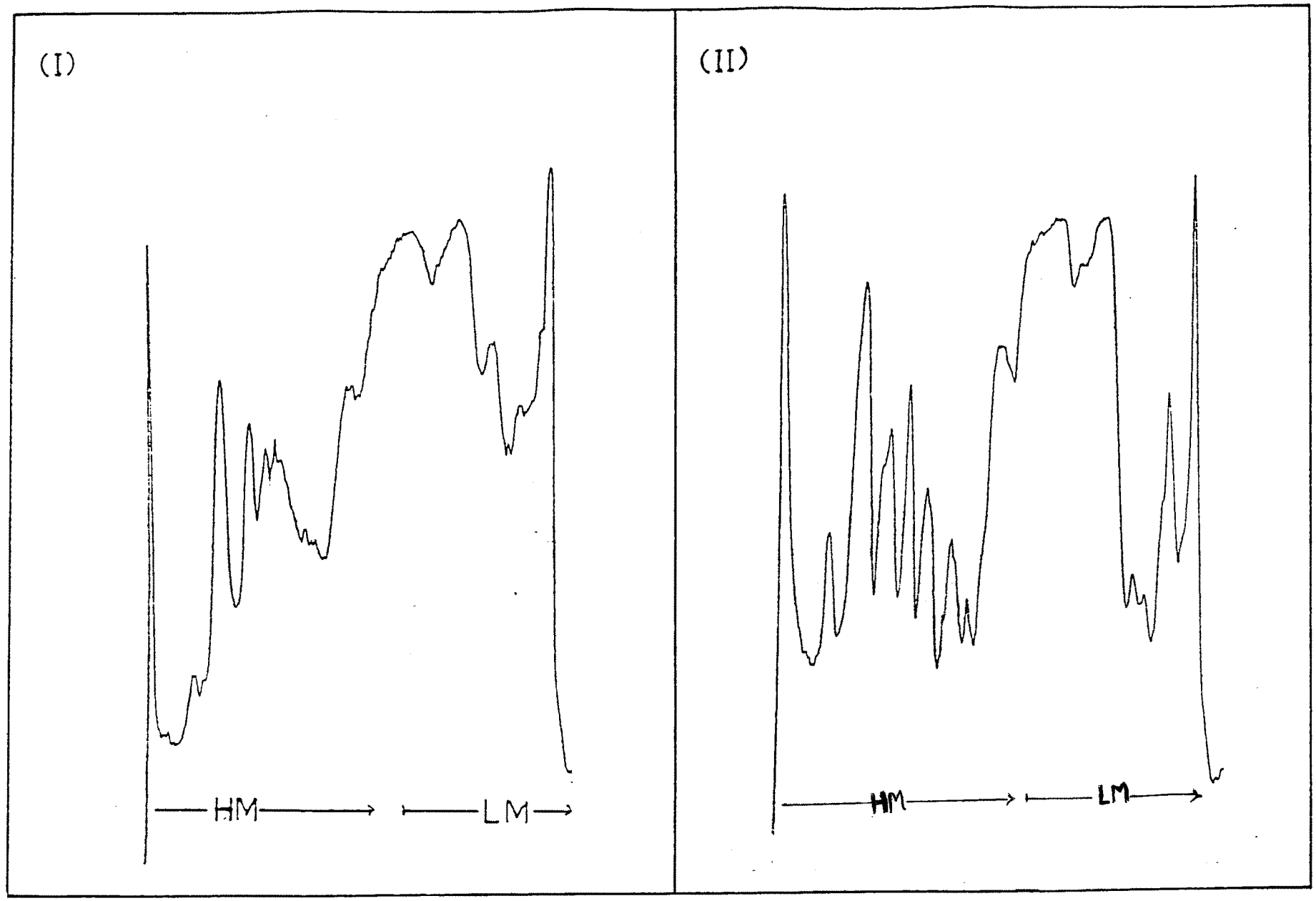


Figure 2 – Densitometer scans of gels of plasma proteins (I) control and (II) UN 48

Table-8

Quantitative alterations in low molecular weight proteins of 48h. Uranyl nitrate treated rats.

Proteins	Control mg/100ml	UN 48 mg/100ml	% Difference mg/100ml
LM_1	190.08	158.42	-16.66
LM_2	93.31	39.74	-57.41
LM_3	58.75	-	-
LM_4	77.18	101.95	+32.09
LM_5	93.60	29.38	-68.62
LM_6	155.52	39.74	-74.44
LM_7	98.49	54.72	-44.44
LM_8	174.53	217.73	+24.75
LM_9	171.65	55.30	-67.79
LM_{10}	107.14	136.51	+27.42
LM_{11}	229.25	359.42	+56.78
LM_{12}	181.20	139.97	-22.75

UN48 = Uranyl nitrate treatment for 48 hours.

-ve % difference = Decrease in % concentration than control.

+ve % difference = Increase in % concentration than control.

LM = Low moelcular weight plasma proteins.

Table-9

Quantitative alterations in high molecular weight protein of 48 h. Uranyl nitrate treated rats.

Proteins	Control mg/100ml	UN 48 mg/100ml	% difference mg/100ml
HM_1	74.30	12.10	-83.72
HM_2	61.06	40.32	-33.96
HM_3	82.94	24.19	-70.83
HM_4	38.02	65.66	+72.73
HM_5	50.69	-	-
HM_6	66.24	13.82	-79.1
HM_7	28.80	78.91	+174.00
HM_8	47.81	127.87	+167.47
HM_9	67.97	114.05	+67.80
HM_{10}	72.58	33.98	-53.17
HM_{11}	88.13	-	-
HM_{12}	74.30	-	-
HM_{13}	130.75	187.78	+43.61
HM_{14}	10.94	54.14	+394.74
HM_{15}	28.80	12.67	-56.00
HM_{16}	4.61	-	-
HM_{17}	8.64	-	-

HM = High molecular weight plasma proteins

UN 48 = Uranyl nitrate treatment for 48 hours.

+ve % difference = Increase in % concentration than control

-ve % difference = Decrease in % concentration than control.

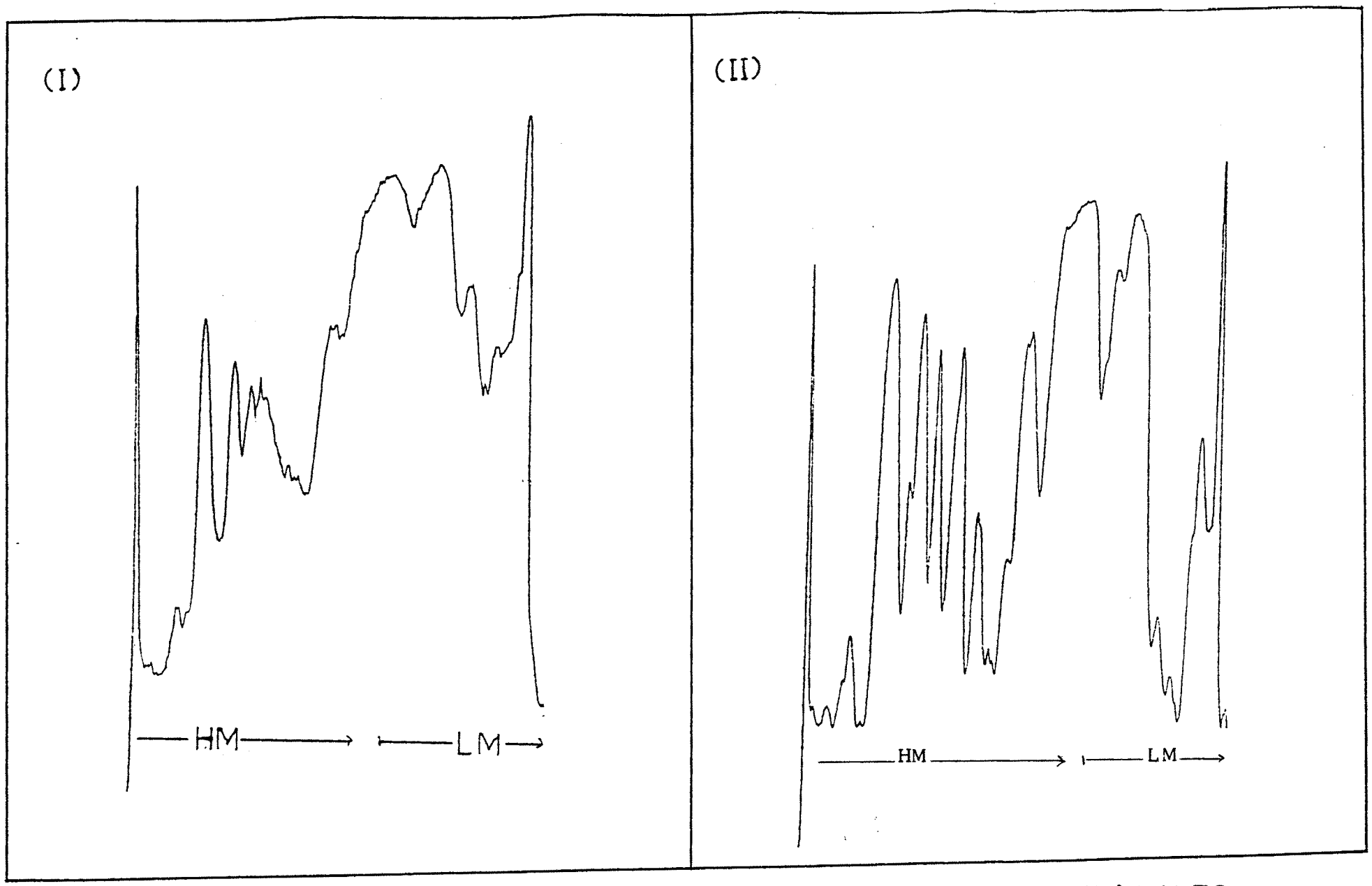


Figure 3 – Densitometer scans of gels of plasma proteins (I) control and (II) UN 72

Table_10

Quantitative alterations in low Molecular weight proteins after 72 Uranyl nitrate treated rats.

Protein	Control	Un 72	% difference
LM_1	190.08	96.77	-49.09
LM_2	93.31	-	-
LM_3	58.75	12.67	-78.43
LM_4	77.18	105.41	+36.57
LM_5	93.60	27.77	-70.33
LM_6	155.52	19.58	-87.41
LM_7	98.49	32.36	-67.25
LM_8	174.53	177.98	+1.98
LM_9	171.65	134.21	-21.81
LM_{10}	107.14	64.09	-40.18
LM_{11}	229.25	305.28	+33.17
LM_{12}	181.20	93.31	-48.50

LM = Low molecular weight plasma proteins.

UN 72 = Uranyl nitrate treatment for 72 hours.

-ve % difference = Decrease in % concentration than control.

+ve % difference = Increase in % concentration than control.

Table _ 11

Quantitative alterations in high molecular weight protein after 72h.

Uranyl nitrate treated rats.

Protein	Control	UN 72	% Difference
HM_1	74.30	45.50	-38.76
HM_2	61.06	33.98	-44.34
HM_3	82.94	11.54	-86.09
HM_4	38.02	194.72	+412.21
HM_5	50.69	13.25	-73.86
HM_6	66.24	55.30	-16.52
HM_7	28.80	91.01	+216.00
HM_8	47.81	-	-
HM_9	67.97	39.17	-42.37
HM_{10}	72.58	134.78	+85.71
HM_{11}	88.13	171.07	+94.12
HM_{12}	74.30	48.66	-34.52
HM_{13}	130.75	211.97	+62.11
HM_{14}	10.94	72.58	+563.16
HM_{15}	28.80	29.95	-4.00
HM_{16}	4.61	10.37	+125.00
HM_{17}	8.64	17.28	+100.00
HM_{18}	-	6.91	-

HM = High Molecular weight plasma proteins.

UN 72 = Uranyl nitrate treatment for 72 hours.

-ve % difference = Decrease in % concentration than control.

+ve % difference = Increase in % concentration than control.

treatment. Some protein fractions exhibit altogether different patterns. They disappeared after 24h, 48 h, UN treatment and reappeared after 72 h. treatment (LM_3). Still some proteins decrease after 24 h. as compared to control then there is no change after 48 h. and disappear after 72 h. (LM_2). There is a group of protein fractions exhibiting gradual decrease from 24 h. to 72 h. as compared to control. Some protein fractions exhibited a gradual rising pattern from 24h. to 72h. as compaired with control. Some protein fractions initially increase during 24h. UN treatment and further increase during 48 h. treatment but after 72 h. treatment it is deplited considerably.

Table 13 shows the patterns of high molecular weight protein changes after UN treatment. The identified protein fractions such as transferrin and plasminogen exhibit gradual decrease from UN 24 to UN 72 as compared to control. Some fraction of protein exhibit different patterns than above described. Initially they were increased during 24 h and further increased upto 72 h. Some fraction of proteins such as IgM (HM_6-94000) were initially increased than control after 24 h. but sharply decreased after 48 h. and then once again increased at 72 h.

The ceruloplasmin, another identified protein component (HMg-120,000) were initially decreased after 24 h. but sharply increased after UN 48 h. Once again it was decreased at 72h. Whereas Ig - G (HM_{12}-150,000) initially decreased after 24 h.

Table 12

The patterns of low molecular weight protein changes after UN treatment.

Protein	Molecular weight (19)	Control mg/100ml	UN 24 mg/100ml	% difference	UN 48	% Difference	UN 72	% difference
LM_1	15000	190.18	124.42	-34.65	158.42	-16.66	96.77	-49.09
LM_2	16000	93.31	39.42	-57.76	39.74	-57.41	-	-
LM_3	21000	58.75	-	-	-	-	12.67	-78.43
LM_4	27000	77.18	152.64	+97.76	101.95	+32.09	105.41	+36.57
LM_5 (Thyroglobulin)	32000	155.52	66.24	-57.41	39.74	-74.44	19.58	-86.41
LM_6	37000	155.52	66.24	-57.41	39.74	-74.44	19.58	-86.41
LM_7	42000	98.49	89.98	-8.65	54.72	-44.44	32.26	-67.25
LM_8	46000	174.53	205.63	+17.82	217.73	+24.75	177.98	+1.98
LM_9	52000	171.65	113.47	-33.89	55.30	-67.79	134.21	-21.81
LM_{10}	55000	107.14	106.56	-0.54	136.51	+27.42	64.09	-40.18
LM_{11}	63000	229.25	232.70	+1.51	359.42	+56.78	305.28	+33.17
LM_{12} (Albumin)	66000	181.20	197.57	+9.03	139.97	-22.75	93.31	-48.50

LM = Low molecular weight proteins of plasma.

UN 24 = Uranyl nitrate treatment 24 hours.

UN 48 = Uranyl nitrate treatment for 48 hours.

UN 72 = Uranyl nitrate treatment for 72 hours.

Table 13

The patterns of high molecular weight protein changes after UN treatment.

Protein	Molecular Weight (D)	Control	UN 24	% Diff.	UN 48	% Diff.	UN 72	% Diff.
HM_1 (Transferrin)	73000	74.30	24.19	-67.44	12.10	-83.72	45.50	-38.76
HM_2 (Plasminogen)	80000	61.06	59.04	-3.30	40.32	-33.96	33.98	-44.34
HM_3	84000	82.94	45.50	-45.14	24.19	-70.83	11.54	-86.09
HM_4	88000	38.02	83.52	+119.70	65.66	-72.73	194.72	+412.21
HM_5	92000	50.69	161.28	+218.18	-	-	13.25	-73.86
HM_6	94000	66.24	150.34	+126.96	13.82	-79.13	55.30	-16.52
HM_7	103000	28.80	185.47	+544.00	78.91	+174.00	91.01	+216.00
HM_8	115000	47.81	74.30	+55.42	127.87	+167.47	-	-
HM_9	120000	67.97	48.96	-27.97	114.05	+67.80	39.17	-42.37
HM_{10}	135000	72.58	214.27	+195.24	33.98	-53.17	137.78	+85.71
HM_{11}	145000	88.13	43.78	-50.33	-	-	171.07	+94.12
HM_{12} (IgG)	150000	74.30	14.98	-79.84	-	-	48.66	-34.52
HM_{13} (IgA)	160000	130.75	66.82	-48.90	187.78	+43.61	211.97	+62.11
HM_{14}	170000	10.94	9.22	-15.79	54.14	+394.74	72.58	+563.16
HM_{15}	185000	28.80	13.82	-52.00	12.67	-56.00	29.95	-4.00
HM_{16} (a_2-Macroglobulin(red)	190000	4.61	16.70	+262.50	-	-	10.37	+125.00
HM_{17}	210000	8.64	26.50	+206.67	-	-	17.28	+100.00

HM = High Molecular weight plasma proteins. UN24 = Uranyl nitrate treatment for 24 hours.

UN 48 = Uranyl nitrate treatment for 48 hours. UN 72 = Uranyl nitrate treatment for 72 hours.

disappeared during 48 h. and reappeared after 72 h. whereas IgA(HM_{13}-160000) is initially decreased after 24 h. but sharply enhanced after 48 h. and still further increase was seen at 72 h. α_2- macroglobulin (red.) (HM_{16}-190000), this fraction was initially increased after 24 h. then disappeared during 48 h. and reappear after 72 h.