C H A P T E R – III

OBSERVATIONS

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The phase specific pattern of neutral lipids and phospholipid components of red blood cell membranes under the toxic influence of uranyl nitrate is illustrated in Fig.1 and 2 respectively. Changes occurring in the hematological parameters such as hemoglobin, red cell count, echinocytes and reticulocytes have been shown in Table No.1. changes occurring in the total lipids, neutral lipids and total phospholipids are shown in Fig.No.3. the changes occurring in the cholesterol and phospholipid concentrations and cholesterol and phospholipid ratio are shown in Fig.No.4. Changes occurring in the individual phospholipid parameters are shown in Fig.No.5. Figure No.6 indicates quantitative variations in the total nonesterified cholesterol in nephrotoxic anemia induced by uranyl nitrate toxicity. Figure No.7 illustrate variations in total phospholipids in uranyl nitrate induced nephrotoxic anemia. Fig. No.8 illustrate the variations in the cholesterol/phospholipid ratios in different phase of intoxification of rat. Total neutral and phospholipid concentrations of red blood cell membrane of control and nephrotoxic rats have been shown in Table No.2. Table No.3 indicates the quantitative variations in cholesterol, phospholipids and cholesterol/phospholipid ratios. while quantitative changes in individual phospholipids of red blood cells during nephrotoxic anemia is shown in Table No.4.

HEMATOLOGIC PROFILE

Consequent hematological changes have been observed in rat blood under the toxic influence of uranyl nitrate. There is significant reduction in the hemoglobin percentage. Control value of 15.5 gm/100 ml reduced to 13.2 on first day further reducing to 12.5 on 2nd day. There was slight elevation in the value of hemoglobin on 3rd day was it not significant as compared to normal. Red blood cell count of control animals was $7.1 \pm 0.782 \times 10^6 /mM^3$ it significantly droped down to $5.6 \pm$ ~~1~~ ~~0~~ 0.03 on 2nd day remained some what constant but slightly rised to 6.1 ± 0.04 as compared to the value of 2nd day red cell count. This value was significantly less than that of control value. The study of peripherial smear indicated absence of echinocyte in control animals. In rats treated with uranyl nitrate the blood smear showed occurrence of echinocytes. The peripherial smears of 2nd day and 3rd day animals also indicated significant number of echinocytes. The reticulocyte number was neglible in the peripherial blood smears of control animals but animals treated with uranyl nitrate showed presence of reticulocytosis in all the phases of toxification.

There was no noticeable effect on behavioural pattern of the experimental animal in the early initiation phase (24 hr) but the blood smear at this stage showed certain morphological changes in the red cell size and shape. Appearance of

echinocytes was clearly observed. There was increase in the mean corpuscular volume of red cells transforming them into macrocytic. Interrestingly enough there was presence of good number of reticulocytes at this stage. The hematological profile was more or less in the late initiation and maintenance phase offcourse there was increased trend of echinocytosis.

One of the significant fact which was noticed during study of pheripherial smear of rat with nephrotoxic anemia, the existance of clumping phenomenon and shape changes of red blood cell. This corpuscular derangement is the early indication of renal malfunction of presence of nephrotoxic anemia. Uranyl nitrate intoxication exhibited sequential changes in red cell membrane morphology. The onset of nephrotoxic anemia begins with appearance of reticulocytes in peripherial blood smear. The phenomenon being the earliest change in red cell structure. In the late initiation phase the peculier red blood cells with wavy outline or crenation on outer surface are seen (enhinocytes). The projections on the outer surface were irregular and had unequal diameter. However, the echinocytes were not uniformally present throughout the sample. The crenated cells resumed their original discoid shape on addition of normal plasma to the smear. During the maintenance phase the crenation on outer surface of red cells increased in length and red cells were fused to each other by cytoplasmic bridges. The echinocytes with cytoplasmic

bridges were seen in patches and not invariable seen through the smear.

Occurrence of reticulocytosis observed as a marked even at early initiation phase of toxification indicated onset of hemolytic anemia. there was as if a shift of marrow reticulocytes into peripherial circulation also indicates increased red cell destruction-a specific sign of nephrotoxic anemia due to uranyl nitrate induced renal dysfunction.

LIPID CHANGES

When the values of the total lipids expressed as moles per mg protein of red blood cell. A notable increase was observed on the first day of intoxification. Subsequently the levels of total lipids in the red cell membrane were maintained high as compare to the control values. Similar trend was observed in total neutral lipids and total phospholipids. The alterations were comparatively significant in case of total neutral lipids as compare to total phospholipids.

Thin layer chromatographic separation of the neutral lipids of red cell samples of control and nephrotoxic rats of different phases indicated that the red cell lipid contained cholesterol as the only neutral lipid component. There were no traces of acylglycerols, free fatty acids or esterified cholesterol.

The quantitative alterations in cholesterol concentrations were interesting. Cholesterol the only neutral lipid component

PLATE NO.1 :Thin layer chromatographic seperation of Red cell membrane neutral lipid, of control and nephrotoxic Rats.

C	:	Control
I	:	24 hours after intoxication
II	:	48 hours after intoxication
III	:	72 hours after intoxication
CH	:	Cholesterol
O	:	Origin

PLATE NO.2 : Thin layer chromatographic seperation of red cell membrane phospholipids of control and nephrotoxic Rats:

C	:	Control
I	:	24 hours after intoxication
II	:	48 hours after intoxication
III	:	72 hours after intoxication
NL	:	Neutral lipids
PE	:	Phosphatidyl ethanolamine
PS	:	Phosphatidyl serine
PI	:	Phosphatidyl inositol
PC	:	Phosphatidyl choline
SPG	:	Sphingomyelin
LPC	:	Lysophosphatidyl choline
O	:	Origin

FIG. - 1

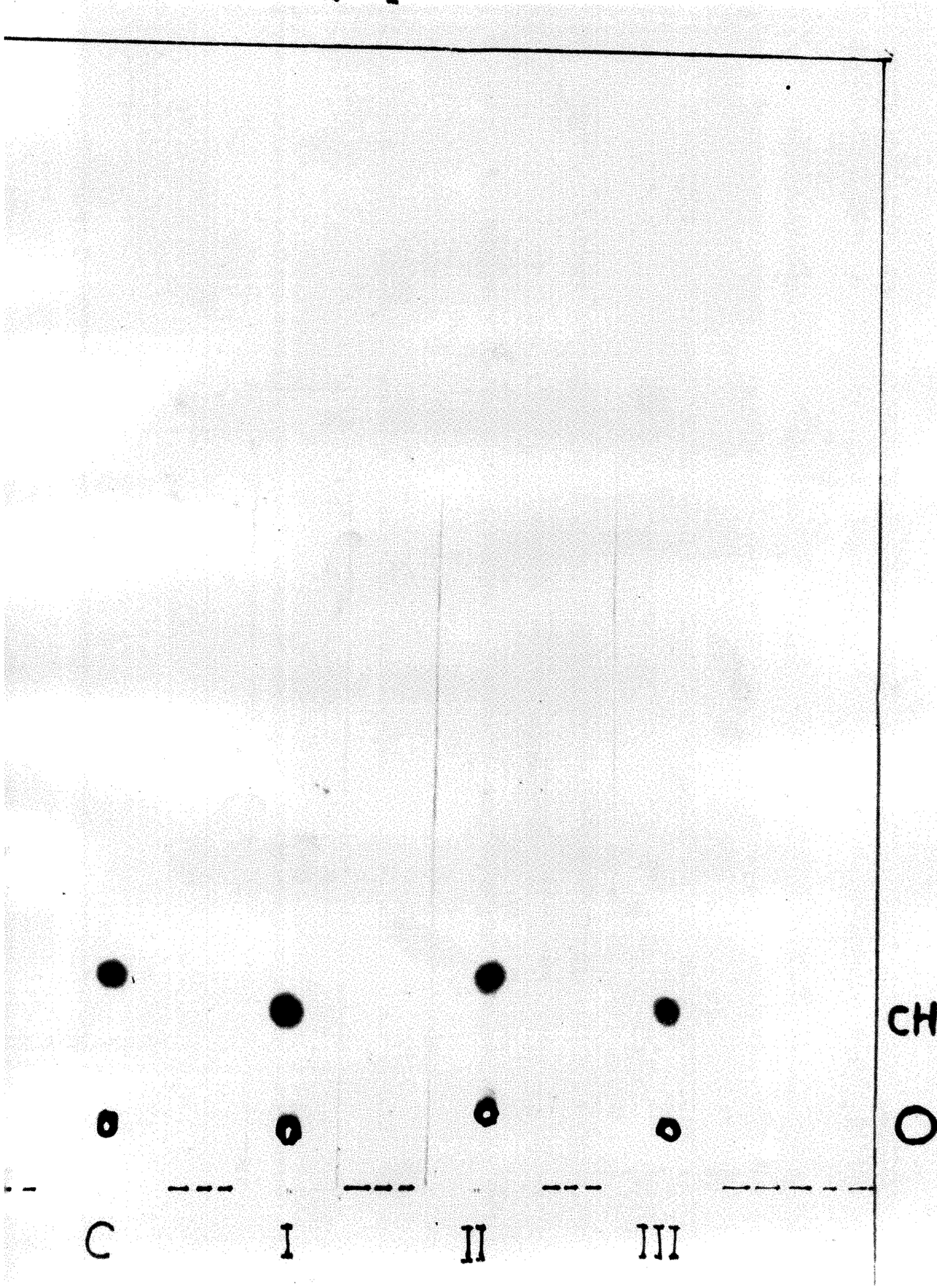
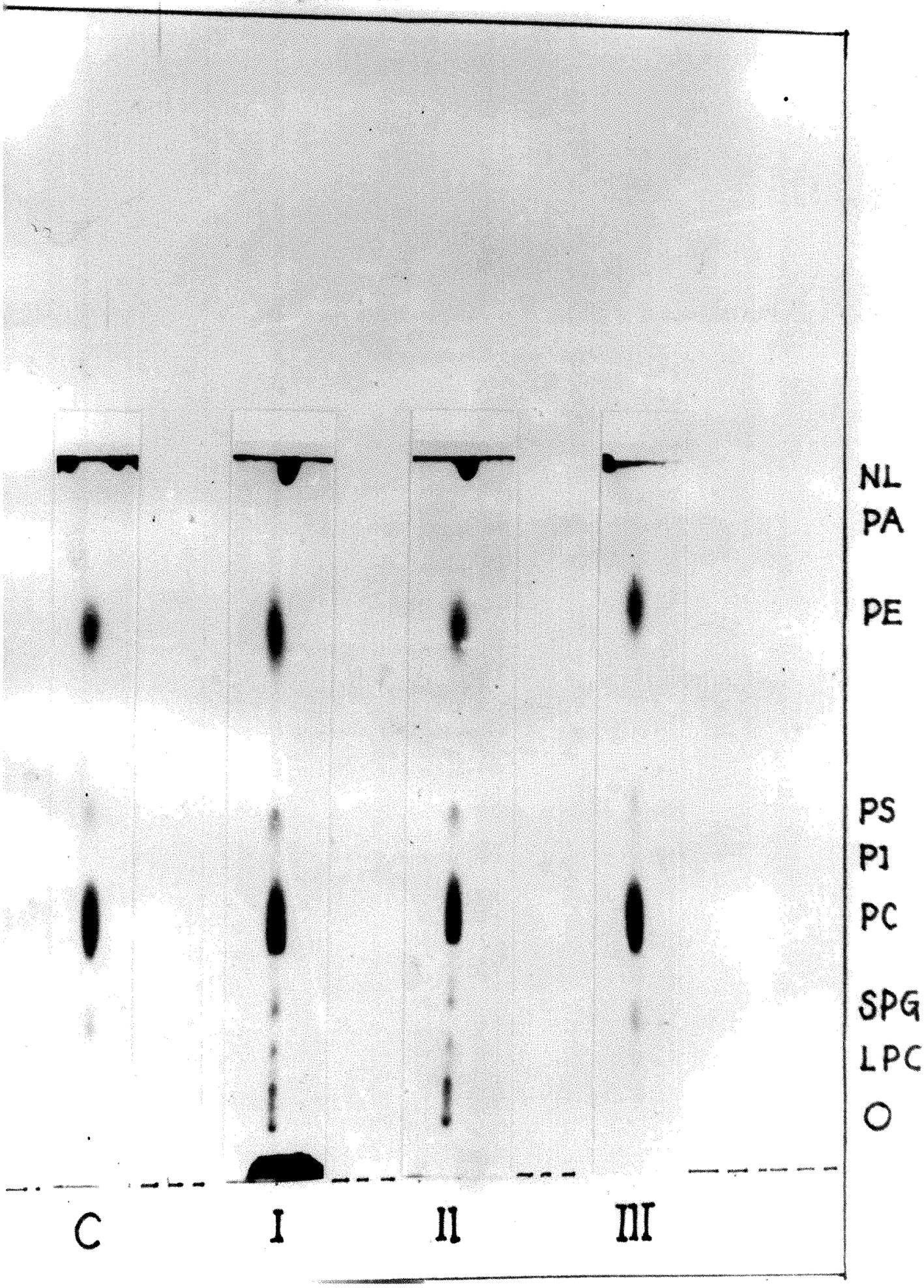


FIG. - 2



of red cell membrane increased by 27 to 28 percent on the first day of intoxication. Subsequently the values of cholesterol concentrations decreased on 2nd and 3rd of intoxication but they were high enough as compared to the values of red cell cholesterol of control animal.

Thin layer chromatographic separation of the phospholipids of the red cell membrane indicated the presence of lysophosphatidyl choline, sphingomyelin, phosphatidyl choline, phosphatidyl serine and phosphatidyl ethanolamine. At comparative level phosphatidyl choline and phosphatidyl ethanolamine were predominant, whereas sphingomyelin, phosphatidyl serine were present in lesser concentration and lysophosphatidyl choline in smaller amounts.

The quantitative alterations in various individual phospholipid components during nephrotoxic anemia of rat paralleled those in the total phospholipids, but the extent of increase and decrease in the individual components differed to great extent. Phosphatidyl choline the major phospholipid component of the red cell membrane increased by 6 mol percent on first day of intoxication and remain in somewhat the same concentration but high enough as compared to control values during 2nd and 3rd day of uranyl nitrate toxification. The values of phosphatidyl ethanolamine and sphingomyelin decreased significantly on 1st, 2nd and 3rd day of nephrotoxic insult.

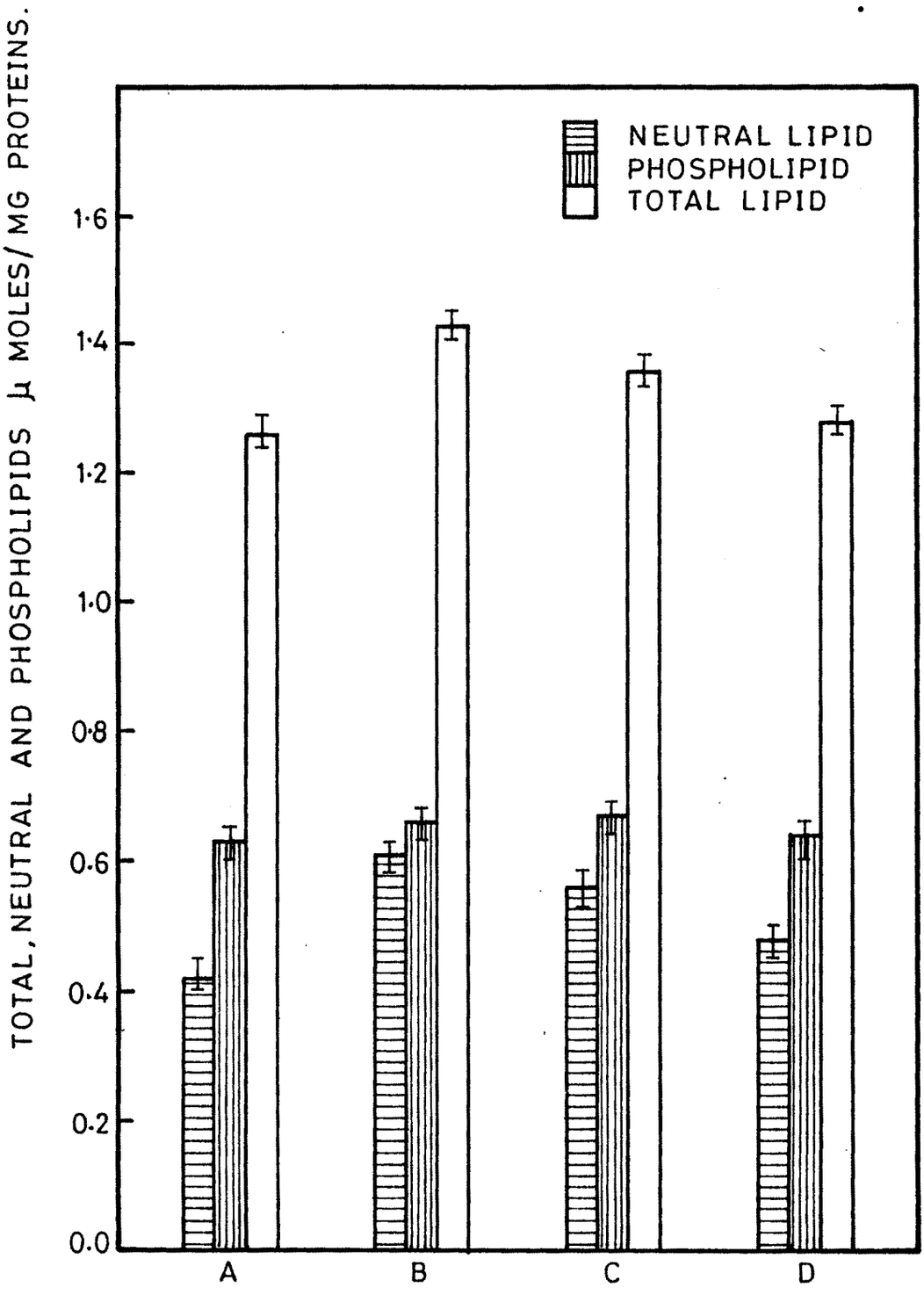


FIG. 3 — TOTAL, NEUTRAL AND PHOSPHOLIPIDS μ MOLES / MG PROTEINS.

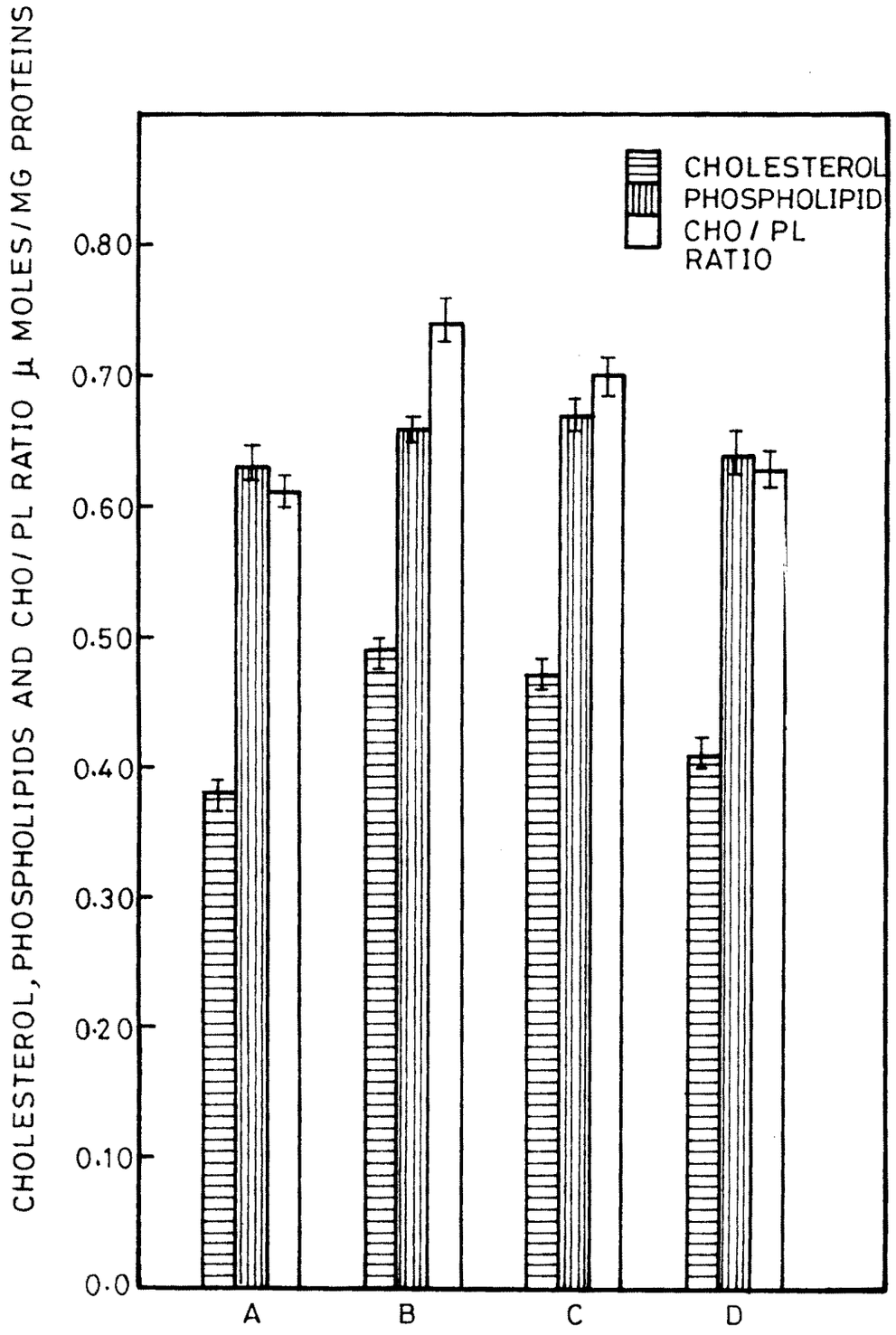


FIG. 4 — CHOLESTEROL PHOSPHOLIPIDS AND CHO/PL RATIO μ MOLES/MG PROTEINS.

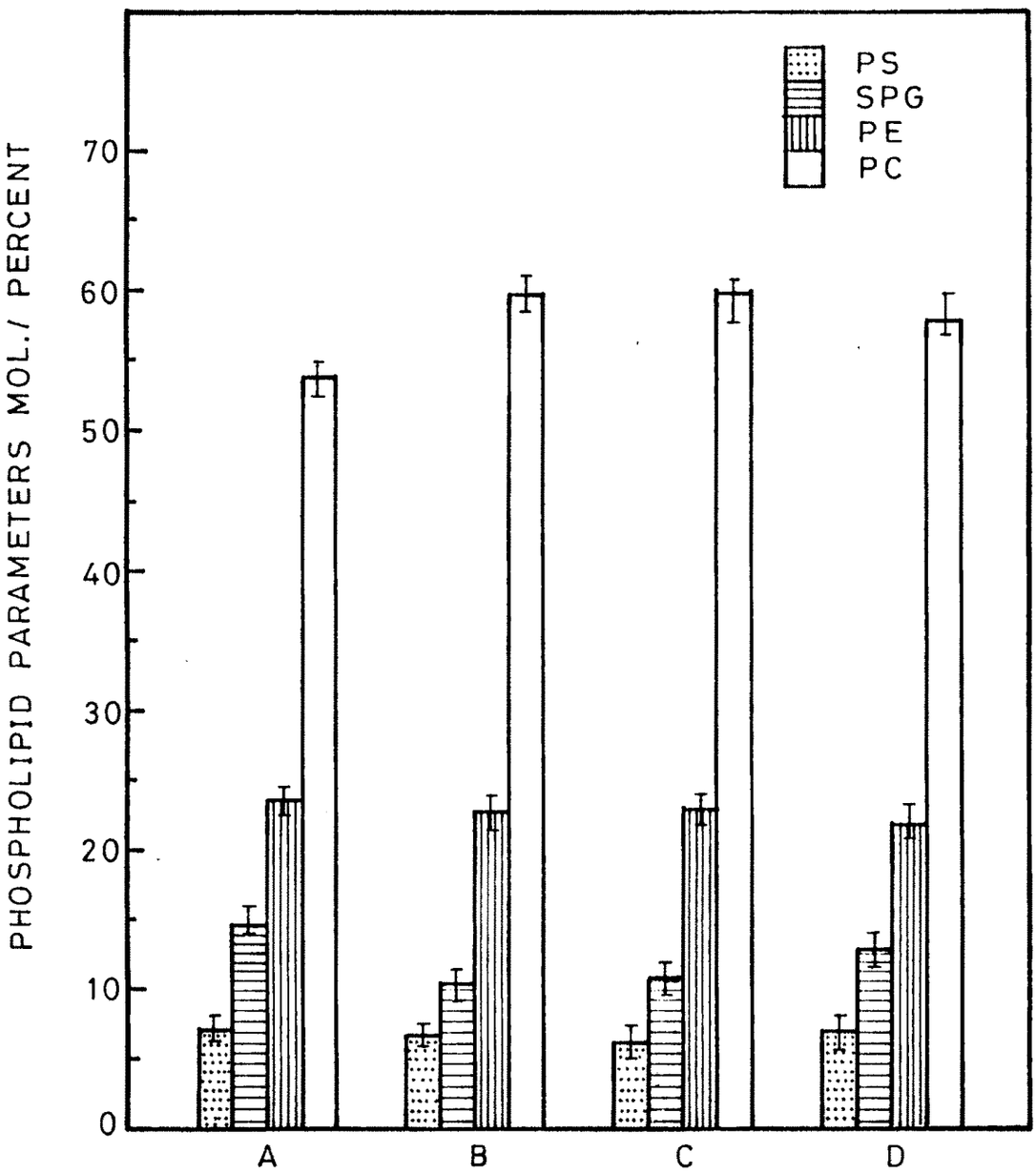


FIG.5 – PHOSPHOLIPID PARAMETERS MOL. PERCENT.

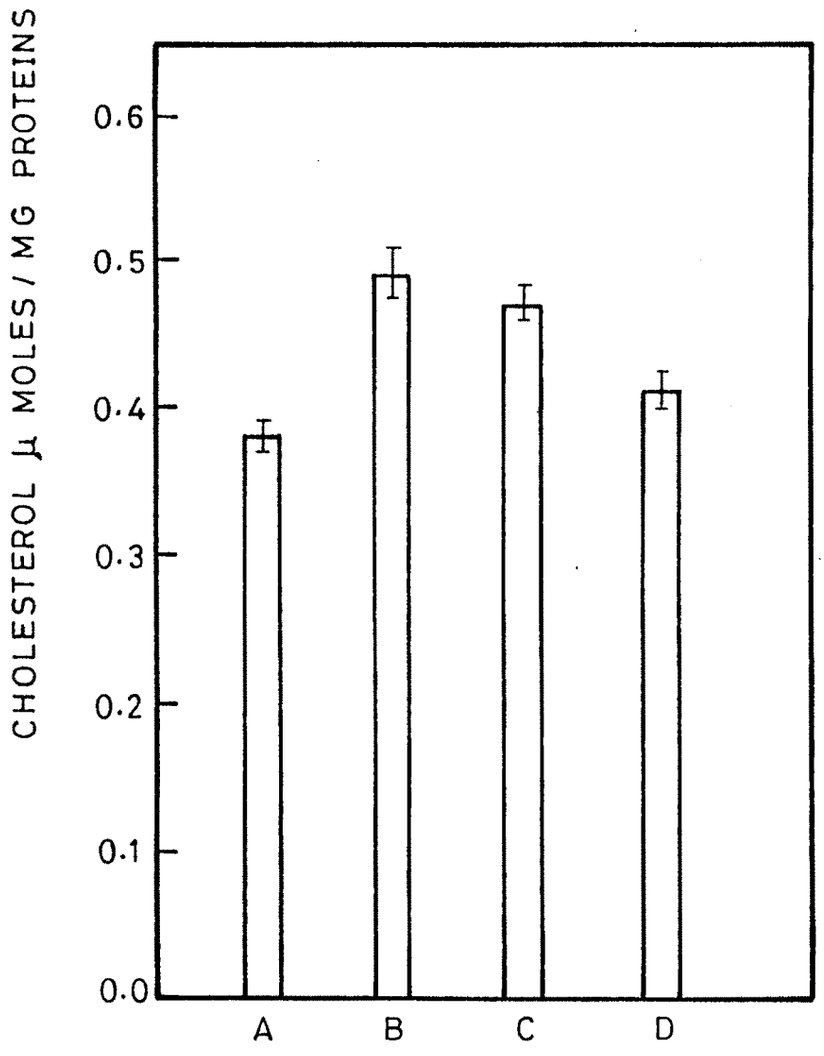


FIG.6 — CHOLESTEROL μ MOLES / MG PROTEINS.

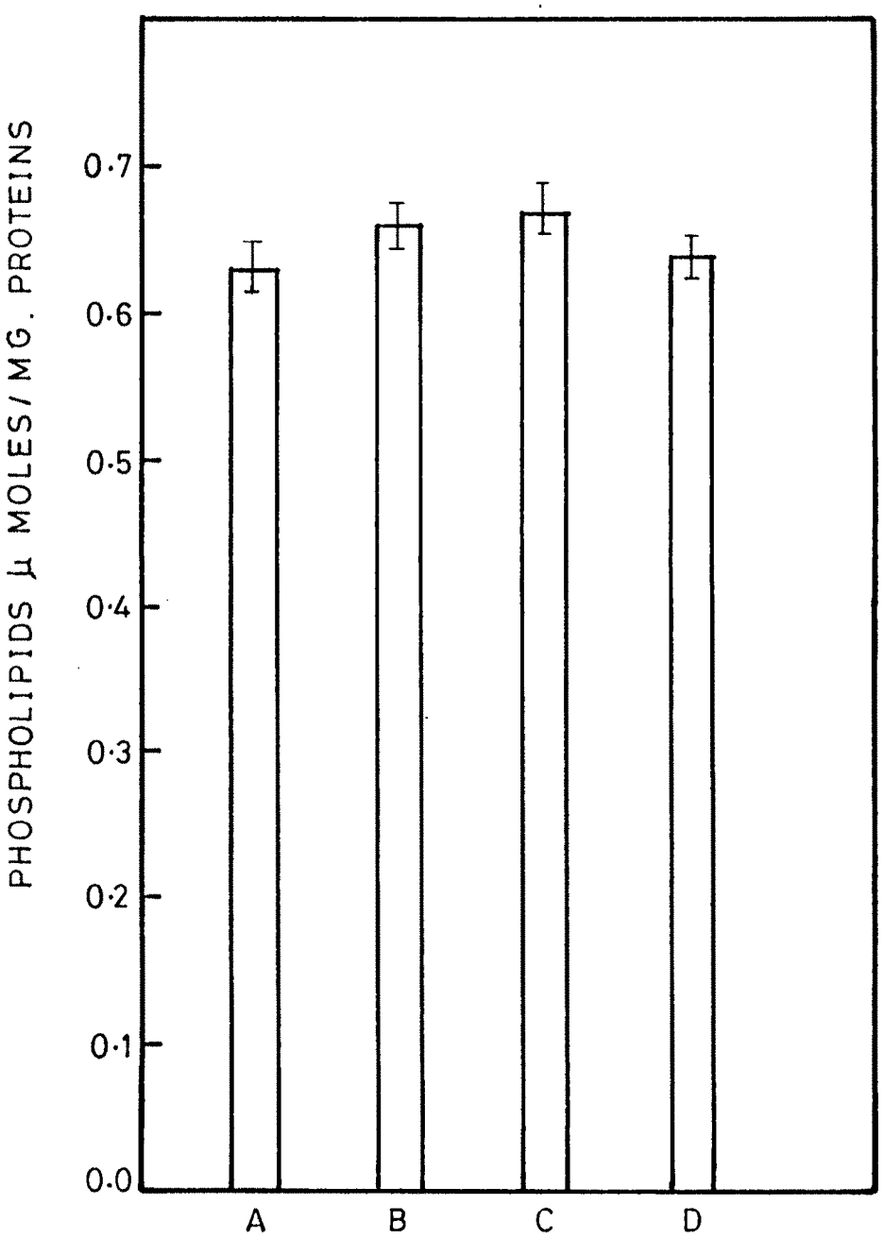


FIG. 7 – PHOSPHOLIPIDS μ MOLES / MG PROTEINS.

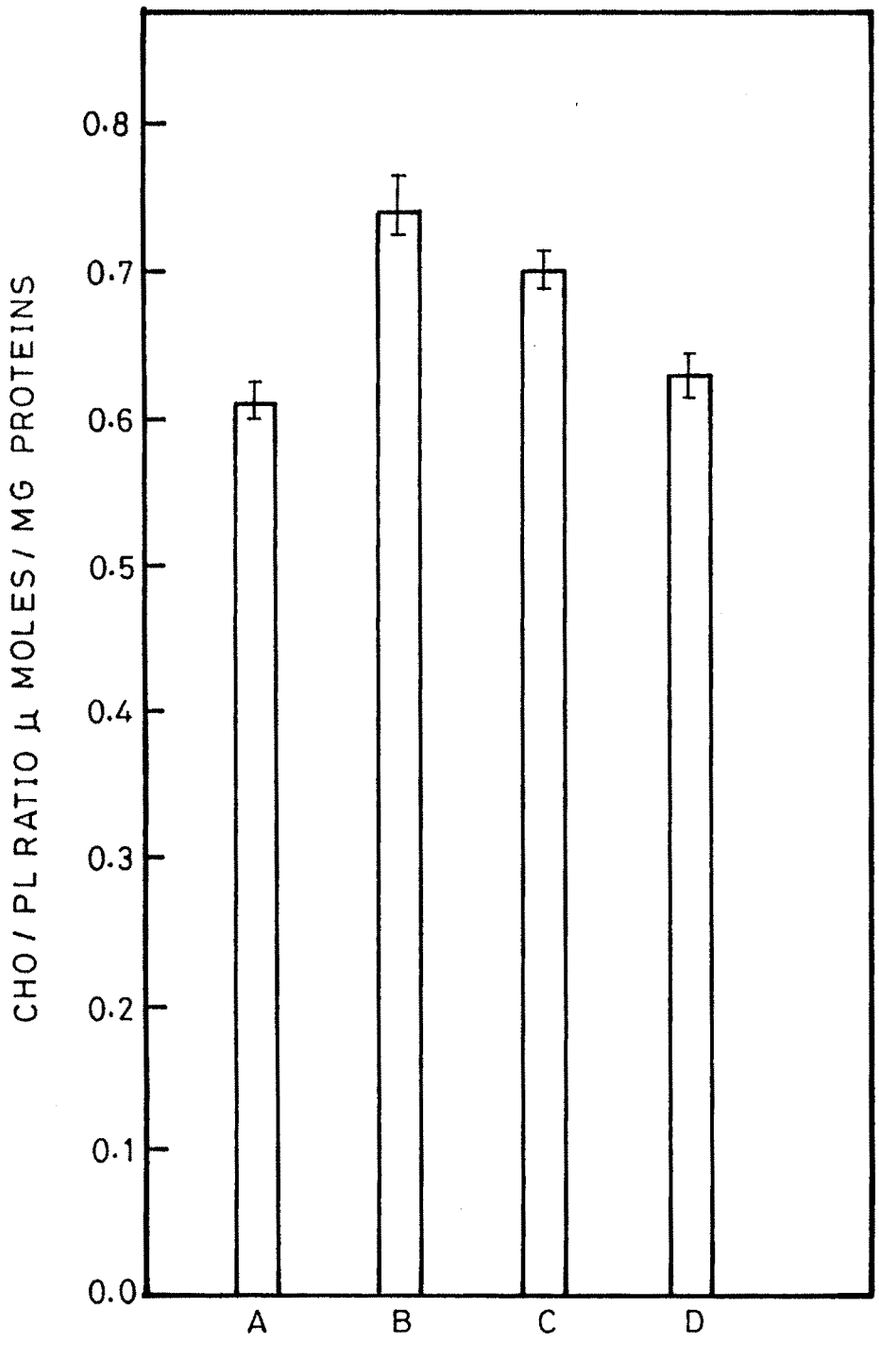


FIG. 8 — CHO/PL RATIO μ MOLES/MG PROTEINS.

Table 1 : Hematologic Parameters of Control and Nephrotoxic Rats

Group	Haemoglobin %	Red cell count X 10^6 /mm^3	Echinocytes %	Reticulocytes %
A	15.5 ± 0.47	7.1 ± 0.782	-	0.2 ± 0.012
B	13.2 ± 0.42	5.6 ± 0.03	5.3 ± 0.062	4.39 ± 0.016
C	12.5 ± 0.08	5.8 ± 0.02	5.8 ± 0.053	5.02 ± 0.02
D	13.2 ± 0.06	6.1 ± 0.04	6.2 ± 0.041	4.96 ± 0.03

All values are ± SE of 5 animals.

Table 2 : Lipid concentration of red cell membrane of control and nephrotoxic rats

Group	Total lipids	Total Neutral lipids	Total phospholipids
A	1.264 ± 0.0428	0.426 ± 0.0246	0.637 ± 0.0421
B	1.437 ± 0.0516	0.618 ± 0.0315	0.663 ± 0.0421
C	1.368 ± 0.0517	0.563 ± 0.0324	0.675 ± 0.0446
D	1.286 ± 0.0468	0.486 ± 0.0295	0.647 ± 0.0478

Values are expressed as μ moles/mg protein

Values are expressed as mean ± S.D. for 5 rats in each group

P value < 0.01

Group A(control), B (24 hrs.), C (48 hrs.), D(72 hrs.).

Table 3 : Red blood cell membrane lipid changes of control and Nephrotoxic Rats.

Group	Cholesterol	Phospholipids	Cho/PL Ratio
A	0.3891 ± 0.0231	0.6371 ± 0.0423	0.6106
B	0.4971 ± 0.0724	0.6631 ± 0.0432	0.7496
C	0.4761 ± 0.0658	0.6751 ± 0.0416	0.7051
D	0.4121 ± 0.0426	0.6471 ± 0.0467	0.6367

Values are expressed as μ moles/mg protein.

Values are expressed as mean ± S.D. for 5 rats in each group

P value < 0.01

Group A (control), B(24 hrs.), C (48 hrs.) ,D (72 hrs.).

Table 4 : Red blood cell phospholipid parameters of control and nephrotoxic rats

Group	LPC	SPG	PC	PI	PS	PE
A	-	14.91 ± 0.86	53.92 ± 2.42	-	7.46 ± 0.89	23.71 ± 1.25
B	Trace	10.52 ± 0.68	59.92 ± 2.85	-	6.8 ± 0.63	22.8 ± 1.16
C	Trace	11.1 ± 0.71	59.42 ± 2.63	-	6.3 ± 0.42	23.2 ± 1.63
D	-	13.0 ± 0.69	58.1 ± 2.72	-	7.0 ± 0.41	22.0 ± 1.59

Values of idnividual phospholipids are expressed as mol. percent.
Values are expressed as mean ± S.D. for 5 rats in each group, $P < 0.01$
Group A (control), B (24 hrs.), C (48 hrs.), and D (72 hrs.)

ALTERATIONS IN LIPID RATIOS

Significant alterations were observed in the cholesterol and phospholipid ratios in red cell membranes during nephrotoxic insult induced by uranyl nitrate. Cholesterol/phospholipid ratio increased on first day of uranyl nitrate treatment. On 2nd and 3rd day of toxification this ratio decreased practically comming close to control value on 3rd day of intoxication.