DISCUSSION
Use of steroid contraceptives leads to hypertriglyceridaemia. An increase in the output of triglyceride (TG) from endogenous sources, decreased TG utilisation or a combination of the two processes may be the underlying basis of steroid contraceptive induced hypertriglyceridaemia. Nevertheless, the exact mechanisms involved therein are not yet clear and cannot be worked out clinically. Various aspects of glyceride metabolism leading to hyperglyceridaemia have been investigated in the present study in an animal model.

**Animal Model**

Two species of laboratory animals, female guinea pigs and female rats, were used, but frank hypertriglyceridaemia developed in female rats only.

**Studies on Guinea Pigs**

Female guinea pigs were given intramuscularly two monthly injections of either medroxy-progesterone acetate (progestin type, DP group) or a mixed type (M group) of steroid contraceptive containing dihydroxyprogesterone acetophenide and estradiol-17-β-enanthate in the ratio of 100:5. Each of the contraceptives was injected in a total volume of 0.1 ml/animal and contained 15 mg of progestin type or 10.5 mg of the mixed type contraceptives. Table II and V show that no hypertriglyceridaemia developed in these animals. However, plasma cholesterol levels in DP group did show a significant increase as compared to the normal guinea pigs (Table III and V). Such an effect
of progestins on the plasma cholesterol levels has been observed clinically by various workers (for references see de Alvare et al. 1973).

Further studies on the lipids of other tissues of guinea pigs gave very interesting results. As shown in Tables VI and IX triglycerides in liver increased significantly after treatment with steroid contraceptives. This may well be a reflection on the derangement of transport of TG from liver. However, the levels of hepatic TG was not so high so as to cause fatty liver. Possibility of the development of fatty liver by a prolonged treatment with contraceptives was not investigated. A defect in the transport of hepatic TG indeed leads to fatty liver as has been shown in animals subjected to choline deficiency (Lombardi et al. 1968), treatment with either ethionine (Farber et al. 1964) or carbon tetrachloride (Racknagel, 1967). A study on the levels of plasma phospholipids showed a significant decrease in guinea pigs treated with mixed contraceptives (Table IV and V). But the total concentration of liver phospholipids did not vary (Table VIII and IX). In view of the evidence that interaction of phospholipids, non-polar lipids and protein are involved in the plasma membranes structures (Vandenheuvel, 1971), differential phospholipids patterns of liver of guinea pig were also investigated. No difference was observed in the pattern of major phospholipids (Table X—XIII). But no attempt was made to fractionate the plasma membrane lipids. An assessment
of lipids of hearts of guinea pigs was also made (Tables XIV and XVII) but no significant changes were observed.

As hypertriglyceridaemia did not develop in female guinea pigs further studies on this species were abandoned and investigations were started on female rats.

Studies on Rats

Female rats were treated with either medroxy-progesterone acetate or mixed contraceptive by two monthly injections and studies were carried out three weeks after the second monthly injection. A perusal of the results presented in Table XIX shows that frank hypertriglyceridaemia developed in female rats treated with mixed type of steroid contraceptives. Increase in plasma triglyceride level was considered hypertriglyceridaemic as its concentration far exceed the upper 95 percent confidence limits, of plasma TG concentration of normal rats. The dosage of each contraceptive was 0.1 ml per rat; containing 15 mg of depot provera or 10.5 mg of mixed contraceptive. As it has been shown that 5-7 times the human dosage is required to inhibit ovulation in rodents on weight to weight basis (Schillinger and Gerhards, 1973), the dosage used in the present study works out to about 2-3 times the effective dose of human beings. As such this dosage is not excessive. Further, the dosage required to inhibit ovulation was necessary as the long acting contraceptives in the dosage used in human beings certainly stop ovulation over and above any other effects.
As the increase in plasma TG levels was remarkable in female rats treated with mixed type of steroid contraceptive, it was decided to study all the aspects of glyceride metabolism. Furthermore, medroxyprogesterone treated rats did not show any hypertriglyceridaemia and were, therefore, used to find out the effects of progestin alone in glyceride metabolism. A group of rats treated with estrogen alone was not studied as pure estrogen contraceptives are not in use (they may be used as morning after contraceptives) and our aim was restricted to study the effect of standard formulations of contraceptives.

Effects on Body and Organ Weights:

Treatment of female rats with either progestin type or mixed type of contraceptives did not lead to any adverse effects on their growth (Table XVIII). In human females too, use of steroid contraceptives may not always lead to increase in the weight due to electrolyte retention (Preedy, 1969). However, liver weight/100 g body weight increased in rats treated with mixed type of steroid contraceptives. Perhaps it is due to a little retardation in their growth (Table XVIII). Analysis of variance showed that body and liver weights in 3 groups were not significantly different. Final body weight of rats (Table XVIII) is similar to the rats used by other workers (Schillinger and Gerhards, 1974)

Lipids Profiles

As plasma triglycerides concentration is, in part
dependent upon the effects of steroid contraceptives on various other lipids, tissues were also assayed for those lipids.

Profiles of plasma cholesterol and phospholipids (XX - XXI) along with phospholipids to cholesterol ratio were assessed. Even though there was some increase in cholesterol levels (Table XX), plasma phospholipids to cholesterol ratio increased significantly only in those rats which were treated with mixed type of contraceptives. It means that the mixed type of steroid contraceptive acts as an hypocholesterolemic agent. Such effects of estrogenic agents have been observed by other too (Schillinger and Gerhard, 1975). Even though there was a significant increase in the levels of plasma free fatty acids of treated rats (Table XXIV), much importance cannot be attached to this parameter alone for various reasons. First of all, turnover of FFA is so rapid (a few minutes) that its chemical concentration may not mean much. FFA reflects to a great extent the equilibrium between lipolysis and formation of TG.

Adipose tissue is one of the most important sources of FFA and long acting steroid contraceptives, being fat soluble, are stored primarily in adipose tissue. Therefore, it is not possible to rule out direct effects of steroid contraceptives on lipolysis and fat storage, work on FFA was not extended further. FFA is present in blood either as free or bound to plasma albumin. The dynamics of
competition between these two separate pools is in fact more important than concentration of total FFA per se.

Differential plasma phospholipids change only in DP group (Table XXV) when percentage of PE increases marginally (P < 0.10). It is difficult to suggest the basis of this increase but it may be a reflection of a possible defect of differential localisation of different phospholipids in erythrocytes but further work is necessary in that direction.

As plasma lipid profiles are dependent upon the tissue lipid patterns, it becomes necessary, therefore, to analyse the tissue lipids also. Concentration of triglycerides (Table XXVI), cholesterol (Table XXVII), phospholipids (Table XXVIII) and FFA (Table XXIX) in liver were not affected by the treatment of steroid contraceptives. The differential phospholipid patterns also showed only marginal alterations (Table XXX). Similarly, hearts of treated rats did not show frank changes in the lipid patterns (Table XXXI - XXXIV). It may, therefore be concluded that treatment of female rats leads to hypertriglyceridaemia and as much significant effects were not observed on the tissue lipid pattern, it is a suitable animal model. It is well known clinically that the derangement of lipid metabolism does take place in TG leading to hypertriglyceridaemia in women using oral contraceptives as was first reported by Aurell et al. (1966) and confirmed by Wynn et al. (1966 a,b, 1969), Haszard et al. (1969 a,b)
and Zorilla et al. (1968). The mechanisms involved in the induction of this hypertriglyceridaemia are not yet clear. Plasma TG levels represent a dynamic equilibrium of TG synthesis, its transport to the blood and its enzymic utilization in the tissues. It is, therefore, necessary to study all these aspects systematically.

**Pathways of TG Synthesis**

Usually incorporation of radioactivity from radiolabelled glucose, acetate and fatty acids like palmitic acid is taken as a measure for TG synthesis.

The important enzyme system involved in TG synthesis is the fatty acid synthetase complex and palmitate is the most important product of this system. Fatty acids of higher chain lengths like lignoceric acid (C24) are synthesized from palmitic acid (C16) by stepwise addition of C2 units. Chain elongation of fatty acids can take place both in microsomes as well as in mitochondria. On the other hand *de novo* synthesis of fatty acids takes place in cytosol and starts with acetyl CoA or malonyl CoA as substrates. Usually chain elongation in mitochondria requires acetyl CoA and in microsomes it is dependent on malonyl CoA. The cofactor for chain elongation in both the subcellular organelles as well as for *de novo* synthesis appears to be NADPH (Wakil, 1970). Microsomal system depends upon malonyl CoA (Stoffel and Ach, 1964, Nugteren, 1965, Mohrhauser et al. 1967). Acetyl CoA is the donor of C2 units from chain length C10 to C22 (Wakil, 1961).
In most of the cells, the fatty acids synthesizing enzymes acetyl CoA carboxylase and fatty acid synthetase are present in cytosol. The "raw material" required for long chain fatty acids is derived principally from ingested carbohydrates. Glucose is broken down to pyruvic acid which is then oxidized to acetyl CoA in mitochondria. The acetyl CoA thus produced condenses with oxaloacetate to form citrate which in turn diffuses out of mitochondria and is recleaved into acetyl CoA and oxaloacetate by an extra mitochondrial system (Srere and Lipman, 1953). Acetyl CoA is utilized for both fatty acid synthesis as well as acyl primer for chain elongation after carboxylation to malonyl CoA (Bhaduri and Srere, 1963, Lowenstein 1963). Obviously any defect in glucose metabolism would lead to a decrease in the incorporation of radioactivity into lipids and would not represent the status of lipid formation per se.

In view of the reports of possible disturbances in the utilization of carbohydrates under the influence of steroid contraceptives (Spellacy, 1969), it was decided to carry out the incorporation studies using acetate-1-C\textsuperscript{14} and palmitate-1-C\textsuperscript{14}.

**Studies on Palmitate-1-C\textsuperscript{14}**

Albumin increases the solubility of FFA to about 1000 times in an aqueous solution (Spector et al. 1969). Most of the albumin bound FFA is palmitic acid (Shafir et al. 1965). In the present study palmitate-1-C\textsuperscript{14} was complexed with bovine serum albumin before administering
intravenously. Palmitate-\( ^{14} \text{C} \) administered by this route is almost immediately oxidized or esterified. The labelled palmitate is also picked up by liver and other tissues. However, due to a possible exchange, some part of labelled palmitate can remain in blood as FFA for about 3 hours (Bragdon and Gordon, 1958, Fredrickson and Gordon, 1958, McCalla et al. 1957). Also more than 50 percent of injected FFA is incorporated into TG (Olivercrona, 1962, Baker, 1967). The incorporation of radioactivity after the intravenous administration of palmitate-\( ^{1} \text{C} ^{14} \) was carried out in TG only. Radioactivity of TG in liver, plasma, and heart was determined one hour after administering of labelled FFA so that reutilization of the isotope does not complicate the findings.

Specific activity (CPM/mg lipid) of TG was taken as a parameter of TG synthesis and it was observed that in liver of rats treated with mixed type of steroid contraceptives TG synthesis was not affected (Table XXXV). However, a significant reduction in specific radioactivity of liver TG of rats treated with DP was observed (P < 0.001 Table XXXV). Unluckily it was not possible to study the kinetics of TG synthesis in the liver. In fasted rats, plasma TG is almost entirely dependant upon its synthesis in the liver. Not surprisingly, therefore no effect on the specific activity of plasma TG was observed (Table XXXVI) in the rats of mixed group. In DP group, there was an apparent decrease in specific activity of plasma TG but this decrease was not found to be statistically significant due to variations in
individual values (Table XXXVI). Again it was not possible to study the rate of transport of TG in this investigation. But it was observed that ratio of sp. activity of TG of liver to that of plasma remain almost constant (Table XXXVIII) suggesting thereby indirectly that possibly pool size of newly formed TG in liver is not changed enormously. Perusal of incorporation data in TG of heart show (Table XXXVII) that in control rats, sp. activity was more than that of plasma but in DP and the mixed groups, there was not much of difference in sp. activity of TG of heart from that of plasma. In fact sp. activity of TG of heart was less than that of plasma. It is likely that under normal conditions TG is synthesised in the heart and in treated rats, heart is dependent upon plasma for its TG. An attempt was also made to study lipogenesis in adipose from palmitate-C₁₄ but TG concentrations and its sp. activity were not affected (Table XXXIX). However, it is very difficult to comment on the significance of these findings without knowing the adipose pool size which is not easy to determine. On the basis of incorporation of radioactivity from palmitate-C₁₄ it may be concluded that the pathway of TG synthesis is intact in the rats of mixed group and hypertriglyceridaemia is not a result of increased TG synthesis. On the other hand in rats of DP group there is a decrease in the synthesis of TG in the liver. Nevertheless to have still better overall picture of synthesis of TG, investigations were carried out to study the incorporation of radioactivity from acetate-1-C₁₄
into different lipids.

Studies on Acetate-$1-^{14}C$.

Acetate can enter the pool of acetyl CoA and thus forms "raw material" not only for TG synthesis but also for cholesterol synthesis as was shown quite early by Bloch and Rittenberg (1942). It was, therefore, decided to study the incorporation of radioactivity from acetate-$1-^{14}C$ into the different lipid fractions. Acetate-$1-^{14}C$ was also administered intravenously. The results show that sp. activity of liver TG of rats treated with mixed type of steroid contraceptives did not differ from that of normal rats (Table XI). It means that even from this precursor TG synthesis is not effected in the livers.

However, there was only a marginal decrease in the sp. activity of TG in livers of female rats treated with progestin type of steroid contraceptives ($P < 0.10 > 0.05$) (Table XII). As the incorporation of radioactivity from acetate-$1-^{14}C$ into cholesterol and phospholipids (Tables XIII and XLIII) of livers of these rats did not change, a reduction in the incorporation into TG does not appear to be due to a preferential utilization of this precursor into lipids other than TG. However, it has been reported that whenever citrate or activators of acetyl CoA carboxylase are not present, fatty acids synthesis from acetate in rat liver preparations at least in vitro is depressed (Morquis et al. 1968). Status of citrate and other activators is still to be looked into in the treated rats. Similarly, further
work on the efficiency of enzyme system and availability of cofactors is also necessary. Results of sp. activity of plasma TG (Table XLIII) are suggestive of comparatively higher output of TG into plasma. In fact there is a significant increase in the mixed group. In rats of DF group increase was not found to be statistically significant evidently due to overlapping of the values. There was no effect on the specific activities of other lipids of plasma. Studies on the lipids of the hearts did not reveal any defects in the incorporation of radioactivity from acetate (Tables XLVI - XLVII). There was a significant decrease in the radioactivity of TG of uterus in rats treated with mixed contraceptives (Table XLVIII). As specific activity of TG in uterus was much lower than that of plasma, most likely uterine TG represents only the transport of TG from plasma. A decrease in TG may be due to a defect of the cellular activity of uterine cells. In rats treated with estrogenic contraceptive, pyometra formation was quite common. Furthermore these contraceptives may also induce uterine hyperaemia (Spasiani and Suddick, 1967 and McKercher et al. 1973). These influences may indeed change the turnover of the cells leading to a lowered TG localization in the uterus. Such a contention is supported by a decrease in the specific activity of other lipids in the uterus. Specific activities of free and esterified cholesterol (Tables XLIX - L) and phospholipids (Table LI) were also decreased.
Transport of Hepatic TG to Peripheral Blood

Triglycerides after their endogenous synthesis in the liver are transported to the peripheral blood. Major transport vehicle of endogenously synthesised TG is VLDL (Bensadoun et al. 1974). Plasma lipoproteins are in a dynamic state being degraded and synthesised continuously. But to study the catabolic status of VLDL is very difficult since an exchange of lipid and protein components between lipoprotein themselves and between lipoprotein and cells cannot be controlled. Thus transfer of TG from VLDL to LDL and HDL has been shown (Nicholas and coworkers, 1965) 1968 and Quarfordt et al. 1971). There are two methods which are popularly used to study the transport of TG from liver. One of the methods involves the perfusion of labelled fatty acids and the specific activity of the labelled TG in the blood is followed. As this method is based upon a number of assumptions like a constant TG pool size and similar distribution of TG in different compartments, it may give variable results. The other method is based upon the fact that plasma TG concentration is the result of a balance between its entry into and removal from blood. If the removal of the TG from blood can be inhibited, the resultant increase in the plasma can then be taken as a function of the transport of TG from its endogenous source. In the fasted animals the only source of endogenous TG is the liver. Detergents have been used as common inhibitors of the transport of TG from blood to tissues. Kellmer and his
coworkers (1950, 1951) were the first to report that intravenous administration of non ionic detergent like triton WR 1339 (oxyethylated tertiary oxytlphenol, formaldehyde polymer), leads to hyperlipaemia. It was further suggested that this detergent changes the lipoprotein (VLDL) structure, so that lipoprotein lipase does not act (Brown et al. 1953). Schots et al. (1957) conclusively showed that the physical alteration of the lipoprotein is the basis of hyperlipaemia. Studies of Seamu et al. (1961 a,b and 1962) confirmed these findings. Very soon the use of triton WR 1339 became a standard method to study the transport of TG from liver to blood (Otway and Robinson, 1967, Baker et al. 1968). In the present study triton WR 1339 was used to investigate the transport of TG from liver to peripheral blood. To avoid the possibility of complications due to changes in plasma volume by repeated bleedings from the same rat, it was decided to bleed rats only once. Therefore different batches of rats were used to determine plasma TG levels with and without triton treatment. Thus results on transport were obtained from pooled data. Triton WR 1339 had no toxic effects on the body and liver weights (Table LII - LIV) and on liver lipid profiles (Table LV - LIVII) of rats in different groups. Similar conclusions have also been drawn by other workers (for references see Recknagel, 1967).

It was observed that packed cells volume of blood of rats in different group did not change and so plasma volume
was presumed to be 4 percent of the total body weights as suggested by Alcindor et al. (1973). Treatment of rats with triton brought about a significant increase in the concentration of plasma TG and from the increase of TG, rate of transport of triglyceride expressed as mg/min/100 g body weight was obtained. The results showed that TG transport remained within normal limits and did not increase. TG transport remained between 0.195 - 0.230 mg/min/100 g body weight in the three groups of rats (Table LVIII). These findings are very much similar to those of Lombardi and Ugazio (1965) who reported that the hepatic triglyceride output was around 100 mg/hour/Kg body weight in fasting state. Backnagel (1967) also observed a rate of 0.097 to 0.123 mg/min/100 g body weight in normal overnight fasted rats. In fact a reduction in the transport of TG to blood can lead to fatty liver formation as has been shown by Lombardi et al. (1968). Furthermore, when transport of TG was expressed as a function of per unit of liver, there was a reduction in the transport (Table LIX). These studies clearly show that hypertriglyceridaemia cannot be the result of an increase in the output of TG from liver to the peripheral blood. Though the uptake of plasma TG by the tissues of tritonized rats is blocked for a few hours the present study was restricted to acute period (1 hour after the administration of triton) under the assumption that increasing concentration of TG per se might tend to decrease the hepatic TG output. The present results, therefore, reflect the true transport rate and there is no
likelihood of underestimating the transport rate in any of the groups, even though the concentration of plasma TG in rats treated with mixed type of contraceptives was higher than that of the other two groups. It has also been reported that VLDL synthesis and secretion in liver may be under the control of fatty acid pool size which induced apoproteins involved in lipoprotein synthesis (Alcindor et al. 1970). These findings over and above of showing no increase in the transport of hepatic TG, therefore, also suggest that availability of FFA for lipogenesis is not effected by treating the rats with steroid contraceptives.

Fat Utilization Studies

The studies so far presented showed that neither the TG synthesis nor its transport from liver to blood was increased in the rats treated with steroid contraceptives, it was therefore decided to investigate TG utilization. The mechanisms of TG disposal can be either hepatic or extrahepatic. It is well recognised that a part of chylomicrons can be 'trapped' by the liver. French et al. (1958) and Rodbell et al. (1964) have shown that Kupffer cells have the capacity to take up artificial fat emulsion without hydrolysis. As reviewed by Robinson (1970), 10-15 percent of the injected dose of chylomicron can be initially trapped in rat liver. The most important sites of fat utilization are however, extrahepatic. Vascular luminal surface lined with endothelial cells entraps the chylomicrons (Schoefl and French 1958, Williamson, 1964). It is believed that the confinement
of chylomicrons in the luminal spaces is necessary before they can travel to extravascular spaces. It appears that some structural change of chylomicron may be necessary before the fatty acid component can be picked up from the blood (Robinson, 1970). Thus a study of the kinetics of removal of TG from the blood is necessary to show that undue 'trapping' of injected fat does not vitiate the results of fat utilization. In the intact animals, TG is present in two types of lipoproteins. Endogenously synthesized TG is present as VLDL and exogenously ingested TG is transported as chylomicrons. However, both these lipoproteins particularly chylomicrons are sequestered at the endothelial surface in tissues rich in clearing enzymes (Schoeffl and French, 1958, Williamson, 1964, Suter and Majno, 1959 and Moskowitz and Moskowitz, 1965). Furthermore, in view of the belief that both the endogenous and exogenous triglycerides compete for the same enzyme system (Brunzell et al., 1973), it is possible to study the systemic capacity of the body to utilize fats. This can be investigated by studying the rate of clearance of suitable fat administered intravenously. Intralipid, an artificial fat emulsion having particle size equivalent to that of chylomicron and used as intravenous nutrition, is almost a physiological triglyceride for this purpose. It has been shown by Nicoll et al. (1977) that intravenous fat tolerance test is a reflection of rate of VLDL catabolism in human beings. When the concentration of TG in the blood is extremely high, intralipid clearance rate
may follow a zero order kinetics but under physiological conditions, fat utilization follows 1 order kinetics (Boberg et al. 1969). It has also been shown by Boberg et al. (1969) that there is no correlation between $K_1$ (zero order kinetics rate constant) and $K_2$ (1 order kinetics rate constant). $K_2$ remains unaffected by exogenous fat infusion and the concentration of plasma triglyceride level (Bouchier and Bronte-stewart, 1963, Feinberg et al. 1961, Mashford and Nestel, 1961, Balodimose et al. 1962). Furthermore in any study on fat utilization, adverse effects of a possible change in plasma total pool size of TG should also be ruled out (Nestel 1964). To obtain meaningful results it is necessary, therefore, to show that the kinetics of intralipid clearance is not changed in rats treated with steroid contraceptives. Results of a typical experiment on plotting as log of nephelometric readings as a function of time (Fig. 4) show that the rate of intralipid clearance follows first order kinetics in all the three groups. Data obtained from different rats (Table IX - LXIII) were used to calculate $t/2$ graphically. A significant increase of $t/2$ for intravenous fat tolerance in rats treated with either progestin or mixed type steroid contraceptive was observed. In view of the absence of frank hypertriglyceridaemia in rats of DP group, a significant increase of $t/2$ of this group is extremely interesting. This means that despite the absence of any increase in blood TG levels, there is a latent defect becoming evident in intralipid utilization studies. In fact Intralipid tolerance should be carried out
to evaluate the presence of latent hypertriglyceridaemia.
Attempts to study the fat tolerance in women using steroid contraceptives have been made earlier. Hazzard et al. (1969) have carried out oral fat tolerance as a measure of fat utilization. Their results were not suggestive of any defect in fat utilization. But oral fat tolerance is not a correct method for the assessment of fat utilization (Fredrickson et al. 1967). Rösner et al. (1971) have observed an increase in plasma TG levels in women after two cycles of contraceptives therapy. They were, however, unable to find a defect in intralipid utilization even though there was a reduction in the levels of post-heparin plasma clearing enzymes. It is likely that these authors carried out the intralipid tolerance after too short a period of contraceptive therapy. Kissenbah et al. (1973) have surprisingly come to a conclusion that intralipid utilization is increased in women using steroid contraceptives. Their findings are extremely difficult to be explained in view of a decrease in clearing enzymes by other workers. However, Kissenbah et al. (1973) did not observe an increase in the clearing enzymes in women using mixed type of steroid contraceptives and it is very difficult to rationalize their findings of accelerated I.V. fat utilization. As mentioned above intralipid utilization competes with endogenous TG for the enzymes involved in their utilization, further studies were therefore planned on lipoprotein lipase.
Studies on Lipoprotein Lipase

Utilization of TG is regulated by an enzyme system. The most important enzyme of this system is lipoprotein lipase and any reduction in this enzyme can obviously lead to hypertriglyceridaemia. Such a possibility in reduction of the activity of the enzyme can be assessed experimentally. Irrespective of their origin, utilization of triglycerides is regulated by lipoprotein lipase which should not be confused with hormone sensitive lipases of adipose tissue (Khoo et al. 1972, 1974). Thus both VLDL and chylomicrons act as substrates for the same enzyme (Brunzell et al. 1973). An increase in t/2 of intravenous intralipid utilization is indeed a reflection of a reduction of the enzyme activity per se.

In this section, therefore, attention was directed to the study of the enzyme lipoprotein lipase.

Normally very little activity of the enzyme is present in blood. Enzyme is secreted in circulation only after intravenous administration of heparin (Hahn 1943). Enzyme so released in the blood is called post heparin lipoprotein lipase (PHLA). Estimation of PHLA after the administration of heparin as a measure of lipoprotein lipase activity has been well established (Fredrickson and Gordon, 1959, Korn, 1959 and Robinson, 1963). Rats in the three groups were administered heparin at a dose level of 500 u/rat and plasma was obtained from them 10 minutes thereafter for the estimation of PHLA. It was observed that there was a significant reduction in the levels of PHLA in the two groups of female rats treated
with steroid contraceptive (Table LXV). A characteristic property of PHLA has been shown to be its inhibition with protamine sulphate or concentrated sod. chloride solution (Rizak, 1961 and Bjorntop and Furman, 1962 a,b). The results of such a study (Table LXIV) show that enzyme was inhibited both by protamine sulphate as well as by a solution of sodium chloride and so the enzyme under study was indeed PHLA. Furthermore the conditions used for the assay of the enzyme (Fig. 1-3) were optimum with regards to the concentration of enzyme and that of substrate. Unluckily there is a little confusion regarding the dosage of heparin which should be administered before estimating plasma PHLA. It has been reported (Elkeles, 1974) that a high dosage of heparin may be inhibitory to the secretion of lipoprotein lipase, therefore, PHLA of plasma of rats after the administration of very low dose of heparin was also studied. Accordingly each rat was administered intravenously 5 units of heparin before collecting plasma for the estimation of PHLA. Again a significant reduction in the levels of PHLA of treated rats was observed. (Table LXVI). Thus there appears to be a genuine reduction in the levels of PHLA in treated rats. Before discussing the various mechanisms which may influence PHLA it is pertinent to understand the mode of action of heparin in stimulating the enzyme secretion in blood. Unluckily exact mechanism of action of heparin is not yet clear. Evidence, however, suggest that heparin or related particles may be forming an integral part of the enzyme (Korn, 1959).
Alternatively it has also been suggested that heparin might be stabilizing lipoprotein lipase or might be competing with endogenous binding moiety responsible for linking the enzyme with its cellular sites (Robinson, 1965 and Korn, 1959). Keeping in view these possible mechanisms of action of heparin, a number of postulates can be considered which may account for the reduction in PHLA in rats treated with steroid contraceptives.

As suggested earlier, pregnancy or treatment with steroid contraceptives can bring about fluid retention (Nelson and Strickland, 1970). This fluid retention may cause a haemodilution of the administered heparin and may lower down the effective concentration of heparin in the treated rats. However, such a possibility does not appear to exist in the present study as PHLA remained depressed after the administration of both the high (500 u/rat) as well as low (5 u/rat) doses of heparin. All the more, recently it has been shown that the final concentration of an injected dose of heparin to women using steroid contraceptives was similar to that of the normal women (Ence et al. 1976). Concentration of PHLA may depend upon the balance of its rate of release and inactivation in plasma (Yoshitoshi et al. 1963). It has been reported that the concentration of thyroid and corticosteroi binding proteins can increase after a treatment with estrogens (Doe et al. 1967). Similarly if the concentration of PHLA binding proteins also increases in the blood then the availability of enzyme would be limited.
Hazzard et al. (1972) have presented the evidence of show that such a possibility does not develop after treatment with steroid contraceptives. Bnce et al. (1976) have also shown that $t/2$ of PHIA is not changed in women using steroid contraceptives. This means that there is no likelihood of formation of PHIA binding proteins after the treatment with contraceptives. That the concentration of PHIA is lowered down after the treatment with steroid contraceptives has also been shown by various workers (Fabian et al. 1971, Hazzard et al. 1969 a,b,1972 and Rosner, et al. 1971).

One more aspect of PHIA action worth considering is the mode of action of this enzyme. Eventhough positional specificity is not yet established, lipoprotein lipase of rat has been reported to release FFA from $C_1$ in preference to $C_2$ and $C_3$ of TG (Assman et al. 1973). PHIA may also contain a phospholipase which acts on $C_1$ of PE or PC (Jackson et al. 1976). Any possibility of a change in the positional specificity of the enzyme or change in nature of substrates is also ruled out as both high as well as low doses of heparin gave similar results. There was no question of a possibility of a change in nature of substrate as Intralipid was used in all the enzymes assays. As PHIA ultimately is located in the cells as lipoprotein lipase it becomes necessary to assess the concentration of enzymes in the tissues.

Lipoprotein lipase is present in heart, adipose tissue, lactating mammary gland and liver apart from other tissues (Robinson, 1963). In adipose tissue the activity
of enzyme goes down in conjunction with a reduction in the triglyceride fatty acid uptake like fasting. It has also been reported that adipose tissue contains hormone sensitive lipases (Vaughan et al. 1964 and Gorin and Shafrir, 1964). The size of adipocytes may also influence the lipoprotein lipase activity (Nestel et al. 1969). Even though the size of adipose cell seems to be similar in human males and females (Sjostrom et al. 1972) further work is still necessary to establish the effect of steroid contraceptives on the size of adipocytes. Similarly liver contains various other lipases also (Robinson, 1965). So the major tissue suitable for carrying out the enzyme levels is heart. Suitability of heart for such a study has been further provided by the fact that TG undergoes hydrolysis before being extracted by myocardium (Schurer and Olsen, 1967). This hydrolysis seems to be brought about by lipoprotein lipase which is susceptible for its release by heparin and is localised on the plasma membranes of the heart cells (Muir, 1968). Therefore, it was decided to estimate the levels of lipoprotein lipase in hearts of rats treated with steroid contraceptives. For the extraction of enzyme fresh tissue was used instead of their acetone powder. Hanosh and Hanosh (1975), did not observe any effect of estrogen treatment on rat lipoprotein lipase when acetone powdered of the tissue was used as the source of the enzyme. Nilsson-Ehle (1974) has shown that the extracting medium containing heparin gives not only preformed enzyme but also the one which can be activated and secreted.
Wilson et al. (1976) showed by using the extraction procedure of Hilson-Ehle (1974) that treatment of male castrated rats with estrogen can bring about an increase in the activity of lipoprotein lipase. The aim of the present investigation was to find out the basis of reduction in the concentration of PHLA and therefore, medium containing heparin was used to extract the enzyme from fresh heart. Surprisingly, however, there was no depression in the levels of heart lipoprotein lipase as a result of treatment with steroid contraceptives (Table LXXII). This means that PHLA level is reduced but lipoprotein lipase in the tissue is not depressed. Under these conditions it was thought that perhaps there was some change in the conformation of affinity of tissue plasma membranes so that enzyme is bound rather strongly in the rat treated with steroid contraceptives. With this in mind attempts were made to improve the utilization of intravenously administered Intralipid.

Studies on the Improved TG Utilization

Haszard et al. (1969 a,b) have suggested that the reduction of PHLA may be due to the development of resistance to heparin under the treatment with steroid contraceptives. However, the nature of resistance is not clear. It was decided to attempt to increase the qualitative nature of stimulus. Heparin stimulates the secretion of lipoprotein lipase from the tissues. Also a physiological stimulus to the activity of enzyme is provided by exogenous TG. It was,
therefore, decided to provide both these stimuli together. Consequently each rat was administered 0.5 ml intralipid containing 1 unit of heparin intravenously and rate of utilization of intralipid was followed. As shown (Fig. 5) intralipid utilization was improved dramatically in treated rats. As a result $k_2$ and t/2 became equal to that of the normal rats. (Table LXVII - LXXI). Thus it appears that the heparin stimulus and physiological substrate can act synergistically.

Though the exact mechanism of the release of the enzyme from the cell surface is not clear, it is believed that it may involve a competition between the soluble heparin and membrane bound polysaccharide on the one hand and conformational changes in the protein molecules on the other hand. It is also widely believed that lipoproteins bind to heparin and the complex so formed is rapidly picked up by the tissues for the action of lipoprotein lipase. Protein part of the membrane serves to anchor the polysaccharide chains and lipoprotein lipase is attached to these chains by electrostatic interaction. It may be necessary for the enzyme molecules to move into the plane of the membrane before acting on lipoproteins (Olivecrona et al. 1977). Under the action of steroid contraceptives, there may be change in the membrane so that the mobility of the enzyme is retarded or else there may be other changes involving the anchoring chains.

**Interrelationship of different parameters**

This study is an outcome of an attempt to underline
various mechanisms involved in the induction of hypertriglyceridaemia caused by the treatment of steroid contraceptives. Obviously such a study requires an animal model which has been produced in female rats. It was shown that treatment of female rats with mixed type of steroid contraceptives can indeed induce hypertriglyceridaemia. On the other hand pure progestins did not induce frank hypertriglyceridaemia but brought about latent hypertriglyceridaemia as shown by a delay in the utilization in intravenously administered intralipid. In fact intralipid utilization is the method of choice in the detection of latent hypertriglyceridaemia. In this study no attempt was made to delineate the effects of estrogens alone as these hormones are always used along with progestins as contraceptives.

In the study of mechanism involved, the first pathway to be studied was obviously synthesis of triglycerides. The method used was to study the incorporation of radioactivity from acetate-1-C\textsuperscript{14} and palmitate-1-C\textsuperscript{14}. It has been reported that estrogens increase TG synthesis (Matkins et al. 1972, Lusky et al. 1974 and Glueck et al. 1974). Investigations carried out in this study showed that there was no effect on synthesis of TG in liver of female rats treated with mixed type of steroid contraceptives. On the other hand a reduction in the TG synthesis in liver after a treatment with medroxyprogesterone acetate was observed. Similar reduction in vitro lipogenesis of adipocytes has been observed by Hertelendy et al. (1976) in female rats treated with medroxyprogesterone
acetate. These workers reported a significant reduction in radioactivity incorporation from glucose-1-C\textsuperscript{14} and glucose 6-C\textsuperscript{14} into lipids, further the ratio of radioactivity incorporated from the two precursors into 14CO\textsubscript{2} was not changed after treatment with the contraceptives. This may mean that a decrease in lipogenesis may be independent of effects on glucose oxidation and there was no preferential input of glucose in EMP or glycolytic pathways.

Results show a significant reduction in the utilization of intralipid in both the groups. Hazzard et al. 1969 a,b did not observe any significant effect on fat utilization in women using steroid contraceptives but they carried out oral fat tolerance which has been shown to be a defective method. Though Kekki and Nikilla (1971) observed no effect of steroid therapy on intralipid utilization their patients were normolipidaemic. Kissenbah et al. (1973) reported paradoxically an increased utilization of TG by mixed type of contraceptives. They also observed an increased rate\textsuperscript{14}C\textsubscript{0} synthesis but at the same time they could not observe an increase in the input of FFA into TG or any effect on PHIA. Thus their findings are not easy to follow.

As the utilization of fat is dependent upon the availability of lipoprotein lipase, a study of this enzyme was carried out in the treated rats. High as well as low doses of heparin were administered before collecting plasma for estimating PHIA. Findings showed a reduction in PHIA level but no change was observed in lipoprotein lipase concentration of heart.
There are a number of reports in which attempts have been made to explain the decrease in PHLA. An increase in turnover of PHLA, or inhibitors of enzymes or heparin are ruled out by experimental evidence (Hazzard et al. 1969 a, b and Enoe et al. 1976). Similarly any possibility of the haemodilution of injected heparin is also ruled out by direct estimation of heparin in women using steroid contraceptives (Enoe et al. 1976) Hazzard et al. (1969 a, b) believe that the defect is not in the depletion of the enzyme but in the release of the enzyme. Our data support such a hypothesis. Furthermore, as TG utilization can be brought back to normal by simultaneous administration of heparin and intralipid, it appears that the binding bonds between the enzyme and the tissue are more rigid in the treated rats. Further work is necessary to study the nature of binding and any conformational changes on plasma membranes where enzyme seems to be linked.