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STUDIES ON THE MODE OF ACTION OF CLOFIBRATE: EFFECTS ON HORMONE-INDUCED CHANGES IN PLASMA FREE FATTY ACIDS, CHOLESTEROL, PHOSPHOLIPIDS AND TOTAL ESTERIFIED FATTY ACIDS IN RATS AND DOGS

BY

A. M. BARRETT AND J. M. THORP

From Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire

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It is well established that clofibrate (ethyl-α-*p*-chlorophenoxy-α-methyl-propionate, Atromid-S) reduces circulating lipid levels although the mechanisms of this effect have not yet been fully defined. One possible explanation is that clofibrate alters the process of lipid synthesis from acetate precursor, which in turn may depend on its rate of production by the metabolism of long-chain fatty acids. It is known that chlorophenoxy-isobutyric acid (CPIB), which is the active form of clofibrate *in vivo*, is strongly bound to plasma albumin at sites concerned with the transport of other anions—for example, plasma free fatty acids (Thorp, 1963). It might be expected that a reduction of circulating levels of free fatty acids would lead to a lower availability of acetate for hepatic lipid synthesis.

Experiments *in vitro* have shown that CPIB will reduce the rate of fatty acid release from epididymal fat pads incubated in plasma. It was concluded that such an effect followed competition for acidic binding sites and might well contribute to the overall hypolipaemic effects *in vivo* (Barrett, 1966a). At the same time it was found that CPIB had little effect on lipolysis *in vitro* stimulated by adrenaline. In other experiments it has been shown that repeated injections of adrenaline, each causing an increase in fat mobilization, will in time produce an increase in the circulating levels of plasma lipoproteins in dogs (Shafrir, Sussman & Steinberg, 1960). Subsequent studies have demonstrated, however, that experimentally induced hyperlipoproteinaemia is not necessarily dependent on increased rates of fatty acid mobilization (Barrett, 1966b). The present experiments were undertaken to determine whether or not clofibrate affected (*a*) the level of plasma free fatty acids at rest and under conditions of stimulation and (*b*) experimentally induced rises in circulating cholesterol, phospholipids and total esterified fatty acids.

METHODS

The animals used in this study were male albino rats (200–240 g) from a specific pathogen free colony, and female beagles (10–12 kg), both species being bred at Alderley Park. The details

concerning housing, feeding and collection of blood samples were as described before (Barrett, 1966b).

The drugs used were clofibrate (I.C.I.), *l*-adrenaline (Burroughs Wellcome & Co.), *l*-noradrenaline (Sterling–Winthrop) and corticotrophin (H.P. Acthar Gel, Armour & Co.). Clofibrate was given to rats at a concentration of 0.25% incorporated in the diet (approximately 200 mg/kg/day), and to dogs orally at 500 mg/day, for a period of 2 weeks before experiment. Rats, anaesthetized with pentobarbitone (60 mg/kg), were infused intravenously with adrenaline or noradrenaline at the rate of 2 μg/kg/min; ACTH was infused at 20 mu./kg/min. Dogs were dosed with adrenaline or noradrenaline at a dose of 0.6 mg/kg subcutaneously (in asceptically prepared oily suspension) and ACTH was given at 1 u./kg subcutaneously. Some dogs were given oral glucose (30 g/kg) at the same time as the ACTH injection and again 2 hr later.

The methods used for the various chemical estimations were as given previously (Barrett, 1966b) with the following exceptions. Clofibrate was estimated as CPIB in plasma. Sample of 1 ml. plasma was extracted with 0.5 ml. 3 N-HCl and 5 ml. of a mixture comprising iso-octane 95 parts and ethyl alcohol 5 parts. After shaking well and centrifuging at 3,000 rev/min for 5 min, the supernatant was removed and read at 226 mμ in a 1-cm silica cell. The concentration in plasma samples was determined by reference to appropriate standard solutions of CPIB prepared at the same time. The method gave a recovery of $64.2 \pm 1.1\%$. The method used for the estimation of plasma free fatty acids (Dole & Meinertz, 1960) is not specific when used for samples obtained from animals previously treated with clofibrate, there being a significant contribution from the drug to the acidity of the final titration mixture. Direct spectrophotometric analysis of the Dole supernatant fluid is not possible at the 226 mμ absorption peak for CPIB because of the poor transmission at this wavelength of the heptane used in the extraction procedure. However, CPIB has a minor absorption peak at 280 mμ. The normal range of concentrations of CPIB found in the plasma of animals given the doses of clofibrate used in these experiments was from 0 to 250 μg/ml. in plasma. At concentrations not exceeding 250 μg/ml., a constant fraction of CPIB was extracted into the final titration mixture for free fatty acid estimation, averaging $11.60 \pm 0.17\%$/ml. of extract. This figure was obtained by spectrophotometric analyses at 280 mμ using a 4 cm. path length. Determination of CPIB extraction by a titrimetric method for the same solutions also gave a constant recovery averaging $11.75 \pm 0.15\%$/ml. Direct determination of CPIB in each plasma free fatty acid extract was not possible except in the few instances where 4 ml. of plasma were available. Theoretically a micro-cell of 4 cm path length could be used for spectrophotometric measurement of the Dole supernatant fluid (4.5 ml./1 ml. of original plasma). In practice great difficulty was encountered in the precise alignment of such cells in the light path. The concentration of CPIB in the titration mixture was therefore calculated from the previously determined plasma concentration, and an appropriate deduction made from the total titration value. The calculations are given below:

1. Let x μg/ml. be the concentration of CPIB in the sample as determined spectrophotometrically.
2. Let y μ-equiv/l. be the total acidity of the sample as determined by titration.
3. Let $a\%$ be the percentage of CPIB which is extracted from the sample into the Dole solvent.
4. Let z μ-equiv/l. be the corrected value for the true free fatty acid concentration in the sample.

Then

$$z = y - \frac{x \times 1000}{\text{Equivalent weight of CPIB}} \times \frac{a}{100}$$

that is,

$$z = y - \frac{x \times 1000}{214.7} \times \frac{a}{100}$$

$$z = y - (0.0466\ a)x$$

or

$$z = y - fx \text{ where } f = (0.0466\ a).$$

Since the value of a is constant for the plasma of a given species, a factor (f) can be calculated for everyday use. The values for a and the appropriate factors are as follows:

Sample	a	Factor
Krebs-Ringer bicarbonate Solution with 5% albumin	51.2 ± 1.29	2.38
Rat plasma	49.2 ± 0.39	2.29
Human plasma	43.0 ± 0.48	2.01
Dog plasma	40.0 ± 1.81	1.86

RESULTS

Administration of clofibrate at the dose levels selected produced adequate blood levels in both species, similar to those attained clinically in man. After treatment for 2 weeks there was a significant reduction in plasma cholesterol ($P<0.001$), as expected from previous work. Early in the experiments it became clear that rats treated with clofibrate had much lower levels of free fatty acids than control animals ($P<0.001$). This difference was not at first apparent in dogs. Whereas rats were allowed access to food at all times and were therefore bled in the fed state, dogs were not given their meal until after the withdrawal of blood samples. When the dogs were bled some 2 hr after eating it was then found that they also had much lower levels of free fatty acids, when on clofibrate, compared with the control periods. Clofibrate treatment also produced a significant fall in plasma lactate in rats ($P<0.05$) although this variable was not measured in dogs. No significant effects were noted on the circulating glucose or adrenocorticosteroid levels. These results are summarized in Table 1.

Rats which have been fasted for 24 hr show significantly higher levels of free fatty acids in plasma than fed animals. Treatment with clofibrate did not reduce the size of the 24 hr increment to fasting, but, because the resting level was lower in treated rats, the absolute value reached was lower (524 ± 111 as compared with 675 ± 48), although this difference is not statistically significant. The same pattern was maintained when adrenaline, noradrenaline or ACTH were used to raise plasma free fatty acid levels in fed rats. The increments were very similar in both control and treated groups but the final level reached in the treated groups was always lower (Table 2).

The failure of clofibrate to modify the fatty acid response to fasting may have been caused by the clearance of most of the drug during the fast period. Rats fed a diet containing 0.25% clofibrate had blood levels of CPIB in the range 150–180 μg/ml. when bled at 10 a.m. After a 24 hr fast the levels observed were 10–30 μg/ml. Therefore, in another experiment, some of a group of rats fasted for 24 hr also received oral clofibrate during this period to ensure the maintenance of high blood levels. This additional treatment did not alter the marked rise in plasma free fatty acids caused by fasting, which was not significantly smaller than that in control rats (Table 3). The free fatty acid increment in response to adrenaline infusion in fasted animals which had received clofibrate in their diet, was almost as great as that in the control group, but was significantly reduced ($P<0.05$) in the group which had received additional clofibrate during fasting.

Dogs treated with adrenaline showed statistically significant increases in plasma free fatty acids ($P<0.05$), glucose ($P<0.001$) and cortisol ($P<0.05$). Treatment with clofibrate had no effect on these variables in control conditions (note that these dogs were bled in

TABLE 1

EFFECT OF CLOFIBRATE ON LEVELS OF VARIOUS PLASMA COMPONENTS IN NON-STARVED RATS (0·25% IN DIET) AND DOGS (500 mg/DAY)

Each value represents the mean with standard error for four animals. The corticoid was estimated as corticosterone for rat and as cortisol for dog.

	Free fatty acids (μ-equiv/l.)		Glucose (mg/100 ml.)		Lactate (mg/100 ml.)		Corticoid (μg/100 ml.)		Cholesterol (mg/100 ml.)		CPIB (μg/ml.)	
Species	Control	Clofibrate	Control	Clofibrate	Control	Clofibrate	Control	Clofibrate	Control	Clofibrate	Control	Clofibrate
	370±13	182±43	118±5·3	123±9·5	—	—	—	—	46·6±3·8	30·3±2·1	—	161±28
	282±17	79±26	—	—	—	—	—	—	48·2±4·4	29·3±4·0	—	166±36
Rat	322±12	88±27	145±6·0	141±10·0	14·8±1·4	10·2±0·8	—	—	—	—	—	151±14
	416±28	198±61	—	—	16·4±1·6	11·4±0·7	—	—	66·0±2·2	46·0±2·1	—	182±20
	—	—	—	—	—	—	5·1±1·9	4·0±1·2	72·5±3·1	55·5±6·1		127±5
Dog	343±32	79±15	76±1·9	73±3·0	—	—	6·1±1·1	6·2±1·2	162±22	121±21	—	191±12
	348±33	91±35	80±1·4	75±2·3	—	—	5·8±0·4	5·4±0·6	181±24	133±20	—	221±21

TABLE 2

EFFECT OF FASTING AND INFUSION OF ADRENALINE, NORADRENALINE AND ACTH FOR 15 MIN ON PLASMA FREE FATTY ACID LEVELS IN CONTROL AND CLOFIBRATE TREATED RATS (0·25% IN DIET FOR 10 DAYS)

Each value represents the mean plasma free fatty acids (μ-equiv/l. with standard error) for the number of rats in parenthesis.

Treatment	Controls	Clofibrate
None	326±21 (8)	130±30 (8)
24 hr fast	675±48 (8)	524±111 (4)
Change	+349±52	+394±115
None	346±10 (8)	135±22 (8)
Adrenaline (2μg/kg/min)	624±56 (8)	430±65 (8)
Change	+278±57	+295±70
None	322±12 (4)	88±27 (4)
Noradrenaline (2μg/kg/min)	610±49 (4)	368±40 (4)
Change	+288±51	+280±48
None	416±28 (4)	198±61 (4)
ACTH (20 mU/kg/min)	714±41 (4)	461±93 (4)
Change	+298±50	+263±111

TABLE 3

EFFECT OF CLOFIBRATE ON ADRENALINE-INDUCED MOBILIZATION OF FREE FATTY ACIDS IN FASTING RATS

Rats were fasted for 24 hr after access for 2 weeks to control diet, or diet containing 0·25% clofibrate. Each animal received either water or clofibrate (200 mg/kg) orally, 15 hr and 1 hr before experiment. Adrenaline was infused for 15 min at the rate of 2 μg/kg/min. Each value represents the mean with standard error for four observations.

Diet before fast	Dosing during fast	Adrenaline infusion	Plasma CPIB (μg/ml.)	Plasma free fatty acid concentration (μ-equiv/l.) Absolute	Increment
Control	Water	No	—	634±47	—
Control	Water	Yes	—	1147±70	+513
0·25% clofibrate	Water	No	13±2	576±24	—
0·25% clofibrate	Water	Yes	29±6	1048±39	+472
0·25% clofibrate	Clofibrate	No	485±41	524±56	—
0·25% clofibrate	Clofibrate	Yes	658±46	713±78	+189

the fasting state). The free fatty acid response to adrenaline was, however, reduced from 85% to 59%. Although this reduction was not significant, the final level reached (1042 ±54 μ-equiv/l.) was significantly lower ($P<0.05$) than that in dogs receiving adrenaline without clofibrate (1426±155 μ-equiv/l.). There was no significant reduction in the response of blood glucose or plasma cortisol to adrenaline in clofibrate treated dogs (Table 4).

Control dogs exhibited a greater response in plasma free fatty acids after injection of noradrenaline than that produced by adrenaline. When these animals had been treated with clofibrate, however, there was a dramatic reduction both in the increment ($P<0.01$) and the absolute level attained ($P<0.001$) after noradrenaline. The reduction in the blood glucose response was not significant, while that in the final cortisol level (4.4 μg/100 ml. lower in dogs treated with clofibrate was only just outside the 5% significance limit (Table 5).

Treatment of dogs with ACTH failed to produce any marked change in plasma free fatty acids or glucose but stimulated a significant increase in cortisol levels ($P<0.01$). The level observed 4 hr after injection of ACTH (11.9±1.3 μg/100 ml.) was lower than

TABLE 4

EFFECT OF CLOFIBRATE TREATMENT (500 mg/DAY FOR 14 DAYS) ON THE CHANGE IN PLASMA FREE FATTY ACIDS, GLUCOSE AND CORTISOL, 4 HR AFTER INJECTION OF ADRENALINE (0·6 mg/kg SUBCUTANEOUSLY) IN DOGS

Each value represents the mean with standard error for four animals.

	Free fatty acids (μ-equiv/l.)		Glucose (mg/100 ml.)		Cortisol (μg/100 ml.)		CPIB (μg/ml.)	
	Control	Clofibrate	Control	Clofibrate	Control	Clofibrate	Control	Clofibrate
Control	770±147	653±34	68±1·4	64±1·2	5·9±3·1	6·1±0·9	—	293±41
4 hr after adrenaline	1426±155	1042±54	131±4·8	126±4·1	14·2±1·1	13·0±1·1	—	240±29
Increment	+656±214	+389±75	+63±5·0	+62±4·3	+8·3±3·3	+6·9±2·0	—	−53±50
% Change	+85	+59	+93	+97	+141	+113	—	−18

TABLE 5

EFFECT OF CLOFIBRATE TREATMENT (500 mg/DAY FOR 14 DAYS) ON THE CHANGE IN PLASMA FREE FATTY ACIDS, GLUCOSE AND CORTISOL, 4 HR AFTER INJECTION OF NORADRENALINE (0·6 mg/kg SUBCUTANEOUSLY) IN DOGS

Each value presents the mean with standard error for four animals.

	Free fatty acids (μ-equiv/l.)		Glucose (mg/100 ml.)		Cortisol (μg/100 ml.)		CPIB (μg/ml.)
	Control	Clofibrate	Control	Clofibrate	Control	Clofibrate	Clofibrate
Control	659±79	397±93	67±7·2	65±1·2	6·6±1·1	5·6±1·5	236±10
4 hr after noradrenaline	3012±178	1470±176	99±4·5	82±3·0	22·3±1·9	17·9±0·3	248±11
Increment	+2353±195	+1073±199	+33±8·5	+17±3·2	+15·7±2·2	+12·3±1·5	+12±15
% Change	+356	+270	+47	+26	+238	+220	+5

TABLE 6

EFFECT OF CLOFIBRATE TREATMENT (500 mg/DAY FOR 14 DAYS) ON THE CHANGE IN PLASMA FREE FATTY ACIDS, GLUCOSE AND CORTISOL, 4 HR AFTER INJECTION OF ACTH (1 i.u./kg SUBCUTANEOUSLY) IN DOGS

Each value represents the mean with standard error for four animals.

	Free fatty acids (μ-equiv/l.)		Glucose (mg/100 ml.)		Cortisol (μg/100 ml.)		CPIB (μg/ml.)
	Control	Clofibrate	Control	Clofibrate	Control	Clofibrate	Clofibrate
Control	766±214	411±94	76±1·8	75±1·4	5·2±1·1	5·9±1·4	249±31
4 hr after ACTH	622±70	421±70	85±7·3	76±1·2	11·9±1·3	16·9±2·4	237±21
Increment	−144±231	+10±122	+9±7·5	+1±1·8	+6·7±1·7	+11·0±2·8	−12±37
% Change	−18·8	+2·7	+12	+1·3	+129	+186	−4·8

that seen after catecholamines but this merely reflected the shorter duration of action of the former because the peak was reached at 2 hr when a value of 15.8 ± 1.1 μg/100 ml. was found. Clofibrate had little effect except to prolong the cortisol response although this was not statistically significant ($t = 1.8515$).

In all the experiments clofibrate treatment produced a significant reduction in cholesterol and phospholipid levels. In most cases there was also a reduction in total esterified fatty acid concentrations but this reflected the drop in cholesterol and phospholipids rather than any change in triglyceride levels. Injection of adrenaline, noradrenaline or ACTH for 3 consecutive days produced a significant increase in lipoprotein levels in control dogs, as reported earlier (Barrett, 1966b). After treatment with clofibrate the response to adrenaline was enhanced in terms of the increments observed (Table 7). Whereas control dogs showed an increase in cholesterol of 25% over an initial value of 171 mg/100 ml., cholesterol concentrations in treated dogs rose from 103 to 184 mg/100 ml.—an increase of 79%. Similar effects were noted on phospholipid changes. Despite the greater effect after clofibrate, however, the absolute levels reached were still lower than in control dogs. When noradrenaline was given to dogs treated with clofibrate the usual increment in cholesterol and phospholipids was diminished in both cases, although this reduction was not statistically significant (Table 8). On a percentage

TABLE 7

EFFECT OF CLOFIBRATE TREATMENT (500 mg/DAY FOR 14 DAYS) ON THE CHANGES IN PLASMA CHOLESTEROL, PHOSPHOLIPIDS AND TOTAL ESTERIFIED FATTY ACIDS WHICH NORMALLY FOLLOW THREE DAILY INJECTIONS OF ADRENALINE (0·6 mg/kg/DAY) IN DOGS

Each value represents the mean with standard error for four animals.

	Cholesterol (mg/100 ml.)		Phospholipids (mg/100 ml.)		Total esterified fatty acids (μ-equiv/l.)		Phospholipid/ cholesterol ratio	
	Control	Clofibrate	Control	Clofibrate	Control	Clofibrate	Control	Clofibrate
Before adrenaline	171±24	103±17	267±21	187±31	11·7±1·2	7·9±1·1	1·56	1·81
After adrenaline	214±26	184±32	343±17	273±40	11·9±0·4	10·8±1·6	1·60	1·47
Change	+43±5	+81±13	+76±21	+86±33	+0·2±0·2	+2·9±0·8	—	—
% Change	+25	+79	+26	+46	+1·7	+36·7		

TABLE 8

EFFECT OF CLOFIBRATE TREATMENT (500 mg/DAY FOR 14 DAYS) ON THE CHANGES IN PLASMA CHOLESTEROL, PHOSPHOLIPIDS AND TOTAL ESTERIFIED FATTY ACIDS WHICH NORMALLY FOLLOW THREE DAILY INJECTIONS OF NORADRENALINE (0·6 mg/kg/DAY) IN DOGS

Each value represents the mean with standard error for four animals.

	Cholesterol (mg/100 ml.)		Phospholipids (mg/100 ml.)		Total esterified fatty acids (μ-equiv/l.)		Phospholipids/ cholesterol ratio	
	Control	Clofibrate	Control	Clofibrate	Control	Clofibrate	Control	Clofibrate
Before noradrenaline	184±14	112±13	270±16	229±25	10·1±0·9	6·5±1·6	1·47	2·04
After noradrenaline	231±16	142±8	341±33	286±18	12·8±1·0	9·6±1·0	1·48	2·01
Change	+47±8	+30±6	+71±16	+57±16	+2·7±1·2	+3·1±0·7	—	—
% Change	+26	+27	+26	+25	+27	+48	—	—

TABLE 9

EFFECT OF CLOFIBRATE TREATMENT (500 mg/DAY FOR 14 DAYS) ON THE CHANGES IN PLASMA CHOLESTEROL, PHOSPHOLIPIDS AND TOTAL ESTERIFIED FATTY ACIDS WHICH NORMALLY FOLLOW THREE DAILY INJECTIONS OF ACTH (1 I.U./kg/DAY) WITH OR WITHOUT AN ORAL GLUCOSE LOAD (2×30 g/DOG) IN DOGS

Each value represents the mean with standard error for four animals.

	Cholesterol (mg/100 ml.)		Phospholipids (mg/100 ml.)		Total esterified fatty acids (μ-equiv/l.)		Phospholipid/ cholesterol ratio	
	Control	Clofibrate	Control	Clofibrate	Control	Clofibrate	Control	Clofibrate
Before treatment	154±11	81±18	273±22	157±16	7·1±1·0	6·2±0·6	1·77	1·94
After ACTH	192±23	70±7	328±27	167±24	11·6±1·6	7·4±0·9	1·71	2·39
Change	+38±2	−11±10	+55±18	+10±8	+4·1±0·6	+1·2±0·3	—	—
% Change	+25	−14	+20	+6	+64	+19	—	—
Before treatment	153±24	99±6	271±19	173±15	12·0±1·3	8·6±1·1	1·77	1·75
After ACTH and glucose	191±24	94±5	340±17	187±8	11·2±0·8	9·4±0·8	1·78	2·00
Change	+38±2	−5±3	+69±12	+14±7	−0·8±0·2	+0·8±0·2	—	—
% Change	+25	−5	+25	+8	−7	+9	—	—

basis the increments to noradrenaline stimulation were similar in control and treated groups. In contrast, following ACTH stimulation the increases in cholesterol and phospholipids were strikingly abolished in dogs pretreated with clofibrate (Table 9). In order to determine whether the hyperglycaemic effect of adrenaline contributed to the failure of clofibrate to reduce changes in lipoproteins induced by adrenaline, the treatment with ACTH was combined with an oral load of glucose. This did not alter the pattern of response (Table 9).

DISCUSSION

The affinity of clofibrate anion for plasma albumin *in vitro* seems to have significant consequences in the whole animal. Oral administration of clofibrate reduces the resting concentration of plasma free fatty acids in both rats and dogs. Although in most cases the increment in fatty acid levels following various stimuli was similar in control and treated groups, the drug does lower the absolute circulating concentrations attained by up to 50%. From these results it is reasonable to conclude that the concentration of free fatty acids delivered to the liver of animals treated with clofibrate will be lower than that in the controls. Adequate evidence is available to show that hepatic extraction of free fatty acids is proportional to the arterial concentration (McElroy, Siefert & Spitzer, 1960). Sustained hypermobilization of free fatty acids by intravenous infusion of noradrenaline in conscious dogs leads to increased liver glyceride concentration and an increase of up to three times in circulating triglycerides after 24 hr (Fiegelson, Pfaff, Karmen & Steinberg, 1961 ; Carlson, Liljedahl & Wirsen, 1965). In man, it has been found that the increment in plasma free fatty acids to an intravenous infusion of noradrenaline is directly related to the fasting triglyceride level (Nestel, 1964). It is therefore highly probable that the substantial reductions in plasma triglycerides observed in men treated with clofibrate (Oliver, 1963 ; Best & Duncan, 1965) are partly the result of the reduction in free fatty acid transport. Evidence to support this suggestion may be cited. For example, Duncan, Best & Robertson (1965) found that clofibrate treatment reduced

the increment in plasma free fatty acids to noradrenaline infusion from 760 μ-equiv/l. to 390 μ-equiv/l.—a factor of 50%. The absolute peak levels were 1,210 μ-equiv/l. in control subjects and 980 μ-equiv/l. in the treated group but because the experiment was carried out 30 min after the usual dose of clofibrate (0.5 g) and no allowance made for contamination, the latter level must have been appreciably lower. Other studies in which an allowance for clofibrate has been made on an arbitrary basis (Macmillan, Oliver, Simpson & Tothill, 1965) or by direct estimation (Rifkind, 1966) also show a significant reduction in plasma free fatty acid levels. The present experiments indicate that the reduction in fatty acid transport is readily reversible and dependent on the circulating CPIB concentration. Reduced free fatty acid transport does not account for the whole of the hypolipaemic action of clofibrate. For example, Duncan, Best & Despopoulos (1964) have shown that the amount of palmitate uptake in an isolated perfused liver preparation is similar in tissues from both control and clofibrate treated rats. Yet the output of triglyceride from the livers of animals given 0.25% clofibrate in the diet was only 50% of that from control livers. Furthermore it is clear that in man clofibrate accelerates triglyceride clearance (Ryan & Schwartz, 1964).

It is not known whether the abnormally raised lipoprotein levels after treatment with catecholamines or ACTH are truly relevant to hyperlipaemic states in man. From their evidence relating to excess fat mobilization and subsequent increases in lipoprotein levels, Shafrir, Sussman & Steinberg (1960) suggested " that the hypercholesterolaemia of stress reported in man may be in part due to the accompanying overactivity of the adrenal medulla and adrenal cortex ". Clofibrate treatment abolished the increments in cholesterol and phospholipids usually seen after repeated doses of ACTH with or without an oral glucose load. The increments after repeated adrenaline administration were, however, larger in dogs treated with clofibrate than under control conditions while the effects of noradrenaline were unaltered on a percentage basis. It is unlikely that these effects are solely related to altered transport of plasma free fatty acids for the greatest effect of clofibrate on these was in the noradrenaline group. Yet in the hypercholesterolaemic responses, clofibrate exerted its greatest effect after ACTH which itself had no apparent action on fatty acid levels. Similarly, the effect of clofibrate cannot be related only to excess cortisol secretion, because this followed all treatments and only in the case of ACTH was there blockade of the increase in circulating lipoproteins. One of the remaining differences between these treatment groups is the degree of hepatic glycogenolysis, which was greatest with adrenaline and lowest with ACTH plus glucose. At the same time, all treatments produce high circulating levels of cortisol which will stimulate hepatic gluconeogenesis. Making the supposition that the present semi-acute changes in plasma lipoproteins derive from an excess of hepatic glucose, a working hypothesis can be constructed. It is suggested that clofibrate causes an interference with the production of glucose from protein, thereby accounting for its inhibitory actions against the predominantly catabolic effects of ACTH. Consonant with this hypothesis is the observed increase in liver protein in rats and dogs, and the reduction in liver glycogen in fed rats, which occurs during clofibrate treatment (Platt & Thorp, 1966). If the excess hepatic glucose is derived from glycogen—for example, following catecholamines—clofibrate is ineffective against the ensuing hyperlipoproteinaemia. Because adrenalectomy invariably leads to a loss of liver glycogen, this working hypothesis would

also explain why excision of these glands prevents adrenaline-induced rises in cholesterol (Shafrir, Sussman & Steinberg, 1960).

It has been proposed that the hepatic level of free fatty acids operates a feed-back control of the extent to which glycolytic and gluconeogenetic pathways of energy production are followed (Weber, Convery, Lea & Stamm, 1966). High concentrations of free fatty acid serve to depress the activity of key enzymes in the glycolytic pathway thereby increasing the degree of gluconeogenesis. Hepatic extraction of free fatty acids is directly proportional to the plasma concentration (McElroy, Siefert & Spitzer, 1960). Considering the lower absolute levels of free fatty acids which have been demonstrated during clofibrate treatment, it is highly probable that there is a relative enhancement of glycolysis. The effects of clofibrate on gluconeogenesis may therefore be mediated indirectly by means of the plasma concentration of free fatty acids. It would be an oversimplification to assume that alterations in fatty acid transport provide a complete explanation of the actions of clofibrate, because this drug is also involved in the re-distribution of other acids transported by albumin—for example, thyroxine (Thorp, 1963; Osorio, Walton, Browne, West & Whystock, 1965)—with effects extending beyond those on the plasma lipids (Platt & Thorp, 1966; Thorp, Cotton & Oliver, 1967).

SUMMARY

1. The effect of clofibrate has been studied on (*a*) the level of plasma free fatty acids at rest and under conditions of stimulation in rats and dogs and (*b*) experimentally induced rises in circulating cholesterol, phospholipids and total esterified fatty acids in dogs.

2. Determination of free fatty acids in the plasma of animals pretreated with clofibrate is complicated by the presence of chlorophenoxyisobutyric acid (CPIB), the active form of clofibrate *in vivo*. A method is described for the estimation of CPIB in plasma and a calculation presented and validated for the estimation of the true level of fatty acids.

3. The concentration of free fatty acids in the plasma of rats and dogs was reduced by pre-treatment with clofibrate.

4. The increment in free fatty acids in the plasma of rats following a 24 hr fast or stimulation by adrenaline, noradrenaline or ACTH was little changed by clofibrate treatment, but because the starting level was lower in treated rats the ultimate level attained was also lower. Production of higher levels of CPIB in the blood did not prevent the response to fasting but did reduce the increment to adrenaline stimulation.

5. Treatment of dogs with clofibrate reduced the peak levels of free fatty acids after adrenaline and noradrenaline by up to 50% without major change in plasma glucose or cortisol responses.

6. Treatment of dogs with clofibrate reduced circulating cholesterol and phospholipid levels but did not prevent the usual rise in the concentration of these lipids following treatment with adrenaline or noradrenaline. In contrast the increases in cholesterol and phospholipids were abolished when ACTH was used as the stimulant.

7. Arguments are presented in support of the hypothesis that the hypolipaemic effects of clofibrate are caused by reduced fatty acid transport and decreased availability of hepatic carbohydrate.

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REFERENCES

BARRETT, A. M. (1966a). The effect of chlorophenoxyisobutyric acid on the release of free fatty acids from isolated adipose tissue *in vitro*. *Br. J. Pharmac. Chemother.*, **26**, 363–371.

BARRETT, A. M. (1966b). The role of plasma free fatty acids in the elevation of plasma cholesterol and phospholipids produced by adrenaline. *J. Endocr.*, **36**, 301–316.

BEST, M. M. & DUNCAN, C. H. (1965). Reduction of serum triglycerides and cholesterol by ethyl-p-chlorophenoxyisobutyrate (CPIB). *Am. J. Cardiol.*, **15**, 230–233.

CARLSON, L. A., LILJEDAHL, S-O. & WIRSÉN, C. (1965). Blood and tissue changes in the dog during and after excessive free fatty acid mobilization. *Acta med. scand.*, **178**, 81–102.

DOLE, V. P. & MEINERTZ, H. (1960). Microdetermination of long-chain fatty acids in plasma and tissues. *J. biol. Chem.*, **235**, 2595–2599.

DUNCAN, C. H., BEST, M. M. & DESPOPOULOS, A. (1964). Inhibition of hepatic secretion of tryglyceride by chlorophenoxyisobutyrate (CPIB). *Circulation* (suppl. 3), **30**, 7.

DUNCAN, C. H., BEST, M. M. & ROBERTSON, G. L. (1965). A comparison of the effects of ethylchlorophenoxyisobutyrate and nicotinic acid on plasma free fatty acids. *Lancet*, i, 191–193.

FEIGELSON, E. B., PFAFF, W. W., KARMEN, A. & STEINBERG, D. (1961). The role of plasma free fatty acids in development of fatty liver. *J. clin. Invest.*, **40**, 2171–2179.

MCELROY, W. T., SIEFERT, W. L. & SPITZER, J. J. (1960). Relationship of hepatic uptake of FFA to plasma concentration. *Proc. Soc. exp. Biol. Med.*, **104**, 20–23.

MACMILLAN, D. C., OLIVER, M. F., SIMPSON, J. D. & TOTHILL, P. (1965). Effect of ethylchlorophenoxyisobutyrate on weight, plasma volume, total body water and free fatty acids. *Lancet*, ii, 924–926.

NESTEL, P. J. (1964). Plasma triglyceride concentration and plasma free fatty acid changes in response to norepinephrine in man. *J. clin. Invest.*, **43**, 77–82.

OLIVER, M. F. (1963). Further observations on the effects of Atromid and of ethyl chlorophenoxyisobutyrate on serum lipid levels. *J. Atheroscler. Res.*, **3**, 427–444.

OSORIO, C., WALTON, K. W., BROWNE, C. H. W., WEST, D. & WHYSTOCK, P. (1965). The effect of chlorphenoxyisobutyrate ("Atromid-S") on the biliary excretion and distribution of thyroxine in the rat. *Biochem. Pharmac.*, **14**, 1479–1481.

PLATT, D. S. & THORP, J. M. (1966). Changes in the weight and composition of the liver in the rat, dog and monkey treated with ethyl chlorophenoxyisobutyrate. *Biochem. Pharmac.*, **15**, 915–925.

RIFKIND, B. M. (1966). Effect of CPIB Ester on plasma free fatty acid levels in man. *Metabolism*, **15**, 673–675.

RYAN, W. G. & SCHWARTZ, T. B. (1964). The dynamics of triglyceride turnover: effect of Atromid-S. *J. Lab. clin. Med.*, **64**, 1001.

SHAFRIR, E., SUSSMAN, K. E. & STEINBERG, D. (1960). Role of the pituitary and the adrenal in the mobilization of free fatty acids and lipoproteins. *J. Lipid Res.*, **1**, 459–465.

THORP, J. M. (1963). An experimental approach to the problem of disordered lipid metabolism. *J. Atheroscler. Res.*, **3**, 351–360.

THORP, J. M. (1964). The influence of plasma proteins on the action of drugs. In *Absorption and Distribution of Drugs*, ed. Binns, T. B., pp. 64–75. Edinburgh & London: E. & S. Livingstone.

THORP, J. M., COTTON, R. C. & OLIVER, M. F. (1967). Role of the endocrine system in the regulation of plasma lipids and fibrinogen, with particular reference to the effects of "Atromid-S". *Prog. biochem. Pharmacol.*, **4**, in the Press.

WEBER, G., CONVERY, H. J. H., LEA, M. A. & STAMM, N. B. (1966). Feedback inhibition of key glycolytic enzymes in liver: action of free fatty acids. *Science, N.Y.*, **154**, 1357–1360.