서울의대잡지 제10권 제4호
The Seoul Journal of Medicine
No. 10, Vol. 4, December, 1969

Effects of Clofibrats on Carbon Tetrachloride-Induced Fatty Liver in Rats

— With Special Reference to Liver Triglycerides —

Won Ik Park

Department of Biochemistry, College of Medicine, S. N. U

<Director: Professor Ki Yung Lee and Assistant Professor Han-Seob Kim>

In 1962, Thorp and Waring[1] reported that administration in rats of clofibrate (ethyl 2-(p-chlorophenoxy)-2-methylpropionate= ethyl p-chlorphenoxyisobutyrate, Atromid-S, see Fig. 1) and the combination of clofibrate with androsterone (Atromid)

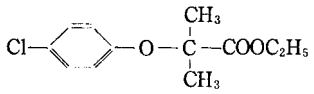

Fig. 1. Chemical structure of ethyl 2-(p-chlorophenoxy)-2-methylpropionate=ethyl p-chlorophenoxyisobutyrate, clofibrate, Atromid-S, a ethyl ester of C.P.I.B. (=chlorophenoxyisobutyric acid).

reduced the plasma cholesterol level. Later in the same year Oliver[2] showed that Atromid lowered elevated serum cholesterol and triglycerides (TG) levels in man. Subsequently, however[3], Oliver himself demonstrated that the administration of clofibrate alone was as effective as Atromid. Since these earlier papers, considerably more experience has been gained concerning the effect of clofibrate, and now it seems well established that clofibrate decrease plasma levels both of TG and cholesterol in humans and rats although the mechanism of this effect has not been fully defined. The active form of clofibrate in vivo seems to be the free chlorophenoxyisobutyric acid (C.P.I.B.) bound to albumin[4].

On the other hand, the induction of a fatty liver in experimental animals following the administration of carbon tetrachloride (CCl_4) has been amply documented[5-7]. The etiology for the accumulation of hepatic fats by CCl_4 has not been settled[5], but chemical investigation revealed that the rise in liver lipids following CCl_4 was primarily due to increased hepatic TG[6]. Most studies field in the past aimed at obtaining the greater knowledge regarding mechanism by which human liver damage is produced, but in recent years this model has been used in study of lipid, especially TG, metabolism[8,9]. At the present time available evidences support the hypothesis that such an accumulation of lipids in liver tissue by CCl_4 is due to a block in the transfer of TG from the liver to the plasma[10,14].

Thus clofibrate and carbon tetrachloride both undoubtedly produce wide spread effect on lipid metabolism in living organism. It would be most interesting at this stage to see the effects of clofibrate on liver lipids, especially TG, of CCl_4 intoxicated rats, which may help for the elucidation of mechanisms involved in the action of clofibrate as well as that of CCl_4.

Effects of clofibrate administration on the lipid content of CCl_4-induced fatty liver in rats

were investigated, and will be presented in the following together with the plasma lipid content determined.

Materials and Methods

Male albino weighing approximately 200-300 g supplied from our regular breeder were distributed into 4 groups, paying attention so as to roughly match with body weight in each group; and they were raised in wire-bottom cages, in each of which 3-4 animals were put together. A period of two weeks was allowed for stabilization on our regular feed, which consisted of, with ad lib water, ground wheat, dry fish-powder (3%) and dry milk (3%); and on bloc group body weight of each animal group noted. To the half of animals or to the two out of 4 groups of animals 0.2% clofibrate in their feed was given for 21 days. Clofibrate (Imperial Chemical Industries Ltd., Great Britanin) was dissolved in ether, mixed with the requisite amount of feed, and the ether allowed to evaporate. On the 2lst day on clofibrate feed the body weights of individual animals were checked; and to the halves of clofibrate and regular feed fed rats, CCl_4 in liquid praffin (3:7 by volume) in amount of 0.5 ml/100 g body weight was given by stomach tube. To the rest halves of clofibrate fed and regularly fed rats, 1.0-1.5 ml of liquid paraffin was given similarly. Thus each group of animals received following treatment:

Group I (controls), regular feed and liquid parafaffin;

Group II (CCl_4), regular feed and CCl_4 (in liquid paraffin);

Group III (clofibrate), clofibrate feed and liquid paraffin;

Group IV (clofibrate & CCl_4) clofibrate feed and CCl_4 (in liquid paraffin).

After an overnight fast following the administration of CCl_4 to the appropriate groups as indicated above, all groups of animals were killed by rapid decapitation, when the blood was cellected into tubes containing EDTA (25 mg), and the liver tissue removed quickly, weighed and stored for a while in the freezing compartment of a refrigerator.

Liver lipids were extracted twice in aglass-homogenizer by the method of Folch et al[15], from accurately weighed liver tissue of approximately 500 mg, with 2 : 1 chloroform-methanol mixture to the final volume of 10 ml. The extract was washed with 2 ml of distilled water and the volume of single phase lipid extract was brought to the 10 ml, in a calibrated test tube, with additional chloroform. Suitable portions of this liver lipid extract were used for each fractional lipid analsis.

Liver and prasma TG were determined by the method of Van Handel[16] and Van Handel et al[17]. Commercial olive oil purified by treating bulk amount of Doucil (W.A. Taylor, U.S.A.) in chloroform, the chloroform being evaporated in vacuo, was used as TG standard. Assessment of plasma free fatty acids (FFA) was done according to Kim et al[18], using the same filtrate of plasma for TG determination. Fatty acid standard used was linoleic acid (Sigma, U.S.A.). Liver and plasma total cholesterol was determined by the method of *Z*ak et al[19], and phospholipids after the method of Connerty et al.[20].

Results

I. Plasma Lipids

The effects of clofibrate administration for 21 days on the mean fractional plasma lipids of rats overnight fast after the force feeding of CCl_4 are summarized in Table I and visua-

Table I. Plasma Lipid Level of Control, Clofibrate Given, CCl_4 Given, and Clofibrate and CCl_4 Given Rats (0.2% Clofibrate in the feed given for 21 days and CCl_4 given once on the las day of clofibrate administration).

Treatment	Rat No.	Triglycerides (mg/100 ml)	Cholesterol (mg/100 ml)	Phospholipids (mg/100 ml)	Free Fatty Acids (μEq/1)
Control (Liquid Paraffin)	1	52.3	96.6	184.0	980
	2	68.1	71.3	164.4	1,200
	3	48.6	85.3	171.3	1,100
	4	70.2	76.3	167.5	1,000
	5	48.0	83.5	150.0	810
	6	59.8	90.2	170.0	890
	7	68.3	84.2	161.4	860
(Mean)		59.33	84.17	166.8	977.1
(S.D.)		9.74	4.42	10.35	138.2
CCl_4	1	60.6	96.6	183.4	1,040
	2	70.2	78.6	149.8	1,420
	3	67.2	81.0	159.3	920
	4	54.5	78.6	162.7	1,200
	5	58.4	91.2	142.0	1,200
	6	42.3	93.0	178.9	840
	7	39.0	88.2	157.5	900
(Mean)		56.03	86.74	161.80	1,074.3
(S.D.)		12.75	7.35	14.88	208.7
Clofibrate (and Liquid Paraffin)	1	44.5	68.3	191.8	696
	2	39.4	52.6	168.7	760
	3	45.5	68.5	149.8	596
	4	51.8	81.0	153.5	566
	5	56.5	77.3	158.1	920
	6	39.9	56.7	125.0	850
	7	38.4	64.1	171.3	900
(Mean)		45.14	67.07	159.76	855.4
(S.D.)		5.62	8.81	20.77	142.5
Clofibrate & CCl_4	1	56.0	88.6	209.0	952
	2	52.2	89.3	150.0	690
	3	38.0	70.6	149.8	728
	4	61.0	70.6	140.4	870
	5	51.4	60.0	168.7	780
	6	59.1	62.6	172.0	860
	7	48.6	71.8	171.3	896
(Mean)		52.33	73.36	165.89	825.1
(S.D.)		7.68	14.14	22.71	160.2

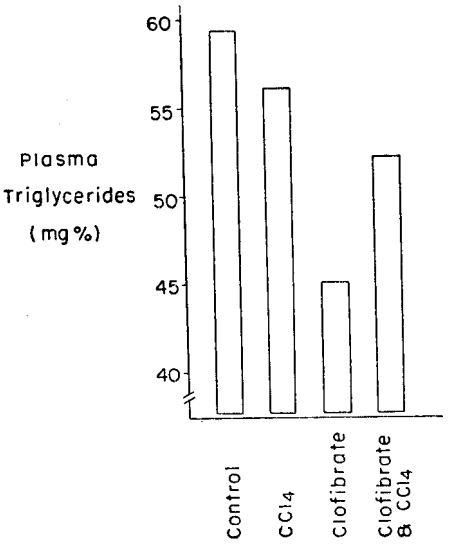

Fig. 2. Effects of CCl_4, clofibrate & CCl_4 on plasma triglyceride level in rats. CCl_4 in liquid paraffin (3:7 by volume) 0.5 ml/100 g body weight by stomach tube; clofibrate at 0.2% in feed for 3 weeks (The same experimental conditions apply to all figures following).

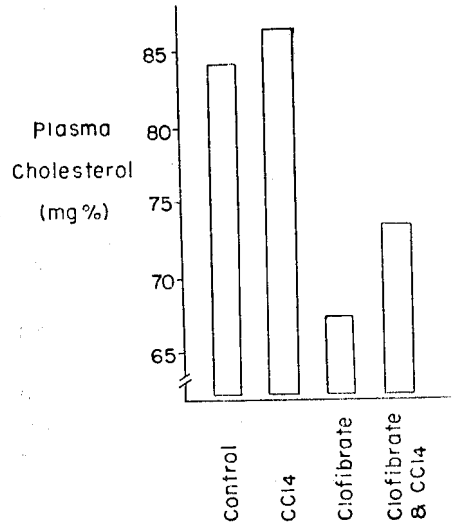

Fig. 3. Effects of CCl_4, clofibrate, and clofibrate & CCl_4 on plasma cholesterol level in rats.

lized in Fig. 2 through 5. Attention should be drawn to the group relationship among the 4 groups of animals in interpreting the plasma, and liver lipid values as well. Thus Group Ⅰ animals served variously as controls for Group Ⅱ with respect to the effect of simple CCl_4 administration; for Group Ⅲ with respect to simple clofibrate effect; and for Group Ⅳ to the combined effects of clofibrate and CCl_4.

In comparison with the mean plasma TG level of 59.33 (±9.74) mg/100 ml of controls (Group Ⅰ), that of the CCl_4 group (Group Ⅱ) showed the value of 56.03 (±12.75) mg/100 ml, with very little change from the controls. The decrease in plasma TG of clofibrate group (Group Ⅲ) was a remarkable one, from 59.33 mg/100 ml of controls down to 45.14 (±5.62) mg/100 ml ($P<0.01$). Combined effects of clofibrate and CCl_4 (in Group Ⅳ) on plasma mean TG level revealed the change (from 59.33 of controls to 52.33 (±7.68) mg/100 ml of test) statistically insignificant ($P>0.05$). The implication of this finding was meaningful in the context of the present experiments, for administration of CCl_4 to the clofibrate fed rats (Group Ⅳ) lessoned the signific ant TG lowering effect of clofibrate, as manifested in Group Ⅲ rats, to the statistically insignificant extent.

The mean plasma total cholesterol level of Group Ⅰ, Ⅲ and Ⅳ rats showed very much similar patterns of group alterations to those seen with plasma TG content (Group Ⅰ versus Ⅲ: $P<0.01$; and Group Ⅰ versus Ⅳ: $P>0.1$), indicating again the effect of CCl_4 to lessen the cholesterol lowering effect of clofibrate.

The mean plasma phospholipid levels of 3 test animal groups were all alike more or less lower than that of controls, but the differences were insignificant.

Plasma FFA level of clofibrate fed Group Ⅲ

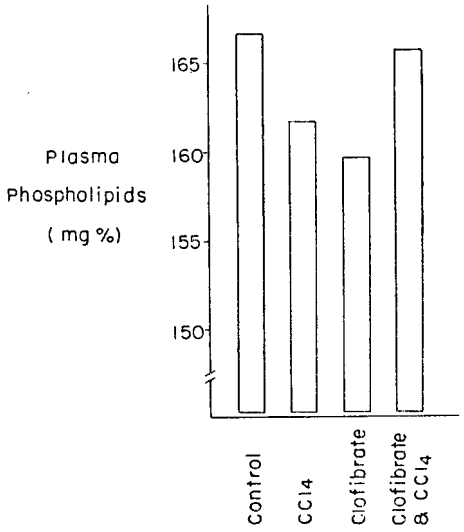

Fig. 4. Effects of CCl_4, clofibrate, and clofibrate & CCl_4 on plasma phospholipid level in rats.

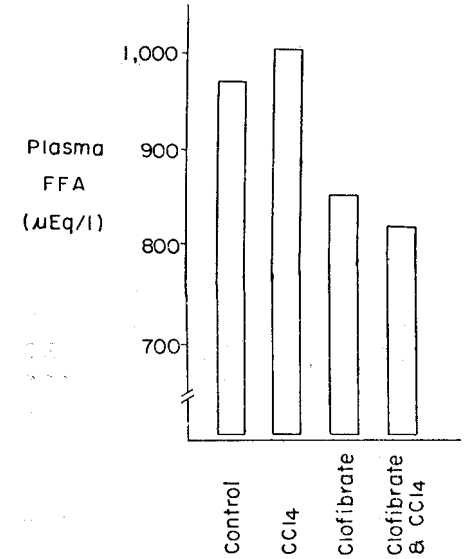

Fig. 5. Effects of CCl_4, clofibrate, and clofibrate & CCl_4 on plasma free fatty acid level in rats.

and Ⅳ animals were considerably lower than that of controls, although the decreases were not statistically significant (P's>0.1). Plasma FFA concentration of CCl_4 given rats (Group Ⅱ) was higher than that of controls, again without statistical significance (P>0.2). But the difference in plasma FFA concentration between Group Ⅲ and Ⅲ or Ⅳ, whatever the meaning of this statistical analysis might be for the moment, was significant with the probability of P<0.05.

II. Body Weight, Liver Weight and Liver Lipids (Table II, and Fig. 6 though 9)

1. Body and Liver Weight The en bloc group body weight at the biginning and individual body weight of the rats at the end of feeding with or without clofibrate for 21 days showed a variable degree of body weight gain by each of the 4groups of experimental animals. The slight disagreeable odor of clofibrate causing aversion for feed by the animals might have inevitably resulted in lesser body weight gain observed in the drug fed groups.

To evaluate the changes in liver weight apart from the body size incurred by experimental conditions set upon each group of animals, fractions of liver to total body weights were calculated, as appear in Table Ⅱ. The increment in liver weight was only nominal in CCl_4 treated rats as compared to controle (P>0.5), but it was definitely significant in clofibrate treated rats (P>0.0001). The liver weight fraction to total body weight of clofibrate and CCl_4 treatedrats (Group Ⅳ) rose to the same degree as that of simply clofibrate treated ones (Group Ⅲ), but it came out statistically insignificant (P>0.1) probably because of the larger standard deviation. It seems that clofibrate induces hepatomegaly as previously observed by others[2, 21], but CCl_4

Table Ⅱ. Body Weight, Liver Weight and Liver Lipids of Control, Clofibrate Given, CCl_4 Given, and Clofibrate and CCl_4 Given Rats (Clofibrate given for 21 days and CCl_4 given once on the last day or clofibrate administration).

Treatment	Rat No.	Body Weight (g)		Liver Weight (g) and % Body Weight	Triglycerides (mg/g)	Cholesterol (mg/g)	Phospholipids (mg/g)
		Initial*	Final				
Control (Liquid Paraffin)	1		275	8.51 (3.1)	5.6	3.6	39.7
	2		250	7.35 (2.9)	6.1	3.1	46.2
	3		288	8.54 (3.0)	6.6	4.1	35.2
	4		250	7.88 (3.2)	7.0	4.0	43.3
	5		225	6.63 (2.6)	8.3	3.5	40.2
	6		225	7.37 (3.3)	7.1	3.2	45.5
	7		300	9.21 (3.1)	—	3.5	35.2
(Mean)		250.0	259.0	7.927 (3.06)	6.78	3.56	40.24
(S.D. #)			29.58	0.855 (0.16)	0.93	0.38	3.7
CCl_4	1		275	7.37 (2.7)	94.5	4.5	42.0
	2		300	9.84 (3.3)	59.0	6.4	35.2
	3		313	9.94 (3.2)	74.8	4.0	35.4
	4		275	7.55 (2.7)	86.2	4.0	36.2
	5		225	9.14 (4.1)	82.0	4.8	32.0
	6		250	8.89 (3.6)	81.4	4.5	36.2
	7		250	6.86 (2.7)	88.3	5.4	44.2
(Mean)		260.6	269.7	8.513 (3.16)	80.89	4.80	37.31
(S.D.)			30.6	1.25 (0.52)	10.59	0.85	4.25
Clofibrate (and Liquid Paraffin)	1		263	9.68 (3.7)	6.7	3.3	42.1
	2		283	10.16 (3.6)	4.0	2.4	37.0
	3		283	10.47 (3.7)	4.5	3.5	34.3
	4		250	8 94 (3.6)	4.7	2.9	41.9
	5		238	7.71 (3.2)	5.6	3.0	44.2
	6		300	8.45 (2.8)	5.1	3.3	41.6
	7		225	8.52 (3.8)	6.9	2.8	40.0
(Mean)		261.9	263.1	9.133 (3.47)	5.36	3.03	40.16
(S.D.)			27.1	1.003 (0.11)	1.10	0.49	4.15
CCl_4 & Clofibrate	1		275	9.10 (3.3)	71.7	3.0	39.7
	2		300	11.12 (3.7)	59.1	4.5	32.0
	3		283	9.81 (3.5)	84.2	2.8	35.2
	4		275	8.30 (3.0)	62.5	3.6	35.2
	5		275	8.04 (2.9)	57.3	3.7	44.2
	6		300	13.44 (4.5)	58.5	2.3	33.5
	7		238	7.88 (3.3)	61.8	4.4	46.2
(Mean)		275.0	278.0	9.670 (3.48)	65.01	3.47	38.0
(S.D.)			20.9	2.016 (0.52)	8.99	0.82	5.17

* Calculated from blanket group body weights.

\# Standard deviation.

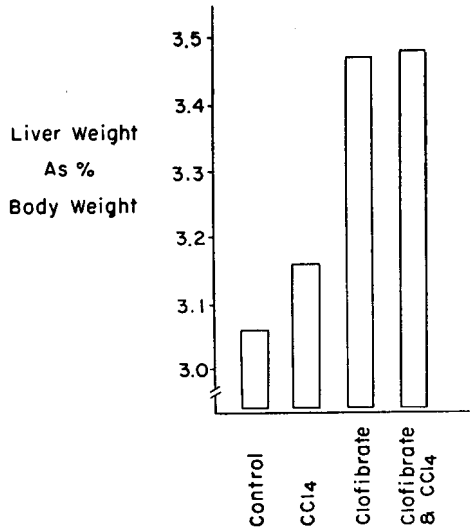

Fig. 6. Effects of CCl_4, clofibrate, and clofibrate & CCl_4 on liver weight as % of body weight of rats.

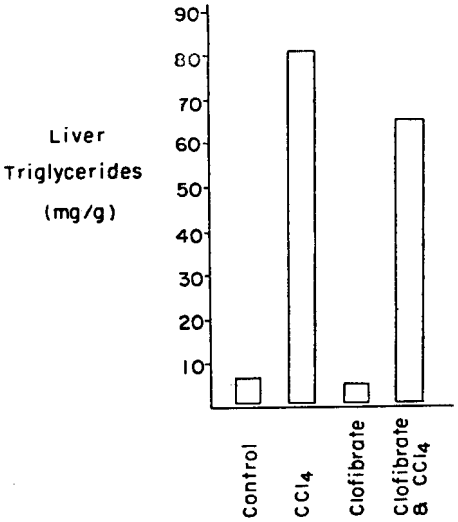

Fig. 7. Effects of CCl_4, clofibrate, and clofibrate & CCl_4 on liver triglyceride content in rats.

blur the clofibrate effect (in Group Ⅳ)by way of marked individual fluctuation in liver size increment.

2. Trigly cerides The mean values were 6.78 (±0.93) mg/g for the controls (Group Ⅰ) and 5.36 (−1.10)mg/g for the clofibrate administered animals (Group Ⅲ), a significant decrease ($P<0.01$). To compensate for the increase in liver weights of clofibrate group (Gronp Ⅲ) described previously, total quantities of TG in the liver were calculated (unit weight liver TG X total liver weight). The mean values indicated 53.75 (±4.73) mg for the controls and 48.95 (±9.56) mg for the the clofibrate fed animals (Group Ⅲ), a nonsignificant decrease. Administration of CCl_4 to regularly fed rats (Group Ⅱ) resulted in a expected large increase in liver TG concentration: from 6.78 mg/g of controls to 80.89 (10.59) mg/g of CCl_4 rats. Total liver TG quanties were also remarkably increased, from 53. 75mg (of Group Ⅰ) to 688.62 (±67.44) mg (of Group Ⅱ). Similar results with total liver TG by the administration of CCl_4 were seen in the clofibrate fed animals: from 48.95 mg of Group Ⅲ to 628.65 (-134.83) mg of Group Ⅳ rats. Clofibrate treatment of rats, however, resulted in significantly less accumulation of mean unit weight liver TG by CCl_4 (in Group Ⅳ) than the non-treated rats (Group Ⅲ): i e, liver TG for the former were 65.01 (±8.99) mg/g, and for the latter 80.89 mg/g ($P<0.02$). The difference between the total liver TG of these two animal groups was not statisticallt significant ($P>0.1$).

3. Cholesterol The mean liver .cholesterol content was significantly increased by CCl_4 administration in the regularly fed animal groups: from 3.56 (±0.38) mg/g controls (Group Ⅰ) to 4.80 (±0.85) mg/g of test (Group Ⅱ) ($P<0.01$); and it was significantly decreased by clofibrate in the diet: from 3.56

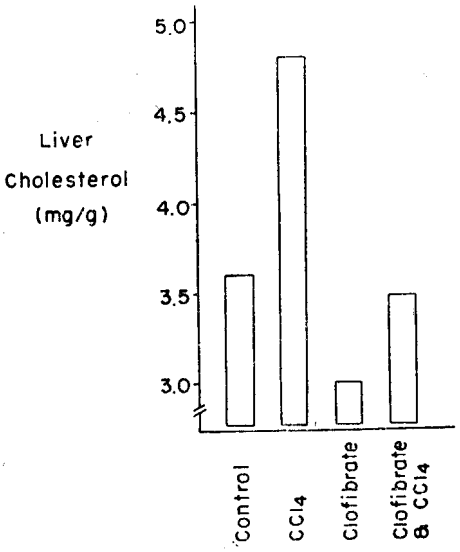

Fig. 8. Effects of CCl_4, clofibrate, and clofibrate & CCl_4 on liver cholesterol content in rats.

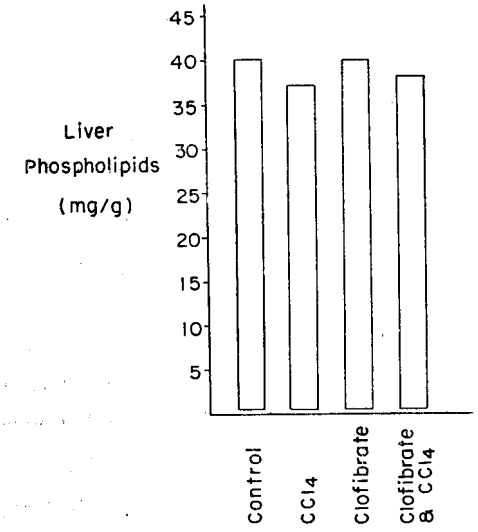

Fig. 9. Effects of CCl_4, clofibrate, and clofibrate & CCl_4 on liver phospholipid content in rats.

mg/g of controls to 3.03 (±0.49) mg/g of test (Group Ⅲ) ($P<0.05$). In the clofibrate fed animal groups, administration of CCl_4 resulted in insignifiant increase in liver cholesterol: from 3.03 mg/g of Group Ⅲ to 3.47(±0.82) mg/g of Group Ⅳ rats ($P>0.05$). Combined effects of clofibrate and CCl_4 again resulted in insignififcant increase in liver cholesterol: from 3.56 mg/g of controls (Group Ⅰ) to 3.47 mg/g of test animals (Group Ⅳ) ($P>0.05$).

4. Phospholipids None of the mean liver phospholipid data of the 4 groups of animals obtained had deviated significantly from each other. The slight decrease of CCl_4 given animals (group Ⅱ) in phospholipid content may represent the increased liver weight of this group due possibly to the accumulation of lipids after CCl_4, although the liver weight gain observed in this group of animals (Group Ⅱ) was only slight, as stated above previously. The liver enlargement observed in the clofibrate fed Group Ⅲ and Ⅳ animals did not cause any reduction in phospholipid content of these animals, however. The mean total liver phospholipid quantities of Group Ⅲ and Ⅳ animals were remarkably similar 364.32 (±58.36) mg/g and 360.61 (±14.09) mg, respectively, whereas those of controls were 320.75 (±27.81)mg. It seems, therefor, that while the CCl_4 play little part in regard to liver phospholipids, clofibrate may actively influence by retention of these lipids in the liver.

Discussion

The reduction of both the plasma TG and cholesterol in man[22,23] as well as in experimental animals[24] by clofibrate is now well established. The significant decreases in the mean plasma concentration of TG and cholesterol after 3 week simple clefibrate administ-

ration in Group Ⅲ animals of the present expeiments, as compared with those of controls (Group Ⅰ), were but an another manifestation of the hitherto known effect of the drug. Although Hellman et al[25] reported 17% decrease in plasma phospholipid content by clofibrate in study with humans, our data with rats showed that the decreases were not statistically significantly different from those in controls. The primary effect of clofibrate, in fact, was to reduce the TG-rich lipoproteins of very low density lipoproteins (Sf 2-400), and to a lesser degree the cholesterol-rich low density lipoproteins (Sf 0-20)[26] In our results plasma FFA concentration of clofibrate fed Group III animals was also decreased, but without statstical significance. However, arguements are still going on over the effect of clofibrate upon plasma FFA, which, in fact, has been implicated in explaining the mechanism of hypolipidemic action of clofibrate by one school[27] as will be discussed later.

In commenting on our plasma lipid data of CCl_4 treated rats, suffice it to cite for the momenta more inclusive work by Stern et al[28], who examined over 72 hours the changes in plasma lipid levels induced by CCl_4. According to them, plasma TG, cholesterol and phospholipid concentration decreased during the first 6hours and remained low for at least 16 houres after CCl_4. Plasma TG level subsequently rose with higher level than control value during the 18-26 hour period, corresponding at which time our experimental animals were at once killed. Plasma cholesterol and phospholipid concentration likewise rose, but did not exceed control levels either at the 18-26 hour pour period on 48 hours after CCl_4 administration. By 72 hours all three plasma lipid values were markedly higher in animals received CCl_4 than in control animals. Plasma FFA concentration did not differ from control values during the 2-6 hour intervals, but rose during the 8-16 hour period and continued to rise throughout the times studied.

The aim of the present investigation was to observe the effect of clofibrate upon serum and especially, liver lipids with in rats fatty liver induced by CCl_4 administration. In evaluating our results it seems appropriate that we should be well aware of the proposed mechanisms of action of clofibrate and CCl_4 known to-day in living organism.

In Thorp and Waring's original study[1], the cholesterol lowering activity of clofibrate appeared to coincide in time with spontaneous seasonal reduction in serum cholesterol of control rats, and they suggested that the clofibrate augmented a rhythmic hypocholesterolemic mechamism. In view of previous findings of Hellman et al[29] that thyroid hormone led to increased androsterone and that this androgen metabolite was hypocholesterolemic in man, they suggested further that androsterone might be the endogenous agent responsible for the phasic changes in cholesterol. They administered clofibrate with androsteron (Atromid) to rats and found that the combination produced a continuous hypocholesterolemic response[1]. The hypolipidemic effect of Atromid was confirmed in man soon thereafter[225]. They postulated that clofibrate either displaced androsterone from plasma protein[1] or acted by competing for the binding capacity of serum albumin, displacing acidic hormones, such as thyroxine, leading to their localization in the liver where it produces alterations in enzymic mechanizm leading ultimately to reduction in plasma concentration of cholesterol and TG[30]. However, all investigators now agree that oral androsterone contribute nothing to the hypolipidemic effect of clofibrate. Furt-

her studies demonstrating the failure of adrenalectomy and gonadectomy to prevent hypolipidemic effect of clofibrate in rats[31] and man[32] suggest that the mechanism of action is not concerned with potentiation of endogenous steroid hormones. Furthermore, clofibrate has been shown to influence thyroxine distribution as suggested by Thorp[30], but by reducing the hepatic content and volume of distribution of thyroxine, not increasing[33], which gives the additional evidence that its mode of action probably is not mediated via thyroid hormone metabolism. Other investigator suggest that clofibrate may act as an inhibitor of cholesterol biosynthesis[25]. Azarnoff et al[34] found that the stage of inhibition of clofibrate is between mevalonic acid and isopentenyl pyrophate. Other studies by the same authors[34] and reported others[35, 36] suggested that the hypolipidemic action of clofibrate also may be due to a partial failure of hepatic secretion of TG.

On the other hand, considerable amount of work has been done ever the last several decades in an attempt to define the biochemical changes associated with the effect of CCl_4 on hepatic lipids. Yet the etiology for the accumulation of hepatic fats by CCl_4 has not been settled. Chemical investigation revealed that rise in liver lipids following CCl_4 was due primarily to increased hepatic TG[6]. The first increase in liver TG after single dose of CCl_4 appears within 1 to 2 hours; the accumulation proceeds rapidly thereafter, and by 3 hours the level of hepatic TG was doubled the control value[6]. During the last several years, ample evidence[10, 13], including that of Stern et al[28] refered above, have accumulated to show that in the early stage of acite CCl_4 intoxication there is a direct relationship between the accumulation of TG in the liver and the decrease in the concentration of plasma TG. These evidences are consistent with the hypothesis, first proposed by Recknagel et al[10], that the development of fatty liver, in CCl_4treated rats, is primarily due to a block in the release of hepatic TG to the plasma. According to this hypothesis, fatty acids mobilized from adipose tissue are transported to the liver where they are rapidly esterified to TG. Carbon tetrachloride, however, interfere with secretion of hepatic TG into the plasma, with consequent accumulation of TG in the liver and a fall of their concentration in the plasma. Evidence for such a blook came from experiments by Heimberg et al[18], in which it was shown that isolated perfused livers, obtained from rats treated with CCl_4 3.5 hours before perfusion, failed to release TG into the perfusate. Interference by CCl_4 with secretion of hepatic TG has also been shown in experiments with labeled fatty acids by Maling et al[11], and by Schotz et al[41], In these experiments in was shown that after intravenons injection of palmitic acid-1-^{14}C, only traces of label appear in the plasma TG of CCl_4 treated rats. Using a complex mathmatical analysis of their results Schotz et al have also estimated that in the CCl_4 treated animals the rats of hepatic TG secretion into the plasma is only 10% of normal.

In view of action mechanism of CCl_4 and of one of the hypothese that partial failure of hepatic secretion of TG by clofibrate, like CCl_4, as described above, we were expecting more marked accumulation of TG in livers of rats given clofibrate and CCl_4 (Group Ⅳ) than in than in those given CCl_4 alone (Group Ⅱ). Our anticipation crumbled at the moment when we opened the abdomens to excise livers of a fewe xperimental animals of Group Ⅱ and Ⅳ. The degree of fatty infiltration

in the two groups were such that it was easily discernible by macroscopic views that fatty infiltration was more marked in the livers of Group Ⅱ animals. Thus in our results it was evident that the liver TG of both clofibrate and CCl_4 given animals (Group Ⅳ) were significantly less than those found in simply CCl_4 given animals (Group Ⅱ), showing apparently the preventive effect of clofibrate on liver TG accumulation by CCl_4. We had at first no feasible explanation for the observed reduction by clofibrate in TG of fatty liver induced by CCl_4. Speculation was than made from our data that the results could be reasonably ascribable to the reduction in plasma FFA of the Group Ⅳ when compared with that of Group Ⅱ animals. It has been well known that the plasma FFA, being building block of TG in the liver, regulate the synthesis of TG in this tissue. Studies of the fatty acid composition of liver TG, plasma FFA, and adipose tissue TG were consistent with the finding that liver TG were synthesized from plasma FFA mobilized from adipose tissue[38]. And adepuate evidence is available to show that hepatic extraction of FFA is proportional to the blood concentration[39]. Sustained hypermobilization of FFA by intravenous infusion of noradrenaline in dogs leads to progressive and rapid increase in liver TG[38]. From these much evidences and from our reduced FFA values observed in Group Ⅳ animals with delivery of low FFA concentration to the liver, it was reasonable to see decreased liver TG in these animals as were in our results. It seems appropriate to recall at this time that, although the reduction in plasma FFA of clofibrate and CCl_4 given animals (Group Ⅳ) was not statistically significant from those of controls (Group Ⅰ), the difference in FFA concentration between the CCl_4 given (Group Ⅱ) and both clofibrate and CCl_4 given (Grovp Ⅳ) rats was statistically significant ($P<0.05$).

As for the lowering of plasma FFA concentration observed in clofibrate treated animals of the present experiments (Group Ⅲ and Ⅳ), we admit there are controversy going over this effect of clofibrate. In fact this problem has to do with the action mechanism, in addition to those cited above, of clofibrate itself by some investigation, as was touched previously. Sachs[22] stated that the plasma FFA was not affected by clofibrate. On the other hand, Barrett and Thorp[27] demonstrated that oral admininistion of clofibrate reduced the resting FFA levels in both rats and dogs. In addition they and others[40] presented experimental evidence showing that C.P.I.B., the active metabolite of clofibrate (clofibrate is a C.P.I.B. ethyl ester), reduces rate of free fatty acid release from epididymal fat pad incubated in plasm in vitro. Barrett et al went further that the final hypolipidemic effect of clofibrate is ultimately related to FFA lowering effect of clofibrate. As Nestel[41] found, the increment in plasma FFA was directly related to the fasting plasma TG level. It is therefore highly probable that the substantial reductions in plasma TG observed in man and experimental animals treated with clofibrate are partly the result of the reduction in plasma FFA. Although there are reports that clofibrate has to do with cholesterol synthesis as stated previously, the more profound decrease in the plasma TG concentration as compared to cholesterol by clofibrate and the observation that the greatest falls in cholesterol values by clofibrate occur in subjects with highest plasma TG[22] suggest that the primary action of clofibrate may be on TG synthesis. However, there have been other reports againtst this, so to speak, "FFA theory" for the

hypolipidemic action of clofibrate. FFA turnover and TG production was found normal during clofibrate administration[42], and the inhibition by clofibrate of catecholamine-induced release of fatty acids from adipose tissue was not responsible for the lower ing of blood lipids[43].

Atttention should be drawn again to our results in that, despite the significant reduction in unit weight liver TG of both clofibrate and CCl_4 administered animals (Group IV)as compared to that of simply CCl_4 treated ones (Group II), the mean total liver TG (and phospholipid) quautities of the animals of the two groups (Group II and IV) came out roughly equal due to the liver enlarging effect of clofibrate observed in drug treated animals ie (Group IV). The increase in liver size in rats by clofibrate has been previously reported by others[2,21], but the direct cause of this hepatomegaly is unknown. It is of inetrest that the administration of clofibrate to man has been observed to produce a transient increase in serum GOT level[2]. It seems that liver enlargement was of hyperplastic in nature, for total liver DNA content was increased in clofibrate treated rats[44]. At any rate, clofibrate was shown in our experiments to lower high lipid concentration induced by CCl_4, but not to lower total quantities in terms of whole liver lipids. If clofibrate has any effect to raise the clearence of TG from the body[45,45] the amount of TG cleared from the body would be equal to plasma TG reduction multiplied by plasma volume, which is a minor fraction of total liver or total body TG. The result that reduction in TG concentration, but not in total TG, of liver by clofibrate in CCl_4 induced fatty liver may pose another problem in attacking the mechanism of clofibrate by other investigators in the future.

Summary

Studies were carried out to see the effect of pre-treatment of rats with clofibrate, now widely used as hypolipidemic agent, on the CCl_4 induced fatty liver, with special reference to TG. Clofibrate incorporated into feed to contain 0.2% was fed to experimental animals for 3 weeks, and CCl_4 in liquid paraffin (3:7 by volum in amount of 0.5ml/100 g body weight (or 1.0-1.5 ml of liquid paraffin) was given by stomach tube on the last day of experimental feeding.

Results obtained were as follows.

Clofibrate feeding significantly reduces TG accumulation produced by CCl_4, preventing the lipid accumulation would result by simple CCl_4 administ ation. This result may cast suspicion against the concept that clofibriate, like CCl_9 as proposed, partially hinders release of TG from the liver. Based on our plasma lipid values determined, the most likely explanation for the lesser accumulation of TG by clofibrate in CCl_4 induced fatty liver, could be found in the low plasma FFA level observed in our clofibrate & CCl_4 administered animal group. While major plasma lipid fractions (primarily TG and cholesterol clofibrate & CCl_4 treated animals showed in general values in between those of each clofibrate alone and CCl_4 alone treated animals, the plasma FFA did not get along this line of alteration. Plasma FFA concentration of CCl_4 group was the highest among those of the experimental groups studied, but the FFA was significantly low in clofibratd & CCl_4 as well as simple clofibrate treated animals as compared with the CCl_4 groups. Previously reported hepatomegalic effect of clofibrate was manifested whether or not experimental animal

were treated with CCl_4. Due to this liver enlargement, the calculated total liver TG (and phospholipids) of clofibrate & CCl_4 treated animals had approximately the same amount of lipids) found in simply CCl_4 given animals, indicating minor clearance of the lipids by clofibrate from the CCl_4 in toxicated fatty liver as a whole.

—國文抄錄—

Clofibrate가 4鹽化炭素(CCl_4)誘發 白鼠 脂肪肝의 脂質量에 미치는 影響

——特히 肝中性脂肪量을 中心으로——

서울大學校 醫科大學 生化學敎室

(指導: 李基寧 敎授 및 金漢燮 助敎授)

朴 源 益

血淸脂質低下劑 clofibrate (ethyl 2-(*p*. chlorophenoxy) -2-methylpropionate (I. U. C. 化學名)=ethyl *p*-chlorophenoxy-isobutyrate (通用化學名), Atromid-S (商標)) 가 1962년 學界에 새로히 紹介된 以來 이에 대한 많은 硏究가 있었으나 그 作用機轉은 아직 뚜렷이 밝혀진바 없다. 한편 實驗動物에 4鹽化炭素 (CCl_4)를 주면 肝損傷, 特히 急性投與時 脂肪肝이 誘發된다는 것은 周知의 事實로서 이러한 模型은 오래前부터 人體肝障碍機轉을 糾明하는데, 또 近來에는 脂質 特히 中性脂肪(triglycerides)代謝를 硏究하는데 利用되어 왔다. CCl_4 誘發脂肪肝의 發生機轉도 아직 뚜렷치는 않으나 現在 學界에서 共認하는 것은 CCl_4가 肝에 直接 作用하여 肝에서 血漿으로 中性脂肪을 分泌하는 정상段階를 抑制한다는 說이다. Clofibrate의 作用機轉에 對하여서는 CCl_4와 같은 肝一血漿 中性脂肪分泌抑制說, cholesterol 生合成阻止說, 內分泌系作用說等이 登頭되어 있으나 學界의 定說로 歸着된 說은 아직 없다. 如何튼 clofibrate나 CCl_4가 生體脂質代謝에 積極的으로 關與함은 疑心할 餘地가 없는바, 此際에 이들 兩者間의 關係를 살펴봄은 生體脂質代謝를 硏究함에 있어서나 이들의 作用機轉을 밝히는데 있어서도 大端히 有益한 일로 思料된다.

實驗動物白鼠는 다음과 같이 4群으로 나누었다.

第1群: 通常白鼠飼料로 3週間 飼育하고 飼育最終日에 paraffin油를 體重에 따라 1.0~1.5 ml 胃管投與(對照群).

第2群: 第1群처럼 飼育하고 飼育最終日에 CCl_4-paraffin油(配合容積比 3:7)를 0.5 ml/100 g 體重으로 胃管投與(CCl_4群).

第3群: Clofibrate를 0.2% 되게 飼料에 混合하여 3週間 飼育하고 飼育最終日에 第1群처럼 處理(clofibrate群).

第4群: 第3群처럼 飼育하고 飼育最終日에 第2群처럼 處理(clofibrate 및 CCl_4群).

CCl_4-paraffin油 또는 paraffin油 投與後 各動物群은 굶기고서 다음날 일제히 屠殺, 肝 및 血漿에서 中性脂肪, cholesterol 및 燐脂質, 그리고 血漿에서는 遊離脂酸(FFA)까지를 각각 定量하였다.

實驗結果 얻어진 知見은 要約 다음과 같다. 即 clofibrate는 CCl_4 誘發脂肪肝의 中性脂肪量을 顯著하게 減少시켜 CCl_4 單獨投與時에 볼 수 있는 中性脂肪의 蓄積을 輕減케 하는 效果를 나타냈다. 이는 最少限 clofibrate의 肝中性脂肪分泌抑制說을 反證하는 結果라 보여진다. Clofibrate가 肝의 中性脂肪分泌를 억제한다면 CCl_4와 함께 投與하였을 때 CCl_4 單獨投與時보다 더 많은 中性脂肪蓄積을 招來했어야 하기 때문이다. clofibrate가 CCl_4 誘發脂肪肝에서 이와 같은 影響을 끼친것은 clofibrate 및 CCl_4 群(第4群)의 血漿遊離脂酸値가 낮았던 사실에 연유하는 것으로 생각된다. 即 各實驗動物群間의 血漿脂質値를 比較할 때 cholesterol 및 CCl_4 群(第4群)은 主要血漿脂質(中性脂肪 및 clofibrate)量에 있어 大體로 CCl_4 群(第4群)과 clofibrate 群(第3群)의 中間値를 보이나 唯獨 혈장 FFA 만은 clofibrate 群(第3群)과 더부러 CCl_4 群(第2群)보다 훨씬 낮았었다.

이미 報告된 것처럼 本 實驗結果에서도 clofibrate의 肝腫大效果가 clofibrate 單獨投與時 뿐만 아니라(第3群) CCl_4를 投與하였을 때(第4群)도 觀察되었다. Clofibrate 및 CCl_4 群(第4群)의 肝腫大를 考慮한 全肝中性脂肪(및 燐脂質)量을 算出, CCl_4 單獨投與群(第2群)과 比較해본 結果는 兩群이 서로 比等한 量을 나타내었다. 即 Clofibrate는 비록 CCl_4 유발지방간의 單位重量當 中性脂肪(및 燐脂質)量을 減少시키기는 하였으나 脂肪肝의 總體的인 脂質量은 減少시키지는 못하였다.

REFERENCES

1. Thorp, J. M., and Waring, W. S.: *Modification of metabolism and distribution of lipids by ethyl chlorophonexyrate. Nature 194. 948, 1962.*
2. Oliver, M. F.: *Reduction of serum lipids and uric*

acid levels by an orally active androsterone. Lancet 1, 1321, 1962.

3. ———: *Further observations on the effects of Atromid and of ethylchlorophonexyisebutyrate on serum lipid leveles. J. Atheroscl. Res. 3, 421, 1963.*
4. Thorp, J. M.: *Experimental evaluation of an orally active combination of androsterone with chlorephenobutyrate. Lancet i, 1323, 1962.*
5. Recknagel, R. O.: *Carbon tetrachloride hepatotoxicity. Pharmacol. Rev. 19. 145, 1967.*
6. Schotz, M. C., and Recknagel, R. O.: *Rapid increase of rat liver triglyceriees following carbon tetrachloride poisoning. Biochim. Biophys. Acta 41, 151, 1960.*
7. Bang, S. W., and Lee, K. Y.: *Effects of 24-hour starvation on the lipid content of carbon tetrachloride-induced fatty liver in mice. Seoul J. Med. 9, 1, 1968.*
8. Schotz, M, C., Baker, N., and Chavez, M. N.: *Effect of carbon tetrachloride ingestion on liver and plasma triglyceride turnover rates, J. Lipid Res., 5, 569, 1964.*
9. Baker, N., Garfinkel, A. S., and Schorz, M. C.: *Hepatic TG secretion in relation to lipogenesis and FFA mobiligation in fasted and glucose. Refed rats J. L. R. 9, 1, 1968.*
10. Recknagel, R. O., Lombardi, B., and Schotz, M. C.: *A new insight into pathogenesis of carbon tetrachloride fat infiltration. Proc. Soc. Exp. Biol. Med. 104, 608, 1960.*
11. Maling, H. M., Frakn, A., and Horning, M. C.: *Effect of carbon tetrackloride on hepatic synthesis and release of triglycerides. Biochim. Biophys. Acta. 64, 540, 1962.*
12. Heimberg, M., Weinstein, I., Dishmon, G., and Dunkerley, A.: *The action of carbon tetrachloride on the transport and metabolism of triglycerides and fatty acids by the isolated perused rat liver and its relationship to the etiology of fatty liver. J. Biol. Chem. 237, 3628, 1962.*
13. Seakins, A., and Robinson, D. S.: *The effct of the administration of carbon tetrachloride on the formation of plasma lipoproteins in the rat. Biochem. J. 86, 401, 1963.*
14. Schotz, M. C., Baker, N., and Chavez, M. N.: *Effect of carbon tetrachloride ingestion on liver and plasma triglyceride turnover rates. J. Lipie Res. 5, 569, 1964.*
15. Folh, J., Lees, M., and Sloane Stanley, G. G.: *A simple method for the isolation and purification of lipides from animal tissues. J. Biol. Chem. 226, 479, 1957.*
16. Van Handel, E.: *Suggested modification of the micro-determination of triglycerides. Clin. Chem. 7, 249, 1961.*
17. Van Handel, E., and Zilvermit, D. B.: *Micro method for the direct determieation of serum triglycerides. J. Lab. Clin. Med. 50, 152, 1957.*
18. Kim, H.—S., Bang, S. W., and Lee, K. Y.: *Colorimetric determination of plasma free fatty acids (FFA). Seoul Univ. J., Series C, 19, 1, 1968.*
19. Zak, B., Dickenman, R. C., White, E. G., Burnet, H., and Cerney, P. J.: *Rapid estimation of free and total cholesterol. Amer. J. Clin. Pathel. 24, 1307, 1654.*
20. Connerty, H. V,. Briggs, A., and Eaton, F. H., Jr.: *Simplified determination of the lipid components of blood serum. Clin. Chem. 7, 37, 1961.*
21. Avoy, D. R., Swyryd, E. A., and Gould, R. G.: *Effects of a-p-chlorophenoxyisobutyrayl ethyl ester (CPIB) with and without androsterone on cholesterol biosynthesis in rat liver, J. Lipid Res. 6, 369, 1965.*
22. Sachs, B. A.,: *Appraisal of clofibrate as a hypollpemic agent. Amer. Heart J. 75, 707, 1968.*
23. Song H. S., Lee Y. W., Suh, J. D., Kim, S. C., and Kim, H. S.: *Effect of clofibrate (Atromid-S) on serum lipids, J. Korean Med. Assoc. 12, 824, 1969.*
24. Oliver, M. F.: *The current status of ethylchlorophenoxyisebutyrate (Atromids). in Progress in Bicchemical Pharmacology, Vol. 2, Paoletti, R., Steinberg, D., and Kritchevaky, eds., Karger, Base 1/New York, 1967, p. 1.*
25. Hellman, L., Zumoff, B., Kessler, G., Kraa, E., RubL. in, I., and Rosenfeld, R. S.: *Reduc-*

tion of cholesterol and lipids in man by ethyl p-chloro-Phenoxyisobutyrate. Ann. Int. Med. 59, 477, 1963.

26. Strisower, E. H. : *The respones of hyperlipoproteinmias to atromid and ethyl chlorophenoxyisobutyrate. J. Atheroscl. Res. 3, 445, 1963.*
27. Barrett, A. M., and Thorp, J. M. : *studies on the mofe of action of clofibrate: Effects on hormone-induced changes in plasma free fatty acids in rats and dogs. Brit. J. Pharmacol. 322, 351, 1968.*
28. Stern, P. H., Frukawa, T., and Broby, T. M. : *Rat liver and plasma lipids after carbon tetrachloride administration. J. Lipid Res. 6, 278, 1965.*
29. Hellman, L., Bradlow, H., Zumoff, B., Fukushima, D. K., and Gallaghar, T. F. : *Thyroid-androgen interrelations, and hypocholesterolemic effect of androsterone, J. Clin. Endocrinol. 19, 936, 1959.*
30. Thorp, J. M. : *An experimental appraoch to the problem of disordered lipid metablism, J. Atheroscler. Res. 3, 351, 1963.*
31. Best, M. M., and Duncan, C. H. : *Ethyl p-chlorophenoxyisobntyrats (CPIB) in the rat, J. Lab. Clin. Med. 64, 634, 1964.*
32. Berry, C., Moxham, A, Smith, E., Kellie, A. E., and Nabarro, J. D. J., : *The effects of atromid on the metabolism of adrenal steroids and on plasma lipid fractions, J. Atheroscler. Res. 3, 380, 1963.*
33. Musa, B. U., Ogilvie, J. T., and Dowling, J. T., *Effects of ethyl chlorophenoxyisobutyrate on thyroine distribution, transoprt and metabolism in man, Metabolism, 17, 909, 1968.*
34. Azarnoff, D. L., Tucker, D. R., and Barr, G. A. : *Studies with ethyl chlorophenoxyisobutyrate (clofibrate), Metabolism, 14, 959, 1965.*
35. Miskel, M. A.., and Webb. W. F. : *the mechanism unerlying the hypolipidaemic effect of atromid-S, nicotinic acid and benzmalecene-1 Biochem. Pharmacol. 16, 897 1967.*
36. Dunscan, C. H., Bert, M. M., and Wespopoulos, A. : *Inhibition of hepatic secretion of triglyceride by chlorsphenoxyisobutyrate (CPIB), circulation (Suppl. 3), 30, 7, 1964.*
37. Poggi, M., and Paoletti, R. : *A new insight on carbon tebrachloride effect on lipid transport. Biochem. Pharmcol. 13, 949, 1964.*
38. Fiegelson, E. B., Pfaff, W. W., Karman, A., and Steinberg, D. : *The role of plasma free fatty acids in development of fatty liver, I. Clin. Invest. 49, 2171, 1961.*
39. McEloy, W. T., Siefert, W. L., and Spitzer, J. J. : *Relationship of hepaatic uptake of FFA to plasma concentration. Proc. Sec. Exp. Biol. Med. 104, 20, 1960.*
40. MacMillan, D. C., Oliver, M. F., Simpson, J. D., and Tothill, P. : *Effect of ethyl chlorophenoxyisobutyrate (Atromid-S) on weight, plasma volume, total body water and free fatty acids Lancet i, 924, 1965.*
41. Nestel, P. J. : *Plasma triglyceride concentration and plasma free fatty acid chnges in response to norepinephrine in man, J. Clin. Invest 43, 77, 1964.*
42. Ryan, W. G., and Schwartz, T. B. : *Dynamics of plasma triglyceride turnover in man,* Metabolism, *14, 1243, 1965.*
43. Wuncan, C. H., Best, M. M., and Robertson, G. L. : *A comparison of the effects of ethylchlorophenoxyisobutyrate and on plasma free fatty acides, Lancet i, 191, 1965.*
44. Goul d, R. G., Swyryd, E. A., Coan, B. J., and Avoy, D. R. : *Effects of CPIB in liver composition and TG synthesis in Rats J. Atheroscler. Res 6, 555, 1966.*
45. Ryan, W. G. and Schwartz, T. B. *The dynamics of triglyceride turnover: Effect of Atromid-S, J, Lab. Clin. Med., 64, 1001, 1964,*