CHAPTER - IV

DISSUSSION AND CONCLUDING REMARKS

DISCUSSION

Though, the plasma protein functions are described in detail by various research workers yet, the effects of various drugs, chemicals, toxic substances still remain to be described. However, some reports on the plasma proteins with some inhibitory or stimulatory substances are available (Brodie et al., 1958), but the literature dealing with above substances is scanty. The work related with plasma protein after the treatment of either stimulatory or inhibitory substances is briefly described below :

I) RELATIVE SPECIFICITY OF ACTION OF HEPATO-TOXINS FOR THE LIVER :

The mechanism underlying the selective damaging action of some chemicals on the liver may relate to the way in which they damage the liver cells. It is quite possible, that the liver is more "sensitive" to the agent, but an alternative possibility is that the liver converts it into a more toxic form, (Stoner and Magee, 1957). Examples of compounds which may undergo such conversions are dimethyl-nitrosamine (Heath, 1962; Magee, 1956), the carcinogenic azo dyes (Mueller and Miller, 1948; Mueller and Miller, 1953) and possibly, even carbon-tetrachloride (Butler, 1961; MoCollister et al. 1951; Paul and Rubinstein, 1963; Reynolds, 1963a; Renolds, 1963b). Beryllium is taken up from the circulation in the colloidal form by the reticulo-endothelial cells

of liver, from which it migrates to parenchymal cells and exerts on them a damaging effect (Magee, 1966).

II) MECHANISM OF HEPATO - TOXINS

The relative action specificity of some toxins is attributable to the active molecules, resulting ultimately in the conversion of them in the liver. The study of the toxicants and their metabolism becomes extremely important. The biochemical leasions can be elucidated only if, the toxic molecule is know. The liver is the organ where the toxicants are metabolized, even though some of them might act as the liver toxicants, the liver has a capacity to render the potent poisons to toxins having less potency. This is possible due to the fact that a chemically reactive molecule is rendered in active. This in turn would explain, why the liver has a selective action on different toxicants including the one under consideration.

The metabolism of foreign compounds has been studied very extensively by Brodie et al. (1958). There is a system of enzymes located in the microsome fractions of the liver, which acts upon a wide range of molecules of foreign origin. The enzyme system has a requirement for reducing pyridine nucleotides. This or similar enzyme systems, have been shown to act on some of the hepato-toxins and carcinogens, and infact the first demonstration of the system was made by Mueller and Miller

(1953), working with azo dyes. The alkylnitrosamines, especially dime-thylnitrosamine have been shown to be metabolized (Heath, 1962; Heath and Dutton, 1958; Magee, 1956). Metabolism was undetectable in the totally hepatectomised rat, suggesting that the liver is the main organ concerned (Magee, 1966). Metabolism also occurs _in vitro_ with rat liver slices and homogenates but, with the possible exception of the kidney, other organs were inactive in this regard (Brouwers _et al._, 1960; Magee, 1958). Brouwers and Emmelot (1960) have studied the metabolism of dimethyl and diethyl nitrose amine in greater detail. The metabolic activity was found in the microsomal fraction of the homogenate and there was a requirement for reduced pyridine neucleotide. Formaldehyde and acetaldehyde were formed from dimethyl and diethyl nitrosamine, respectively.

There is an evidence that carbon-tetrachloride and chloroform are metabolized in the liver, . (Magee, 1966). The first observation with carbon-tetrachloride (CCl_4) was made by McCollister, (1951), who showed that a small proportion of the CCl_4 administered to monkeys by inhalation were converted to CO_2 and that a nonvolatile product of CCl_2 was found in urine, Butler (1961) showed that Chloroform ($CHCl_3$) is found in the expired air CCl_4 and that it is also produced when CCl_4 is incubated with tissue homogenates of reduced glutathione or cysteine. He suggested that this reaction was not enzymatic but resulted from the cleavage of CCl_4 with the formation of free radicals which

may react with proteins and amino acids. Paul and Rubinstein (1963) studied the metabolism of CCl_4 and $CHCl_3$ labelled with 14_C in the rat. They found that 85% of the $^{14}CCl_4$ had been excreted unaltered in the expired air and 1% had been converted to $^{14}CO_2$, 18 hours after the labelled compound had been administere by duodenal intubation. After similar administration of ^{14} $CHCl_3$, 70% had appeared unchanged in the expired air and 4% as $^{14}CO_2$. Liver slices also form CO_2 more rapidly from $CHCl_3$ than CCl_4. The metabolism of both compounds is much reduced if the tissue are homogenized. The production of CO_2 from the Chloro-methane by liver slices is stimulated by citrate and acetate and inhibited by iodoacetate, fluoride, cyanide, and other exzyme inhibitors and also by acid, alkali and heat treatment of the liver slices. In contrast to Butler's (1961) finding, Paul and Rubinstein (1963), concluded that $CHCl_3$ probably is an intermediate in the metabolism of CCL_4. Metabolism of $^{14}CCl_4$ was also shown by Reynolds (1963b) who found non-volatile incorporation of radioactivity into lipids and proteins of liver from rats treated with the compound.

A) **ELECTRON MICROSCOPIC CHANGES IN THE LIVER :**

A common change in toxic liver injury, which occurs well before necrosis, is an alteration in the character and loss of cytoplasmic basophil material. Livers at the same stage of injury have been studied electron microscopcally in a number of

hepatotoxins effect and there is a remarkable degree of similarity in the results. Oberling and Rouller (1956), described changes in the parenchymal cells of rat liver 1 hour after injection of carbon-tetrachloride. Some of the cells are normal at this time but others show cytoplasm filled with vacuoles. The cells with minimal vacuolization show that their ergastoplasm is abnormal. The membranes are less well defined and there are dilated sacs. After 90 min, the effects are more pronounced. The ergastorplasmic granules, described earlier disseminate in the cytoplasm. After 2 h, the ergastoplasmic changes are more advanced. There is an evidence of mitochondrial swelling, and some nuclei loose their homogeneous structure and show irregular granulations. Similar ultrastructural changes induced by carbon-tetrachloride are reported by Bassi(1960) and by Smuckler et al. (1962). They describe the dilation of cisternae of the endoplasmic reticulum and dislocation of ribonucleo-protein (RNP) particles from the membrane surfaces. Very similar but advanced ultrastructural changes have been described by Emmelot and Benedetti (1960), in rat liver due to dimethyl-nitrosamine. These include vacuolization of the endoplamic reticulum and detachment of RNP particles detectable at 3 h, rapidly followed by depletion of glycogen and accumulation of lipid droplets. Porter and Bruni (1959), observed much the same type of changes in livers of rats treated with carcinogenic dyes, and similar responses have been reported with thioacetamide and ethionine. In view of the

difference in the response of liver to these agents, their relationship to necrosis is doubtful. The electron microscopic changes are almost certainly related to the inhibitory effects on protein synthesis.

B) INHIBITION OF PROTEIN SYNTHESIS BY HEPATO-TOXINS AND THEIR POSSIBLE RELATIONSHIP TO NECROSIS :

The effects of several hepato-toxins on amino acid incorporation into liver proteins *in vivo* and *in vitro* have been rather widely studied. Several compounds, including ethionine (Simpson *et al*. 1950), dimethylnitrosamine (Magee, 1958), CCl_4 (Rees, 1964; Robinson and Seakins, 1962; Smuckler and Barker, 1964) have been shown to inhibit the incorporation of labelled amino acids into the liver protein of rat, and it has been suggested that these inhibitory effects may play a part in the production of liver necrosis. Unfortunately, as with so many hypotheses put forward to explain the necrosis, it cannot, in this simple form, be correct.

Ethionine was the first of the compound under consideration which was found to inhibit amino acid incorporation (Simpson *et al*., 1950). There is a sex difference in the inhibiton, males being much less sensitive (Farber and Corban, 1958). The defect in protein synthetic ability is reported to be localized in the microsomes rather than the supernatent fraction

of the liver homogenate. It is important that ethionine added *in vitro* had no effect on incorporation of amino acids by cell-free preparation from untreated females, and the addition of methionine to similar preparation from treated females, does not affect the inhibition of incorporation (Farber and Corban 1958) of amino acids.

At this stage, dimethyl-nitrosamine is shown to inhibit protein synthesis in the liver of rats of either sex. However, the inhibitory action of incorporation of amino acids is not indicated in kidney, spleen or pancrease proteins (Magee, 1958). The effect was well marked 3 h. after administration of median lethal dose of the compound but was not detected after 1 h. The inhibitory effect of dimethyl-nitrosamine is demonstrated on liver slices *in vitro* (Brouwers *et al.*, 1960; Hultin *et al.*, 1960) when the slices are preincubated, and incorporated into the protein treated slice in comparison with control. As with ethionine, the inhibitory effect was localized in the microsomes, rather than the cell sap in preparation from rats treated with dimethyl-nitrosamine *in vitro*.

Inhibition of protein synthesis in rat liver by carbon-tetrachloride is demonstrated by Smuckler *et al.* (1962; 1962). The authors, primarily interested in the mechanism of production of fatty liver by CCl_4, studied the incorporation of (^{14}C) leucine into lipoproteins and residual proteins of plasma. They

reported a marked reduction of incorporation into these proteins as early as 2h after treatment. Liver slices from treated animal showed a similar reduction in incorporation into plasma proteins (Robinson and Seakins, 1961; Seakins and Robinson, 1963). Smuckler and his colleagues (1961; 1962) made a combined electron microscopic and biochemical study of liver in the early stage of the acute injury. They observed decreased amino acid incorporation into the liver produced protein, albumin and fibrinogen. This functional impairment can be correlated with the membranes of the endoplasmic reticulum. They concluded that the failure of incorporation into plasma proteins must be an impaired capacity of the poisoned liver cells to synthesize the plasma proteins. Further they claim that the cell death can be attributed to the altered cellular function, depending, directely or indirectely, on the protein synthesis (Smuckler et al., 1961; 1962). They still further suggeste that since, the well known biochemical changes in mitochodrial functions appear relatively late in the sequence of CCl_4 intoxication and later than the inhibition of protein synthesis, CCl_4 affects the integrity of the mitochondrial structure and function by impairing the replenishment of necessary protein containing ingrediants. They finally concluded that the decreased protein synthesis may be the cause of fatty change and necrosis of the liver cell.

Recently attempts have beem made to explain the inhibition of protein synthesis in molecular terms; that is to

elucidate the biochemical lesion. The most successful attempt so far, has been that of Farber et al., (1964) with ethionine, which has been thoroughly discussed by them. Essentially, the inhibition due to ethonine, is attributed to the failure of synthesis of messenger RNA which, in turn is caused by deficiency of the available adenosine triphosphate (ATP) in the cell. This ATP deficiency arises because, the ethionine replaces mithionine in S-Adenosylethionine which is considerably more stable metabolically than the normally occuring methyl compound and this leads to trapping of much of the cellular adenine, so that the rate of ATP breakdown exceeds that of synthesis and thus, the ATP level falls. The failure of protein synthesis can be reversed by administration of ATP or adenine to the animal so that the biochemical lesions can be cured by either methionine, as mentioned earlier, or by adenine (Farber and Corban, 1964). This explanation, in molecular terms, of the mechanism of one and possibly more of the biochemical lesions induced by ethionine is very attractive. However even here, there are indications, that the things may be a little more complicated. It is not possible at the moment to explain in molecular terms the protection by ATP against the above agents. The implications leading to necrosis are certainly not obvious. Nevertheless, the results involve a similar reaction in necrogenic and non-necrogenic situations are therefore, important.

The work of Villi-Trevino et al. (1964) followed from earlier observation by the same group reveals that the nuclear RNA synthesis is inhibited in the ethionine treated rat. Soon after the fall in ATP level, the fall in protein synthesis occurs. This led to the hypothesis that there is an interference with messenger - RNA synthesis, probably by the failure of the supply of ATP. Clearly, if there is failure of messenger. RNA synthesis, it would be followed by the disaggregation of the ribosomal aggregates, the so called polysomes and this would inturn, lead to inhibition of protein synthesis.

The present view is that, each polysome is held together by a continuous stand of messenger-RNA and that if, this is not resynthesized, disaggregation into ribosomal monomers and consequent inhibition of protein synthesis can be expected to ensue. This hypothesis has been tested by Zone-centrifugation of the deoxycholat-treated post mitochondrial fraction of rat liver after treatment of the animals with a single dose of ethionine. The expected progressive breakdown of the polysomes has been observed in preparations from the treated animals. Furthermore, a rapid reassembly of the ribosome into aggregates would be induced by administration of adenine alone or in combination with mithionine.

Smuckler and Benditt (1965) have used a similar approach to the molecular mechanism of inhibition of protein

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synthesis in liver by CCl_4 and have obtained apperently rather different results.

Morgan (1966), indicates that the transferrin and albumin increase in the treated rat by injection of iron dextran, cobalt, phenylhydrazin, methyl celluose and terpentine or colloidal thorium dioxid. According to him the increase is due to increased transferrin synthesis. Relatively more transferrin than albumin is reported in the livers in most of the treatment groups. In the present investigation after treatment of UN, the albumin in the plasma is increased after 24 h. and then it decreased gradually during 48 and 72 h. Whereas, transferrin exhibits an opposite pattern. Initially, it decreased after 24 h treatment, then it is further decreased after 48h treatment and lastly it is enhanced after 72h. treatment. Thus, results obtained coincide very well with the earlier observations (Morgan, 1966).

The increase in the transferrin synthesis reported in the cobalt, phenyl-hydrazine, methyl cellulose, terpentine and thorium dioxide treated rats thus, appears to be specific for transferrin, since, the absolute turnover rate of albumin was not significantly increased in any treatment group.

Koj and McFarlane in 1968, injected an endotoxin in rabbits and measured plasma albumin and fibrinogen synthesis 14h

to 48h. After the injection of endotoxin there was a marked decrease in the fractional rate of loss in the first few hours of injected radio-iodin labelled fibrinogen and to a smaller extent of similarly labelled albumen in the plasma. The absolute rate of synthesis of fibrinogen is reported to be increased in endotoxin treated rabbits by more than 400% and the rate of synthesis of albumin increased by about 60%. In the present investigation in UN treated rats after 48 h, albumin increased by 9%. But later on as the period is increased, the percentage of it gradually decreased during 48 h and 72 h. Hence, the UN acts antagonistically to endotoxin.

John and Miller (1969) worked on rats. They studied the synthesis of five specific plasma proteins. Their values were estimated with the serological measurements. The influence of insulin, cortisol and growth hormone as well as nutritionally complete mixture of amino acids on the net synthesis of plasma proteins has been evaluated. Control perfusions (no hormones or amino acids added) resulted in relatively linear net synthesis of the specific plasma proteins through-out the estimates. In experiments with full supply of hormones and amino acids, fibrinogen and haptoglobin synthesis increased three fold between 2 to 6h., $alpha_1$ acid - glycoprotein synthesis increased 3.5 fold between 4 to 8h., and $alpha_2$ (acute phase) - globulin synthesis increased 11 folds between 8 to 10h. Increase in the later four proteins (fibrinogen, haptoglobin, $alpha_1$ and $alpha_2$

globulins) was critically dependent upon the presence of cortisol and represented the first demonstration in vitro of hormonal effects on net plasma protein synthesis. In the present investigation, after UN treatment the haptoglobulin (HM_3-84000) gradually descreased from 24h. to 72h, indicating the UN acts as an inhibitor, as far as, haptoglobin synthesis is concerned. On the other hand, the albumin initially increased in the first 24h. but later on it, gradually depleted from 48h. to 72h.

Verbin et al. (1969) worked on male rats by administering 1.5 mg/kg of cycloheximide, and observed markedly decreased hepatic protein synthesis over a 7h. period. It had no effect on ribonecleic acid synthesis after 2h. Despite the severly depressed level of protein synthesis, the liver showed only minimal ultrastrutural changes. Thus, the structural response of liver to sever disturbance of protein metabolism appears to be minimal during the initial few hours. In the present investigation, the results on the total protein agree very well with the above reports. From the work of the investigators it is evident that, the hepatic parenchymal cells in rat can tolerate a considerable curtailment of their protein synthetic activity for atleast several hours without developing any evidence of fatty liver and necrosis. A therotical factor operating in case of the cycloheximide may be the presence of some additional metabolic property which prevents the liver cell from reacting to the concomitant interference with protein synthesis.

Lopez-Mendosa and Villa-Trevino(1971) studied that rat liver protein by hydrazine induced inhibition of amino acid incorporation. The effect of hydrazine upon in vivo incorporation of amino acids into the protein of the liver and other rat tissues has been explored. It has been suggested that hydrazine inhibition of protein synthesis may produce fatty liver. It is true that an inhibition of incorporation of the amino acids alone can not be taken as an inhibition of protein synthesis but the observation that the specific activity of the labelled amino acids is increased after hydrazine treatment excludes the possibility that the inhibition of amino acid incorporation is a dilution of the level in the amino acid pool and suggests protein synthesis inhibition. Recent observations that a cell free system isolated from the liver of hydrazine treated rats also reveals the inhibition of the incorporation of the amino acids are consistant with the idea of a true defect in the mechanism of protein synthesis.

Other workers using liver slices from hydrazine treated rats found no inhibition of the incorporation of labelled amino acids (Amenta and Johnston, 1963). No explanation of this discrepancy can be offered at this stage. Furthermore, after a series of non-isotopic experiments (Banks and Stein, 1965) report an increase in the total liver protein, 24 h. after the administration of hydrazine. In the present investigation the albumin and $alpha_2$ macorglobulin also increase after 24h. after UN

treatment. This could be explained on the basis of an increase in protein synthesis. However, other explanations are also possible, such as a defect in the excretion of proteins (Lombardi and Oler, 1967) as has been suggested for choline-deficiant animals. The reported accumulation would also be the result of a decrease in protein break down.

The acute effect of ethanol on albumin, fibrinogen and transferrin synthesis in rat has been studied by Jeejeebhoy et al., (1972). They report that a decrease of absolute synthesis of albumin and fractional synthesis of transferrin occurs within 3 h of orally administered ethanol (4ml/kg) to rats maintained on 40% protein diet. In contrast, the synthesis of fibrinogen is not affected. They suggest that ethanol may interfere with hepatic plasma protein synthesis and the ultrastructure change through the disturbance of amino acid metabolism than through a direct physical damage to the hepatocytes. In the present investigation, it is observed, that the transferrin decreases after UN treatment within 24h. Hence, the present observation lends support to the earlier observations.

Jeejeebhoy et al. (1972) worked on the factors stimulating the protein synthesis in the rat liver. Dakashinamurti and Livak, (1970) studied the effect of biotin on the protein synthesis. Biotin administration in the biotin deficient rats enhanced the protein synthesis. The time sequense studies indicated that, RNA synthesis is stimulated, immediately 1h after biotin administration. The synthesis of RNA of liver was stimulated by biotin administration. Nuclear RNA isolated from biotin-injected rats is more active in a protein-synthesizing system, in vivo than a similar preparation from the deficient rate liver. It is suggested that biotin has a role in the synthesis of RNA.

The intracellular aspect of transferrin and albumin synthesis has been studied by Morgan and Peters (1971). Both proteins are apparently synthesized directly from the pool of free leucine in the liver, but it takes about 2 min to form a molecule of transferrin in contrast to 1 Min for an albumin molecule. Like albumin, transferrin remains bound to cytoplasmic membranes until its secretion into the circulation.

The secretion of newly formed transferrin is a slower process than that of albumin requiring a minimum time ot about 30 min and an average of about 80 min. The amount of

transferrin found in microsomes is correspondingly larger in relation to its rate of production than the amount of albumin. The levels obtained by an immuno-chemical technique were 320 and 390 g/gm of liver respectively. These results indicate that transferrin and albumin are formed by a similar process. However, synthesis and secretion consume more time in the case of transferrin. Both the proteins occur in membrane bound form, isolated with microsome fraction, from the time of synthesis until secretion into the blood stream. Rat transferrin is reported to have the same molecular weight as rat albumin (Charlwood, 1963). Hence, a difference in molecular size cannot explain this delay. Unlike albumin, transferrin is a glycoprotein and the extra time may be required to incorporate the corbohydrate residues into the molecule. The too proteins are probably synthesized within the same cell. Secretary pathway of transferrin and albumin might be segregated within the same cell, each utilizing a separate membranous channel and being packed into distinctive vesicles in the Golgi apparatus.

The secretion of the serum proteins and synthesis in regenerating rat liver has been investigated by Schreiber _et al._ (1971). They observed the regulation of albumin synthesis and serum protein excretion. The time between intracava; injection of ^{14}C- leucine and the appearence of radio active proteins in the blood secretion, decrease from 15 min to a minimum of 10 min at 48 h after removal of 70% of the liver. They also

indicate that the half life of albumin is 2.66 days in normal rats and 2.13 days in the case of rats after 48h after partial hepatectomy.

Jeejeebhoy et al. (1970) worked on rat plasma protein by injecting cortisol. They report that with a decrease in the absolute synthesis of albumin, no change in that of fibrinogen and an increased fractional synthesis of transferrin occur 3h after intraperitoneal administration of pharmacological dose of 5 mg cortisol to 220 gm rats. In post absorptive state and previously kept on a diet of 40% protein. The concentration of liver total free amino acids remains practically unchanged at this time. The intraperitoneal administration of a mixture of amino acids with the cortisol raises this concentration and is accompanied by an almost complete depression of the synthesis of albumin with no real effect on the fibrinogen. In considerable contrast, in rats studied at 24h after intraperitoneal administration of cortisol, which were fed once in the interium (but which had received no amino acid intraperitoneally), there is a marked increase in absolute synthesis of albumin and fibrinogen, with an increase in fractional synthesis that is less proportional but still very significant and it includes transferrin.

The present studies resolve apparently conflecting results of the effect of cortisol and the synthesis of albumin reported by others. The albumin increased after 24h, but later on it

decreases gradually after 24 h. and 48 h. Surprizingly the process enhances after 72 h. of UN treatment. The IgG also follow the similar pattern of transferrin. On the other hand IgA is decreased after 24h. but later on it rises sharply during 48h. and 72h. after UN treatment.

Kim et al. (1976), studied the effect of excess vitamin A on protein turn over in rat liver and they report that the protein synthesis in the livers of hyper-vitaminotic animals is partially impaired, as shown by the shift of polysomal profile towards lighter aggregates.

CONCLUDING REMARKS

The earlier part of the discussion deals with toxic, nacrotic, inhibitory and stimulatory substances and plasma proteins. This part of the discussion deals with the Uranyl nitrate intoxication in the rat (*R. narvegicus*). The effect of UN on the plasma protein via liver is presented as follows. The control rat plasma proteins and UN treated rat plasma proteins at various time interval i.e. for 24h., 48h. and 72h. are reported in the present investigation. The plasma proteins in all above cases were separated by using SDS-PAGE and 29 fraction of plasma protein were separated. Out of 29 fractions were identified and condirmed by using the molecular weights. 3 fractions of plasma proteins were identified using approximate molecular weights, but are yet to be confirmed. The remaining 19 plasma protein fractions could neither identified nor confirmed by the presently used electrophoretic method. Their identification and confirmation needs further research work.

The protein fractions, identified and confirmed were (LM_5-Thyroglobulin; LM_{12}-Albumin; HM_1 - Transferrin; HM_2 - Plasminogen, HM_{12} - Immunoglobulin-G; HM_{13} - Immunoglobulin-A and HM_{16}- a_2 Macroglobulin (reduced), only identified but not confirmed were (HM_3 - Haptoglobin, HM_6 - IgM and HM_9 - Ceruloplasmin) and neither identified, nor confirmed were the remaining 19 fraction. The patterns of all, varied during

different time intervals and these are highlighted below;

Albumin (LM_{12}) exhibits an interesting pattern. Initially after 24 h. its concentration is enhanced than control and lateron as the period prolongs i.e. 48 h. and 72 h. it gradually decreases. So the reason for the initial increase might be that the initial toxic effect of UN tried to nullify the defense mechanism of the body. But later on as the dose interval prolongs, UN may be affecting the plasma protein synthesis in the liver consequently depleting the albumin concentration.

Transferrin (HM_1) shows decreasing pattern from 24 h. to 72 h. One of the properties of the transferrin is binding with parenchymal cells and sometimes it may not be released into the circulation very quickly. The effect of UN on the transferrin synthesis in the liver may be impaired and hence its concentration is gradually decreased after UN treatment. Similarly Thyroglobulin (LM_5) exhibits the gradual decrease in its concentration.

Even if the synthesis of immunoglobulin takes place mainly in lymph nodes, their pattern shows some change after UN treatment. IgG (HM_{12}) shows sharp decrease after UN24 and completely disappears in UN48 stage, but again reappears in UN72 sample of plasma proteins.So initially due to defence of the body against UN intoxication, it decreases and at 48 h. UN intoxication

is proved to be more powerful than IgG hence the protein disappears completely. In the body co-ordination for maintaining the equilibrium, recovery is fast and IgG rate of synthesis may be increased. So in 72 h. UN treated rat plasma again makes its appearence.

IgA (HM_{13}) decreases sharply after UN24, but later on it gradually increases at UN48 and UN72. The possible mechanism may be that the IgA synthesis rate is stimulating the internal body mechanism for the explaination of which the further research work is needed.

Plasminogen (HM_2) exibits a gradual decrease after UN24 through UN48 and UN72. This decrease may be due to the UN intoxication at liver level, which may be impaired.

α_2 - macroglobulin (reduced) (HM_{16}) initially increase. 4 fold - after UN24 as compared to control, but later on it disappears after UN48 and finally 2 fold increase occurs at UN72 compared to control. This may be due to the inhibitary effects on the α_2 - macroglobulin synthesis as was seen due to other inhibitors.

The remaining three plasma proteins were separated by SDS-PAGE Method and by using their approximate molecular weights, were identified. For their confirmation, further work

and more elaborate techniques are required. In the present investigation only their pattern due to the UN treatment is studied. Haptoglobin (HM_3) exibits gradual decrease from UN24 through UN48 to UN72. Whereas IgM (HM_6) increases approximately 2 folds than control at UN24, but suddenly decreases after UN48 and further increases but is little less than control after UN72.

The ceruloplasmin (HM_9)exhibits slighlty different pattern than the above two protein fractions. Initially, it is decreased than control after UN24, but a sudden increase occurs after UN48 and then again a decrease after UN72 is seen. The changes in the pattern of the above 3 protein fractions at physiological level can not be explained at this stage due to the lack of data on physiological changes.

The remaining low molecular weight protein fractions (LM_1 to LM_4 and LM_6 to LM_{11}) exhibit very interesting pattern during UN treatment from 24h. to 72h. Some protein fractions (LM_1, LM_6, LM_7 and LM_9) have shown decrease in quantity than the control concentration of protein fractions such as LM_2 show decrease after UN24 and UN48 than control and then disappear during UN72. The other factor i.e. LM_3 which disappears during UN24 and UN48 reappears during UN72. In these cases UN may be acting as a poison or intoxicant impairing the protein synthesis machinary and hence the protein fractions either decrease or disappears from the plasma.

The remaining low molecular weight protein fractions such as LM_4, LM_8 and LM_{11} showed a totally different pattern. They gradually increased from UN24 to UN72. Why this increase after UN treatment (which considered toxic) is occuring can not be answered at this stage.

The second group of protein fractions with high molecular weight also exhibit peculiar changing patterns during UN treatment from 24h. to 72h. Some of the protein fractions such as HM_4, HM_7, HM_{10} and HM_{14} exhibit increasing pattern throughout the treatment, than the control. Whereas HM_{15} protein fraction show decreasing trend as compared with control. Some protein fractions increase gradually and some are decreasing after UN treatment. These trends can not be explained positively with present information. The remaining protein fractions such as HM_5, HM_8 and HM_{17} increase initially during UN24, then disappear during UN48 and reappear at UN72. One protein fraction i.e. HM_{11} is initially decreased after UN24 completely disappears from plasma after UN48, but is sharply increased (2 fold) after UN72. Also there is a possibility of the appearence of some new protein in the later stage after UN treatment i.e. UN72. Such changing patterns can not be explained positively. For the answers to the above unexplained observations, future research in methodology as well as in biochemistry is needed. The present work undertaken still opens several avenues for further research on the following aspects.

(I) Why some protein fractions are sharplty increased after UN24 than control, but decreased at UN48 and once again increased after UN72 ?

(II) Why some protein fractions disappear after UN24 and UN48 to reappear after UN72 ?

(III) In some protein fractions there is no change after UN24 but increase in concentration occurs after UN48 and then decrease in quantity after UN72 is seen. Why ?

(IV) Some protein fractions are increased after UN24 but then are gradually decreased during UN48 and UN72. Why ?

(V) Why some protein fractions are initially decreased in UN24 than control and then is increased in UN48 with sharp increase after UN72 ?

(VI) Some protein fractions decrease initially than the control after UN24, sharply increased after UN48 to again decrease after UN72. Why ?

(VII) Some protein fraction increase sharply than control after

UN24, then completely disappear after UN48, but once again reappear in UN72. Why ?

With all shortcomings in mind, the auther feels grateful that she had atleast presented, though preliminary, but informative study of plasma proteins after UN treatment.

"To make an end is to make a beginning
The end is where we start from. "

T.S. Eliot.