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

— With Special Reference to Liver Triglycerides —

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

\[
\text{CH}_3\hspace{1cm}\text{Cl}\hspace{1cm}\text{O}\hspace{1cm}\text{C}\hspace{1cm}\text{COOC}_2\text{H}_5
\]

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\(^2\), 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\(^6\), but chemical investigation revealed that the rise in liver lipids following CCl\(_4\) was primarily due to increased hepatic TG\(^8\). 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\(^9\)–\(^9\). 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 Britain) was dissolved in ether, mixed with the requisite amount of feed, and the ether allowed to evaporate. On the 21st day on clofibrate feed the body weights of individual animals were checked; and to the halves of clofibrate and regular feed fed rats, CCl\textsubscript{4} in liquid paraffin (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 paraffin;
- **Group II (CCl\textsubscript{4})**, regular feed and CCl\textsubscript{4} (in liquid paraffin);
- **Group III (clofibrate)**, clofibrate feed and liquid paraffin;
- **Group IV (clofibrate & CCl\textsubscript{4})** clofibrate feed and CCl\textsubscript{4} (in liquid paraffin).

After an overnight fast following the administration of CCl\textsubscript{4} to the appropriate groups as indicated above, all groups of animals were killed by rapid decapitation, when the blood was collected 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 a glass-homogenizer by the method of Folch et al\textsuperscript{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 analysis.

Liver and plasma TG were determined by the method of Van Handel\textsuperscript{16} and Van Handel et al\textsuperscript{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\textsuperscript{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 Zak et al\textsuperscript{19}, and phospholipids after the method of Connerty et al.\textsuperscript{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\textsubscript{4} are summarized in Table I and visual-
Table I. Plasma Lipid Level of Control, Clofibrate Given, CCl\textsubscript{4} Given, and Clofibrate and CCl\textsubscript{4} Given Rats (0.2% Clofibrate in the feed given for 21 days and CCl\textsubscript{4} given once on the last day of clofibrate administration).

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<th>Rat No.</th>
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<th>Cholesterol (mg/100 ml)</th>
<th>Phospholipids (mg/100 ml)</th>
<th>Free Fatty Acids ((\mu)Eq/1)</th>
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<td>73.36</td>
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<td>7.68</td>
<td>14.14</td>
<td>22.71</td>
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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 CCl₄ administration; for Group III with respect to simple clofibrate effect; and for Group IV to the combined effects of clofibrate and CCl₄.

In comparison with the mean plasma TG level of 59.33 (±9.74) mg/100 ml of controls (Group I), that of the CCl₄ 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 CCl₄ (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 CCl₄ to the clofibrate fed rats (Group IV) lessened the significant TG lowering effect of clofibrate, as manifested in Group II rats, to the statistically insignificant extent.

The mean plasma total cholesterol level of Group I, II and IV rats showed very much similar patterns of group alterations to those seen with plasma TG content (Group I versus II: P<0.01; and Group I versus IV: P>0.1), indicating again the effect of CCl₄ 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 II
and ♂️ animals were considerably lower than that of controls, although the decreases were not statistically significant (P’ ≤ 0.1). Plasma FFA concentration of CCl₄ given rats (Group ♂️) was higher than that of controls, again without statistical significance (P > 0.2). But the difference in plasma FFA concentration between Group ♂️ and ♂️ or ♂️, 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 beginning 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 4 groups 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 I. The increment in liver weight was only nominal in CCl₄ treated rats as compared to control (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 CCl₄ treated rats (Group ♂️) rose to the same degree as that of simply clofibrate treated ones (Group ♂️), 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 others²⁷,²¹, but CCl₄
Table 1. Body Weight, Liver Weight and Liver Lipids of Control, Clofibrate Given, CCl₄ Given, and Clofibrate and CCl₄ Given Rats (Clofibrate given for 21 days and CCl₄ given once on the last day or clofibrate administration).

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<th>Treatment</th>
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<th>Liver Weight (g) and % Body Weight</th>
<th>Triglycerides (mg/g)</th>
<th>Cholesterol (mg/g)</th>
<th>Phospholipids (mg/g)</th>
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<td>4.15</td>
</tr>
<tr>
<td>CCl₄ &amp; Clofibrate</td>
<td>1</td>
<td>275</td>
<td>9.10 (3.3)</td>
<td>71.7</td>
<td>3.0</td>
<td>39.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>300</td>
<td>11.12 (3.7)</td>
<td>59.1</td>
<td>4.5</td>
<td>32.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>283</td>
<td>9.81 (3.5)</td>
<td>84.2</td>
<td>2.8</td>
<td>35.2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>275</td>
<td>8.30 (3.0)</td>
<td>62.5</td>
<td>3.6</td>
<td>35.2</td>
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<tr>
<td></td>
<td>5</td>
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<td>8.04 (2.9)</td>
<td>57.3</td>
<td>3.7</td>
<td>44.2</td>
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<td>13.44 (4.5)</td>
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<td>2.3</td>
<td>33.5</td>
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<td></td>
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<td>238</td>
<td>7.88 (3.3)</td>
<td>61.8</td>
<td>4.4</td>
<td>46.2</td>
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<tr>
<td>(Mean)</td>
<td></td>
<td>275.0</td>
<td>9.670 (3.48)</td>
<td>65.01</td>
<td>3.47</td>
<td>38.0</td>
</tr>
<tr>
<td>(S. D. )</td>
<td></td>
<td>20.9</td>
<td>2.016 (0.52)</td>
<td>8.99</td>
<td>0.82</td>
<td>5.17</td>
</tr>
</tbody>
</table>

* Calculated from blanket group body weights.
# Standard deviation.
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 II), a significant decrease (P<0.01). To compensate for the increase in liver weights of clofibrate group (Group II) 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 II), a nonsignificant decrease. Administration of CCl₄ to regularly fed rats (Group I) 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 CCl₄ rats. Total liver TG quantities were also remarkably increased, from 53.75 mg (of Group I) to 688.62 (±67.44) mg (of Group II). Similar results with total liver TG by the administration of CCl₄ were seen in the clofibrate fed animals: from 48.95 mg of Group II 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 CCl₄ (in Group IV) than the non-treated rats (Group II): 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 statistically significant (P>0.1).

3. Cholesterol The mean liver cholesterol content was significantly increased by CCl₄ 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
mg/g of controls to 3.03 (±0.49) mg/g of test (Group Ⅱ) (P<0.05). In the clofibrate fed animal groups, administration of CCl₄ resulted in insignificant increase in liver cholesterol: from 3.03 mg/g of Group Ⅱ to 3.47 (±0.82) mg/g of Group Ⅱ rats (P>0.05). Combined effects of clofibrate and CCl₄ again resulted in insignificant increase in liver cholesterol: from 3.56 mg/g of controls (Group Ⅰ) to 3.47 mg/g of test animals (Group Ⅱ) (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 CCl₄ given animals (group Ⅱ) in phospholipid content may represent the increased liver weight of this group due possibly to the accumulation of lipids after CCl₄, 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 Ⅱ and Ⅲ animals did not cause any reduction in phospholipid content of these animals, however. The mean total liver phospholipid quantities of Group Ⅱ and Ⅲ 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, therefore, that while CCl₄ 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 man²²,²³ as well as in experimental animals²⁴ by clofibrate is now well established. The significant decreases in the mean plasma concentration of TG and cholesterol after 3 week simple clofibrate adminis-
ration in Group II animals of the present experiments, as compared with those of controls (Group I), were but another manifestation of the hitherto known effect of the drug. Although Hellman et al\textsuperscript{23} 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)\textsuperscript{26} In our results plasma FFA concentration of clofibrate fed Group III animals was also decreased, but without statistical significance. However, arguments 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\textsuperscript{27} as will be discussed later.

In commenting on our plasma lipid data of CCl\textsubscript{4} treated rats, suffice it to cite for the momenta more inclusive work by Stern et al\textsuperscript{28}, who examined over 72 hours the changes in plasma lipid levels induced by CCl\textsubscript{4}. According to them, plasma TG, cholesterol and phospholipid concentration decreased during the first 6 hours and remained low for at least 16 hours after CCl\textsubscript{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\textsubscript{4} administration. By 72 hours all three plasma lipid values were markedly higher in animals received CCl\textsubscript{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\textsubscript{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\textsubscript{4} known to-day in living organism.

In Thorp and Waring's original study\textsuperscript{15}, 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 mechanism. In view of previous findings of Hellman et al\textsuperscript{23} 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 androsterone (Atromid) to rats and found that the combination produced a continuous hypocholesterolemic response\textsuperscript{13}. The hypolipidemic effect of Atromid was confirmed in man soon thereafter\textsuperscript{225}. They postulated that clofibrate either displaced androsterone from plasma protein\textsuperscript{13} 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 mechanism leading ultimately to reduction in plasma concentration of cholesterol and TG\textsuperscript{250}. However, all investigators now agree that oral androsterone contribute nothing to the hypolipidemic effect of clofibrate.
her studies demonstrating the failure of adrenalec-
tomy and gonadectomy to prevent hypolipi-
demic effect of clofibrate in rats\textsuperscript{11} and
man\textsuperscript{12}) suggest that the mechanism of action
is not concerned with potentiation of endoge-
nous steroid hormones. Furthermore, clofibrate
has been shown to influence thyroxine dis-
tribution as suggested by Thorp\textsuperscript{30}, but by redu-
cing the hepatic content and volume of dis-
tribution of thyroxine, not increasing\textsuperscript{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\textsuperscript{35}). Azarnoff et al\textsuperscript{34}
found that the stage of inhibition of clofibrate
is between mevalonic acid and isopentenyl
pyrophosphate. Other studies by the same authors\textsuperscript{34}
and reported others\textsuperscript{30, 30} 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 bioche-
mical changes associated with the effect of
CCl\textsubscript{4} on hepatic lipids. Yet the etiology for
the accumulation of hepatic fats by CCl\textsubscript{4} has
not been settled. Chemical investigation reve-
aled that rise in liver lipids following CCl\textsubscript{4} was
due primarily to increased hepatic TG\textsuperscript{40}. The
first increase in liver TG after single dose of
CCl\textsubscript{4} appears within 1 to 2 hours; the accumu-
luation proceeds rapidly thereafter, and by 3
hours the level of hepatic TG was doubled the
control value\textsuperscript{6}). During the last several years,
ample evidence\textsuperscript{10, 13}, including that of Stern
et al\textsuperscript{24} refered above, have accumulated to
show that in the early stage of acute CCl\textsubscript{4}
toxication there is a direct relationship be-
 tween 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\textsuperscript{10}, that the development of fatty liver, in
CCl\textsubscript{4} treated rats, is primarily due to a block
in the release of hepatic TG to the plasma.
According to this hypothesis, fatty acids mo-
bilized 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 block came from experi-
ments by Heimberg et al\textsuperscript{18}, in which it was
shown that isolated perfused livers, obtained
from rats treated with CCl\textsubscript{4} 3.5 hours before
perfusion, failed to release TG into the perfu-
sate. Interference by CCl\textsubscript{4} with secretion of
hepatic TG has also been shown in experiments
with labeled fatty acids by Maling et al\textsuperscript{119},
and by Schotz et al\textsuperscript{41}). In these experiments
in was shown that after intravenous injection
of palmitic acid-\textsuperscript{14}C, only traces of label
appear in the plasma TG of CCl\textsubscript{4} treated rats.
Using a complex mathematical analysis of their
results Schotz al have also estimated that
in the CCl\textsubscript{4} treated animals the rats of hepatic
TG secretion into the plasma is only 10% of
normal.

In view of action mechanism of CCl\textsubscript{4} and of
one of the hypothes{e that partial failure of
hepatic secretion of TG by clofibrate, like
CCl\textsubscript{4}, as described above, we were expecting
more marked accumulation of TG in livers of
rats given clofibrate and CCl\textsubscript{4} (Group II) than
in than in those given CCl\textsubscript{4} alone (Group
I). Our anticipation crumbled at the moment
when we opened the abdomens to excise
livers of a fewe xperiential animals of Group
I and II. 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 I animals. Thus in our results it was evident that the liver TG of both clofibrate and CCl₄ given animals (Group II) were significantly less than those found in simply CCl₄ given animals (Group I), showing apparently the preventive effect of clofibrate on liver TG accumulation by CCl₄. We had at first no feasible explanation for the observed reduction by clofibrate in TG of fatty liver induced by CCl₄. Speculation was than made from our data that the results could be reasonably ascribable to the reduction in plasma FFA of the Group II when compared with that of Group I 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. And adequate evidence is available to show that hepatic extraction of FFA is proportional to the blood concentration. Sustained hyper-mobilization of FFA by intravenous infusion of noradrenaline in dogs leads to progressive and rapid increase in liver TG. From these much evidences and from our reduced FFA values observed in Group II 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 CCl₄ given animals (Group II) was not statistically significant from those of controls (Group I), the difference in FFA concentration between the CCl₄ given (Group I) and both clofibrate and CCl₄ given (Group II) 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 II 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 stated that the plasma FFA was not affected by clofibrate. On the other hand, Barrett and Thorp demonstrated that oral administration of clofibrate reduced the resting FFA levels in both rats and dogs. In addition they and others 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 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 FFA 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 suggest that the primary action of clofibrate may be on TG synthesis. However, there have been other reports against this, so to speak, "FFA theory" for the
hypohipidemic action of clofibrate. FFA turnover and TG production was found normal during clofibrate administration\(^{(43)}\), and the inhibition by clofibrate of catecholamine-induced release of fatty acids from adipose tissue was not responsible for the lowering of blood lipids\(^{(43)}\).

Attention 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) quantities 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 i.e. (Group IV). The increase in liver size in rats by clofibrate has been previously reported by others\(^{(20, 21)}\), but the direct cause of this hepatomegaly is unknown. It is of interest that the administration of clofibrate to man has been observed to produce a transient increase in serum GGT level\(^{(1)}\). It seems that liver enlargement was of hyperplastic in nature, for total liver DNA content was increased in clofibrate treated rats\(^{(45)}\). 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 clearance of TG from the body\(^{(47, 45)}\) 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 vol.) in amount of 0.5 ml/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\(_4\) administration. This result may cast suspicion against the concept that clofibrate, 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 CCl₄. Due to this liver enlargement, the calculated total liver TG (and phospholipids) of clofibrate & CCl₄ treated animals had approximately the same amount of lipids) found in simply CCl₄ given animals, indicating minor clearance of the lipids by clofibrate from the CCl₄ in toxicated fatty liver as a whole.

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Clofibrate 가 4 몽아르만(CCl₄)诱导 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 ---

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(指導：李基亭 教授 및 金濟徳 助教授)
朴源益


実験動物実験는 다음과 같이 4群으로 나누었다.
第1群：通常白鼠飼料で 3週間 食育하고 食育最終日に paraffin 油を 体重에 따라 1.0~1.5ml 背管投与(對照群).
第2群 : 第1群飼育 食育하고 食育最終日に CCl₄-paraffin 油(配合容積比 3:7)を 0.5ml/100g 体重で 背管投与(CCl₄群).
第3群：Clofibrate를 0.2% 混合 飼料에 混合하여 3 週間 飼育하고 食育最終日に 第1群飼育 處理(clofibrate群).
第4群：第3群飼育 食育하고 食育最終日に 第2群飼育 處理(clofibrate 및 CCl₄群).

CCl₄-paraffin 油 또는 paraffin 油 投與後 各動物群은 3주일에 1회 休眠, 肝 및 血漿에서 中性脂肪, cholesterol 및 磷脂質, 그리고 血漿에는 遊離脂肪酸(FFA)가 각각 定量하였다.

実験結果 얻어知은 知로 다음과 같다. 即 clofibrate는 CCl₄ 诱导脂肪肝의 中性脂肪量을 顯著하게 減少し CCl₄ 単獨投與시에 보는 中性脂肪의 增加를 抑制하여 완全하게 效果를 나타냈다. 이는 最少限 clofibrate의 肝中性脂肪分泌 抑制説을 反証하는 결과로 보였다.

Clofibrate가 肝의 中性脂肪分泌를 抑制한다면 CCl₄와 함께 投與하였을 때 CCl₄ 単獨投與보다 更 많은 中性脂肪量을 招来해야 하기 때문인다. clofibrate가 CCl₄ 诱导脂肪肝에서 이와 같은 影響을 及ぼ한 것은 clofibrate 및 CCl₄群(第4群)의 血漿遊離脂肪酸値이 增加된 사

実験動物群間の 血漿脂質値を 比較して 채sterol 및 CCl₄群(第4群)은 主要血漿脂質(中性脂肪及 clofibrate)値에 있어 大幅

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