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CHANGES IN THE WEIGHT AND COMPOSITION OF THE LIVER IN THE RAT, DOG AND MONKEY TREATED WITH ETHYL CHLOROPHENOXYISOBUTYRATE

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Abstract—Ethyl chlorophenoxyisobutyrate (CPIB) has been shown to cause an increase in rat liver weight and liver protein concentration. Furthermore, it has been shown to reduce liver non-fasting glycogen levels in the rat. Results of similar experiments with monkeys and dogs are presented. The theory of action of CPIB is discussed at length and evidence is presented to indicate that the activity is derived from its binding to a specific fraction of the plasma protein, thereby causing a redistribution of endogenous hormones and cofactors, principally thyroxine, into the liver. The end result is one of an altered dynamic equilibrium due to deposition of extra-protein with concomitant falls in liver carbohydrate levels.

THE EFFECTS of ethyl α-(4-chlorophenoxy)-α-methylpropionate (CPIB, clofibrate Atromid-S*) on blood lipids and clotting factors, in man, have been the subject of many publications (e.g. reference 1). In studying the mode of action of CPIB, we have extended our original observations[2, 3] in experimental animals. Best and Duncan[4] pointed out that CPIB produced hepatomegaly in rats, the significance of which was obscure, as also was the nature of the electron microscopic changes in rat liver observed by Paget.[5] Thorp and Waring[2] found an increase in rat liver protein concentration following treatment with Atromid.*† We have, therefore, examined the time-course and extent of these changes during continuous treatment, as well as the speed of their reversibility.

In this paper, we report details of the changes of weight and composition of rat, dog and monkey liver following administration of CPIB. The changes in serum and liver lipids are considered elsewhere.[2-4, 6, 7] The results are discussed in relation to the mode of action of the compound.

METHODS

Rats were of the Wistar-derived, specific pathogen free, Alderley Park strain. Beagles and Rhesus monkeys were those used in toxicity trials of CPIB either without added androsterone (Atromid-S) or with added androsterone (Atromid).

Rats and monkeys were fed powdered or cubed diets *ad lib.* containing varying concentrations by weight (0–2%) of Atromid or Atromid-S. The basic diet, type O, with vitamin supplementation, is that of Scottish Agricultural Industries. Dogs received

* I.C.I. Trade Marks.

† Atromid is a combination of CPIB with androsterone (2·2% w/v).

oral doses of Atromid-S in the form of capsules at levels of 35–45 mg/kg on alternate days.

Standard procedures were used for the measurement of liver weight, liver water, protein[25] and glycogen[26] concentrations, and the rate of incorporation *in vitro* of ^{14}C-leucine into protein by isolated rat liver ribosomes.[27] Standard statistical procedures were used for the assessment of standard errors and Student's *t* test was applied as a test of significance.

RESULTS

Liver weight changes

Rat. Most of the observations in rats have been made with the dose at 0·25 per cent in the diet, since this is the effective dose with reference to serum lipid changes. Only representative examples of the numerous experimental results are given, each being the actual observation made in one experiment. The liver weight changes of CPIB-dosed animals are compared with those control groups examined simultaneously.

In Table 1 are given typical results following the administration of 0·25% CPIB in the diet to male and female rats. In all cases, the mean level of liver weight in the

TABLE 1. THE LIVER WT. OF RATS TREATED WITH CPIB (0·25 PER CENT IN THE DIET) FOR SHORT PERIODS (17–36 DAYS)

Sex	Initial body wt. range (g)	Duration of experiment (days)	Liver wt.			
			Controls g/100 g body wt.*	Treated g/100 g body wt.*	% controls	*P*
♂	140–160	17	4·42 ± 0·18(5)	6·48 ± 0·18(5)	147	<0·001
	180–210	22	4·15 ± 0·06(10)	5·65 ± 0·13(10)	136	<0·001
	310–350	29	3·52 ± 0·04(9)	4·66 ± 0·15(9)	133	<0·001
	410–490	22	3·26 ± 0·08(5)	3·86 ± 0·09(4)	119	<0·001
♀	180–200†	35	3·90 ± 0·26(4)	4·74 ± 0·19(4)	121·5	<0·001
	300–350	36	3·33 ± 0·05(3)	3·61 ± 0·16(3)	108	<0·20

* Mean ± S.E.M. (number of animals).
† 0·2% CPIB in the diet to this group.

treated group is significantly higher than in the control group. The body weight of the rat at the start of treatment influences the extent of change in the liver weight; the older and heavier the rat, the smaller the effect. The results in Table I are in agreement with the observations of Best and Duncan,[4] and we also confirm their finding that the presence of androsterone in the diet has no additional effect on liver weight change.

The change in liver weight could be detected within the first three days of diet feeding, although maximal effects were not seen until the tenth to fifteenth day of treatment. A typical result is shown in Fig. 1, and it will be seen that once the new level of liver weight has been attained, it is maintained for at least 60 days of treatment. As in Table 1, the initial body weight of the rat influences the position of the new equilibrium. The effect, therefore, may be summarised as comprising an initial phase, in some way related to body weight and age, during which liver weight increases, followed by a second stage, the preservation of the new equilibrium.

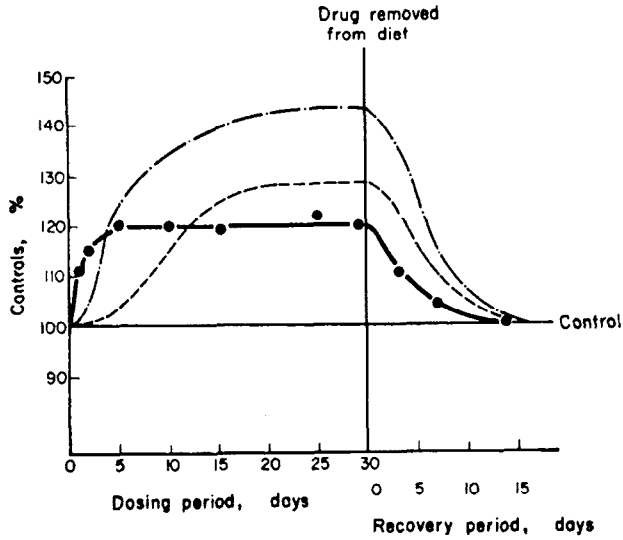

FIG. 1. Comparison of liver wt and protein concentration of rats treated with CPIB (0·25% in the diet) for short periods.

The graphs of liver wt. represent the mean of several experiments. Liver wt. of treated group (g/100 g body wt.) expressed as % controls. Liver protein of treated group (g/100 g fresh liver wt.) expressed as % controls. —•—•— Liver wt. (rats <200 g body wt. initially); ---- Liver wt. (rats >300 g body wt. initially); ●—● Liver protein concentration (irrespective of initial body wt.).

To the above pattern of response may be added a third stage, that of a diminution or apparent diminution of effect after prolonged dosing, particularly in male rats, as indicated in Table 2, which shows the effect of CPIB (together with 2·2% w/v androsterone) given at levels of 0·1–0·4 per cent in the diet for 2 yr.

The rapid reversibility of the liver weight change is illustrated in Fig. 1, normal levels being achieved usually within 10 days from cessation of CPIB treatment.

TABLE 2. THE LIVER WT. OF RATS TREATED WITH CPIB (+ ANDROSTERONE) FOR 2 YR*

Sex	Dose level (%)	No. of rats	Mean terminal body wt.† (g)	% controls	Mean liver wt. g/100 g body wt.†	% controls	P‡
♂	0	20	480 ± 11·25	100	3·52 ± 0·159	100	
	0·1	24	500 ± 10·30	104	3·50 ± 0·082	99·5	>0·2
	0·2	11	454 ± 10·60	95	3·56 ± 0·091	101	>0·2
	0·4	16	426 ± 11·20	89	3·57 ± 0·095	101·5	>0·2
♀	0	22	315 ± 8·50	100	3·66 ± 0·094	100	
	0·1	16	297 ± 6·43	95	3·83 ± 0·106	104·5	>0·2
	0·2	15	281 ± 9·22	89	4·26 ± 0·088	116·5	<0·001
	0·4	20	246 ± 9·10	78	4·31 ± 0·103	117·5	<0·001

* CPIB (containing androsterone 2·2% w/v) was added to the diet of rats at the levels shown.
† Mean ± S.E.M.
‡ Treated vs. control group.

Monkey. The results of 2 yr treatment with Atromid and of 3 months treatment with Atromid-S are given in Table 3. As with a 2 yr trial in male rats, the monkeys from the 2-yr trials showed normal liver weights. The younger monkeys treated for 3 months showed significant elevations in liver weight. There appears, therefore, to be a good correlation with rats in that the effect is detectable in young monkeys, whereas with

TABLE 3. THE LIVER WT. OF MONKEYS TREATED WITH CPIB (WITH OR WITHOUT ADDED ANDROSTERONE*) FOR 3 MONTHS OR 2 YR

Sex and duration	Dose* level (%)	No. of animals	Mean terminal body wt. (kg)	Liver wt. g/100 g body wt.†	Liver wt. % controls	Liver wt. P‡
♂ 3 months	Control	4	3·75	2·37 ± 0·06	100	
	2·0	4	3·48	2·80 ± 0·20	118	0·075
♀ 3 months	Control	4	3·61	2·39 ± 0·09	100	
	2·0	4	3·29	3·19 ± 0·09	133	0·001
♂ 2 yr	Control	5	7·25	2·04 ± 0·19	100	
	0·5	3	6·17	2·05 ± 0·06	101	⩾0·20
	1·0	4	7·00	1·74 ± 0·14	85	⩾0·20
	2·0	5	6·05	2·46 ± 0·19	121	<0·20
♀ 2 yr	Control	4	4·88	2·34 ± 0·28	100	
	0·5	4	5·12	2·01 ± 0·08	86	⩾0·20
	1·0	1	4·50	2·34 ± 0·56§	100·5	⩾0·20
	2·0	4	5·50	2·64 ± 0·12	113	⩾0·20

* CPIB (containing androsterone 2·2% w/v in the 2 yr groups) was added to the diet of monkeys at the levels shown.

† Mean ± S.E.M.

‡ Treated vs. respective control group.

§ Assuming standard deviation to be the same as for control group.

TABLE 4. THE LIVER WT. OF DOGS TREATED WITH CPIB FOR 3 MONTHS

Sex	Dose (mg/kg p.o. every second day)	No. of animals	Mean terminal body weight (kg)	Liver weight g/100 g body wt.*	Liver weight % controls	Liver weight P†
♂	Controls	10	15·2	2·65 ± 0·11	100	
	35	4	14·9	2·94 ± 0·14	111	0·150
♀	Controls	10	12·2	2·93 ± 0·11	100	
	45	4	10·1	3·67 ± 0·10	125	<0·005

* Mean ± S.E.M.

† Treated group vs. respective control group.

increasing age and body weight, as in prolonged dosing, the effect on liver weight decreases or disappears. It is unlikely that the presence of androsterone was responsible for altering the liver weight response to CPIB in the 2-yr experiment.

Dog. The results of 3 months treatment of dogs with CPIB are shown in Table 4. The increased liver weight in the females is significantly different from the controls. Table 5 summarizes the liver weight changes found in rat, monkey and dog.

Liver protein concentration

Rat. Typical results of administration to male rats of 0·25% CPIB in the diet on the liver protein concentration are shown in Table 6. The significant increase in liver protein due to CPIB agrees with the previous values published[2] for CPIB containing 2·2% w/v androsterone. Other observations (unpublished) support the conclusion

TABLE 5. SUMMARY OF THE EFFECTS OF CPIB TREATMENT FOR SHORT OR LONG PERIODS ON RAT, DOG AND MONKEY LIVER WT

Species and duration of treatment	Dose (%)	Males Mean terminal body wt. (kg)	Males Mean liver wt. g/100 g body wt.*	Males Mean liver wt. % controls	Females mean terminal body wt. (kg)	Females mean liver wt. g/100 g body wt.*	Females mean liver wt. % controls
Rat: Short (2–8 weeks)	0	0·2	4·42 ± 0·18(5)	100	0·2	3·90 ± 0·26(4)	100
	0·25		6·48 ± 0·18(5)	146		†4·74 ± 0·19(4)	121·5
	0	0·45	3·26 ± 0·08(5)	100	0·35	3·33 ± 0·05(3)	100
	0·25		3·86 ± 0·09(4)	119		3·61 ± 0·16(3)	108
Rat: Long (2 yr)	0	0·5	3·52 ± 0·159(20)	100	0·3	3·66 ± 0·094(22)	100
	0·1		3·50 ± 0·082(24)	99·5		3·83 ± 0·106(16)	104·5
	0·2		3·56 ± 0·091(11)	101		4·26 ± 0·088(15)	116·5
	0·4		3·57 ± 0·095(16)	101·5		4·31 ± 0·103(20)	117·5
Monkey: Short (3 months)	0	3·75	2·37 ± 0·06(4)	100	3·6	2·39 ± 0·09(4)	100
	2·0	3·50	2·80 ± 0·20(4)	118	3·3	3·19 ± 0·09(4)	133
Monkey: Long (2 yr)	0	7·25	2·04 ± 0·19(5)	100	4·90	2·34 ± 0·28(4)	100
	0·5	6·20	2·05 ± 0·06(3)	101	5·12	2·01 ± 0·08(4)	86
	1·0	7·00	1·74 ± 0·14(4)	85	4·50	2·35 ± 0·56(1)	100·5
	2·0	6·05	2·46 ± 0·19(5)	121	5·50	2·64 ± 0·12(4)	113
Dog: Short (3 months)	0	15·2	2·65 ± 0·11(10)	100	12·2	2·93 ± 0·11(10)	100
	35–45 mg/kg p.o. on alternate days	14·9	2·94 ± 0·14(4)	111	10·2	3·67 ± 0·10(4)	125

* Mean ± S.E.M. (number of animals).
† 0·20% CPIB in the diet of this group.

that the presence of androsterone plays no significant part in the effect of CPIB on rat liver protein concentration. The time course of the effect of CPIB on protein concentration is similar to that on liver weight; first a period of rising protein concentration to a new equilibrium level, which is then maintained during further treatment (at least up to 2 months). Liver protein determinations are not available for the rats dosed for 2 yr.

Figure 1 shows the result of a comparison of the time-courses of the response of liver weight and protein concentration to CPIB. The change in protein concentration

appears to precede the change in liver weight, reaching a maximum usually within 7 days, when liver weight is still increasing. Complete reversal of the protein change occurs within 7 days of cessation of CPIB treatment as shown in Fig. 1. Again, liver weight reversal is apparently preceded by the liver protein change.

TABLE 6. THE LIVER PROTEIN CONCENTRATION OF MALE RATS TREATED WITH CPIB (0·25% IN THE DIET) FOR SHORT PERIODS

Initial body wt. range (g)	Duration of dosing (days)	Liver protein concentration*			
		Controls g/100 g fresh liver wt.‡	Treated g/100 g fresh liver wt.	P†	% controls
<180	8	18·0 ± 0·13(147)	21·3 ± 1·07(2)	0·005	118·5
180–210	22		21·4 ± 0·49(10)	<0·001	119·0
375–410	29		22·3 ± 0·63(6)	<0·001	124·0

* Mean ± S.E.M. (Number of animals).

† Treated group vs. combined control group.

‡ Controls from all experiments combined since the mean for any one group did not differ significantly from the combined mean, irrespective of body wt.

TABLE 7. THE LIVER PROTEIN CONCENTRATION OF MONKEYS TREATED WITH CPIB (WITH OR WITHOUT ADDED ANDROSTERONE*) FOR 3 MONTHS OR 2 YR

Sex and duration	Dose levels (%)*	No. of animals	Liver protein concentration		
			g/100 g fresh liver wt.†	% controls	P‡
♂	Control	4	18·6 ± 0·60	100	
3 months	2·0	4	19·2 ± 0·64	103	>0·2
♀	Control	4	17·7 ± 0·32	100	
3 months	2·0	4	18·9 ± 0·72	107	0·10
♂	Control	5	19·1 ± 0·48	100	
2 yr	0·5	3	19·4 ± 0·39	101·5	⩾0·2
	1·0	4	19·2 ± 0·77	100·5	⩾0·2
	2·0	5	18·4 ± 0·61	96·3	⩾0·2
♀	Control	4	18·3 ± 0·64	100	
2 yr	0·5	4	18·6 ± 0·31	101·5	⩾0·2
	1·0	1	18·6 ± 1·28§	101·5	⩾0·2
	2·0	4	17·2 ± 0·44	94	0·20

* CPIB (containing androsterone 2·2% w/v in the 2 yr groups) was added to the diet of monkeys at the levels shown.

† Mean ± S.E.M.

‡ Treated group vs. respective control group.

§ Assuming a standard deviation equal to the female control group.

Monkey. Liver protein concentrations were determined in the same groups of animals mentioned previously. The results are shown in Table 7. Only in the relatively young females, treated for 3 months, was a change detectable, of marginal significance only. The slight increase in this particular group may be related to the rise in liver weight also found (Table 3).

Dog. Liver protein concentration was determined in the same group of dogs mentioned previously, and the results are indicated in Table 8. In both male and female dogs, three months treatment with CPIB has led to an increase in liver protein concentration.

TABLE 8. THE LIVER PROTEIN CONCENTRATION OF DOGS TREATED WITH CPIB FOR 3 MONTHS

Group	Dose (mg/kg p.o. every second day)	No. of animals	Liver protein concentration (g/100 g fresh liver wt.)		
			Mean*	% controls	P†
♂ and ♀	Control	3	15·50 ± 0·10	100	
♂	35	4	17·05 ± 0·52	110	0·05
♀	45	3	17·10 ± 0·53	110·5	<0·05

* Mean ± S.E.M.

† Treated group vs. mean of male and female controls (assumed that control male liver protein concentration is not significantly different from female level).

TABLE 9. THE RATE OF INCORPORATION OF ^{14}C-LEUCINE BY THE RIBOSOMES OF LIVER FROM MALE RATS TREATED WITH CPIB (0·25% IN THE DIET).

Duration of dosing (days)	Rate of incorporation of ^{14}C-leucine			
	Controls counts/min/mg* ribosomal protein	Treated counts/min/mg* ribosomal protein	% controls	P†
1	248·5 ± 6·2(8)	404 ± 91(2)‡	163	0·10
3		371 ± 69(2)‡	149	0·10
7		305 ± 111(2)	123	>0·2
9		284 ± 14·5(2)	115	0·05

* Mean ± S.E.M. [number of animals (15,000 *g* homogenate preparation used)].

† Treated group vs. combined control group.

‡ When combined, give mean of 387·5 cpm/mg ± 47·6, $P = 0·01$.

Protein synthesis by rat liver ribosomes

In order to determine the role of protein synthesis in the increase of liver weight due to CPIB, the rate of incorporation of ^{14}C-leucine into protein by liver ribosomes *in vitro* was measured by our colleague Dr. T. J. Franklin. This experiment was designed to ensure that animals treated for different lengths of time should be killed in parallel, on the same day, to minimize experimental variation as far as possible. The results shown in Table 9 demonstrate that the rate of incorporation of ^{14}C-leucine by liver ribosomes is stimulated by prior treatment of the animal with CPIB. Maximal stimulation occurs within 24 hr; probably within 12 hr, since the dose was mainly consumed during the nocturnal feeding immediately preceding sacrifice on the morning of the second day of the experiment. The large scatter reffected by the standard errors in Table 9 may be attributed to varying intakes in individual animals during the relatively short experimental period.

The stimulatory effect of CPIB gradually declines, reaching normal levels by about the tenth day. It is noteworthy that the rate of amino-acid incorporation into protein returns to normal as the level of total protein reaches a new equilibrium, i.e. at about 5–10 days after the start of treatment.

Water and glycogen content of rat liver

The degree of hydration of rat liver was determined following doses of 0·25% or 0·50% CPIB in the diet. Table 10 shows no consistent alteration of liver water content in agreement with a previously reported result,[2] where Atromid rather than Atromid-S was used.

TABLE 10. THE WATER CONTENT OF RAT LIVER TREATED WITH CPIB FOR SHORT PERIODS

Dose* (%)	Duration (days)	Liver dry wt. : Liver fresh wt. (%)			
		Controls %†	%†	Treated % controls	P‡
0·50	10–25	29·9 ± 0·16(20)	29·9 ± 0·18(15)	100	>0·2
0·50	7–11	30·1 ± 0·17(19)	30·0 ± 0·24(10)	99·5	>0·2
0·25	22	29·7 ± 0·42(10)	30·6 ± 0·13(10)	103	0·05

* CPIB added to the diet of rats at the levels shown.
† Mean ± S.E.M. (number of animals).
‡ Treated vs. control group.

Treatment of rats with 0·25% CPIB leads to a reduction of the liver glycogen concentration; control rats showed a glycogen concentration (at 11 a.m.) of 2·5 ± 0·12% of the fresh liver weight, and rats treated with 0·25% CPIB for 14 days showed levels of 1·4 ± 0·17% of the fresh weight at the same time of day. The glycogen depletion is readily visible in liver sections, and resembles that due to thyroxine treatment (Fig. 2).

DISCUSSION

It has been demonstrated that CPIB, when administered in the diet to rats, causes an increase in the weight of the liver, an increase in the concentration of liver protein, a decrease in non-fasting glycogen content, but has no effect on the liver water content. The effects on liver weight and protein content follow a similar pattern, although changes in liver protein precede the changes in overall liver weight. The pattern is one of an initial stimulatory period, where these parameters undergo maximum change, followed by an equilibrium phase maintained throughout drug administration; the parameters return rapidly to normal after removal of the drug from the diet. A third stage becomes apparent with age where, particularly in senile male rats (Table 2), continuous treatment for 2 yr leads to an apparent "loss" of effect. Marked stimulation of amino-acid incorporation by the liver ribosomes has been shown within 24 hr of CPIB feeding, followed by a steady decline to the control level, which is reached coincidentally with attainment of maximum liver protein concentration. The presence of androsterone in the CPIB preparation has no additional effect on these findings, supporting the observations of Best and Duncan.[4]

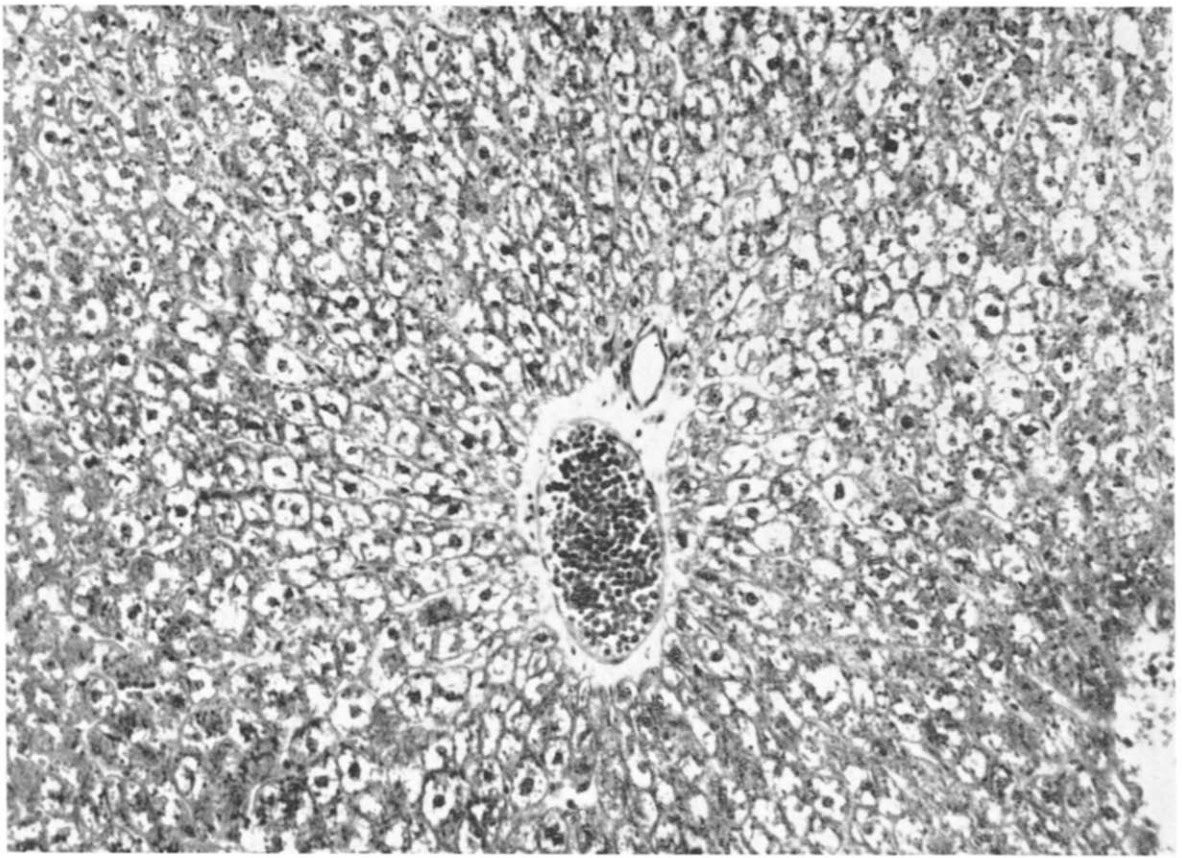

FIG. 2a, b and c. Comparison of the effects of L-thyroxine administration and CP1B administration on glycogen vacuolation of rat liver cells. a. Normal liver.

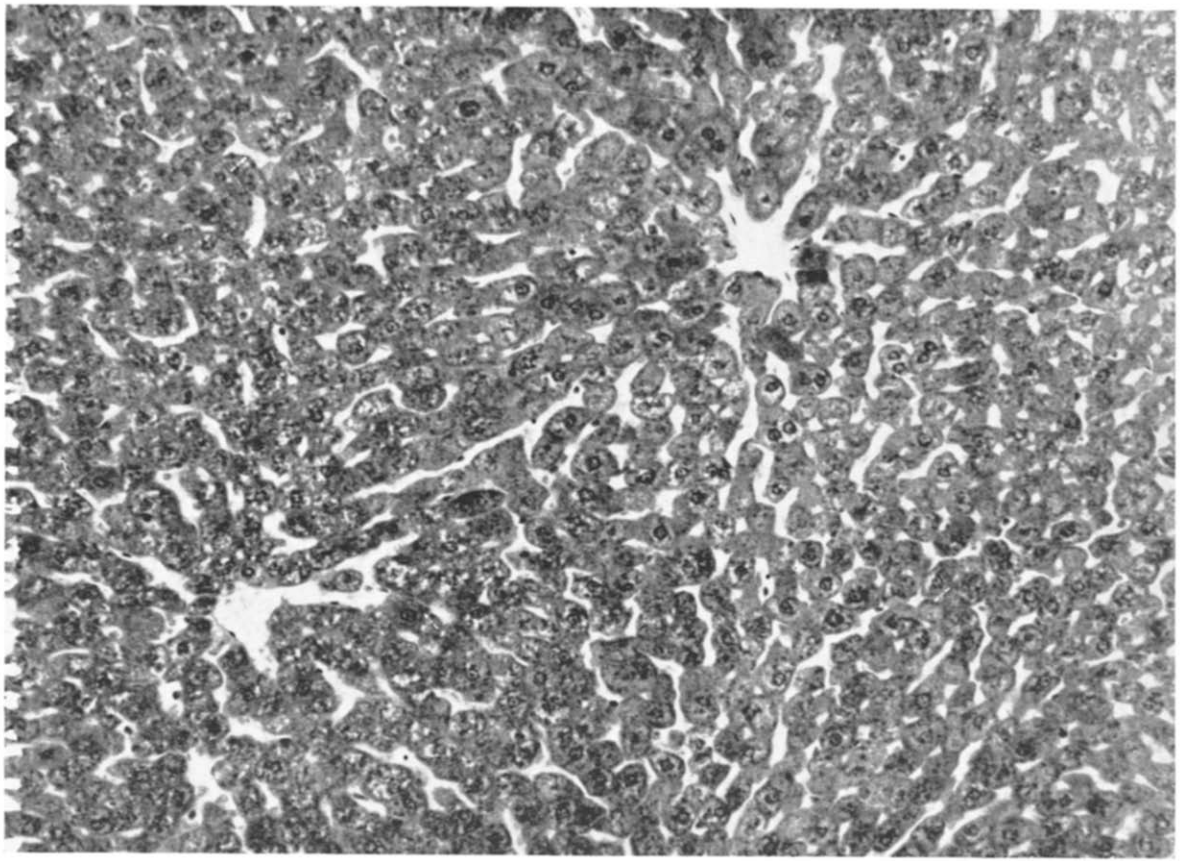

FIG. 2b. CPIB treated (0·25% w/w in diet for 15 days).

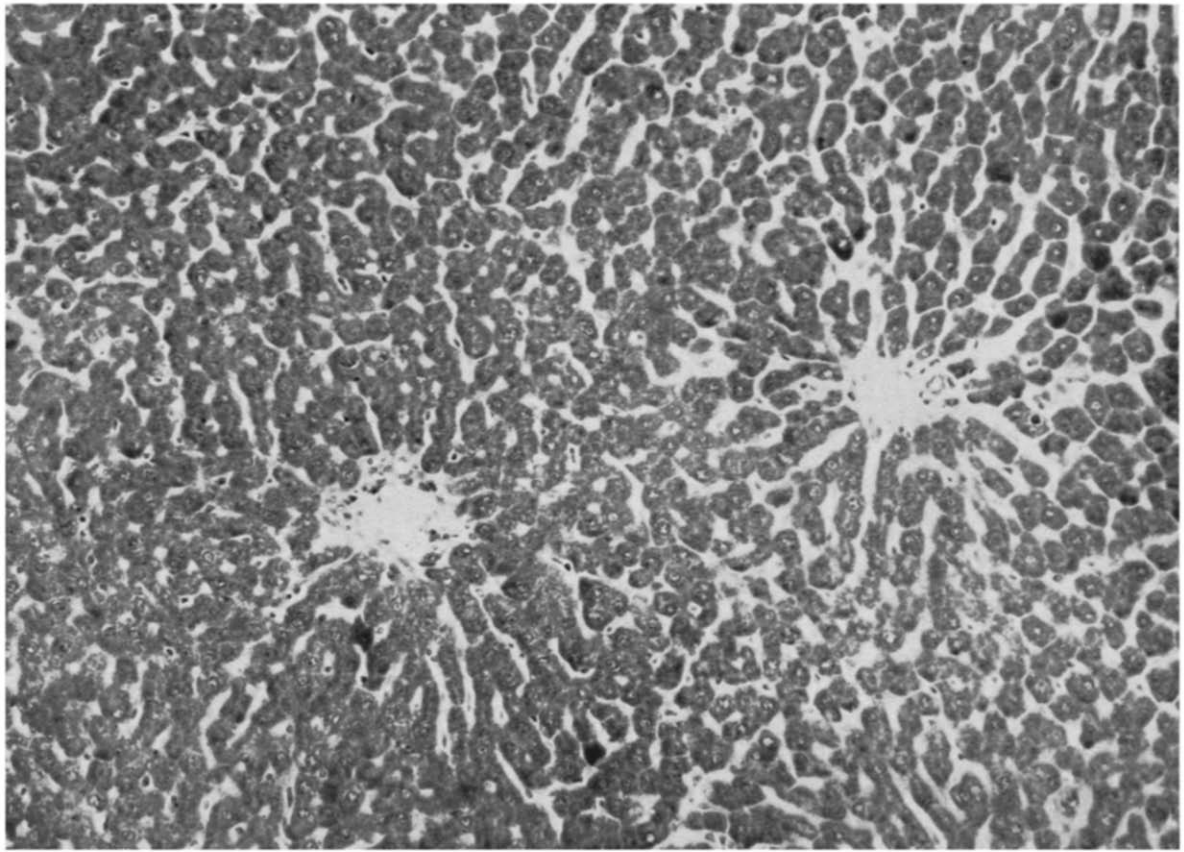

FIG 2c. Thyroxine treated (0·001 % w/w in diet for 28 days). Note reduction of glycogen vacuolation and basophilic granulation in CPIB and thyroxine treated rats (H and E stain; × 150).

Investigations in the dog have shown that CPIB raises both liver weight and protein concentration, but in monkey only the smaller animals, treated for a short period (3 months), showed an effect on liver weight which was increased; liver protein concentration was not significantly changed in this group. Treatment of monkeys for two years as in the rat, leads to an apparent loss of effect on liver weight. Best and Duncan[4] found that adrenalectomy and gonadectomy in rats abolished the effect of CPIB, and that thyroidectomy reduced rat liver weight. They found that CPIB partially restored the liver weight of thyroidectomized rats towards normal, but it is significant that, in this condition, the effect of CPIB was markedly curtailed compared to that in a euthyroid rat. Any attempt, therefore, to explain the action of CPIB on the liver must take into consideration these changes.

Since, at a dose level of 0·25 per cent in the diet, CPIB fails to enter rat liver cells,[9] it is unlikely that the effects on the liver are direct effects; the evidence points to an indirect effect as a result of intermediary factors. Perfusion of dog liver for 4 hr at levels of 200 μg CPIB/ml. fails to demonstrate any penetration of the drug into dog liver cells.[9]

It has been established[6] that CPIB is bound at a particular site of serum albumen, which is the same as that occupied by such endogenous factors as androsterone and dehydroepiandrosterone sulphates, L-thyroxine, pyridoxal phosphate, non-esterified fatty acids and L-tryptophan.[8] Thorp[9] and Osorio *et al.*[10] have also shown that L-thyroxine, displaced by CPIB is, taken up specifically by the liver, and this evidence supports the interpretation that CPIB potentiates the hepatic effects of endogenous hormones and other factors, as originally postulated.[2, 3] Essentially, therefore, the degree of response to CPIB depends upon the plasma level attained, this is turn leading to a redistribution (rather than a complete displacement) of several factors, principally between plasma and liver. This is in contrast to the usual pattern, where binding of a drug leads to its inactivation. The predictable outcome, therefore, of overdosage is to produce deficiency of one or more of these factors, and in fact in the dog classical signs of vitamin B_6 deficiency were found after prolonged high dosage. The half-life of CPIB in dog serum is over 40 hr compared to less than 12 hr in all other species, including man. Because of the general nature of the factors displaced by CPIB, it would be wrong to assume that CPIB only affects fat metabolism and this is supported by the observed alterations in liver protein and glycogen levels.

We believe, from the evidence available, that the effects noted are primarily due to the displacement of thyroxine into the liver. It has been shown[4, 9] that thyroidectomy of the rat effectively abolishes the effect of CPIB on serum cholesterol and, furthermore, that the maximal effect of CPIB on rat serum cholesterol corresponds with the periods of maximal thyroid and adrenocortical function.[6, 11] Considerable evidence is available[12–15] that thyroxine at physiological levels stimulates amino-acid incorporation by rat liver ribosome preparations within a few hours of treatment. Moury and Crane[15] maintain that the effects of thyroxine are restricted to synthesis of certain specific proteins and the evidence for stimulation by CPIB cannot be said to apply to the general synthesis of all proteins.[24] The normal endogenous secretion of thyroxine is of the order of 1 μg/100 g body wt./day,[16] and thus a small change in its distribution would be expected to have readily observable effects. Yatvin *et al.*[17] *inter alia* have shown that thyroidectomy in rats leads to a decrease in liver weight, in the protein: DNA ratio and in the rate of protein biosynthesis; these effects are the direct anti-

thesis of the actions of CPIB reported in this paper (histological evidence has shown no change in liver cell number following CPIB).[5] Thyroxine administration also leads to a reduction in liver glycogen, e.g. reference 6.

Because of the displacement of thyroxine by CPIB into the liver, the overall effect is to produce an hyperthyroid effect in the liver and an hypothyroid effect in the periphery. Eiler *et al.*[18] have pointed out that the early effects of thyroxine in the liver are modified by the secondary effects, such as those on peripheral metabolism and the evidence in this paper also indicates modification of the initial response, leading to an equilibrium phase. This must arise, for the reasons given, from the intervention of other factors and it is, therefore, not possible to parallel the effects of CPIB in the liver by manipulating thyroxine administration.

In the case of CPIB treatment, these secondary factors probably include tryptophan, pyridoxal phosphate and androsterone, although the anabolic effects of androsterone may be involved in the initial stimulation of protein biosynthesis. Tryptophan[19, 20] and pyridoxal phosphate[18, 21–23] have been shown to antagonise or modify the effects of thyroxine, but the exact nature of the interplay in the normal state is not understood. It would seem that the pattern of response to CPIB treatment represents the end result of a differential redistribution of these factors from the plasma to the liver and that the species variation in response is a matter only of degree, depending upon relative metabolic rates and the extent to which each of these factors is redistributed and the resultant interplay of their effects in the liver. The basic mode of action of CPIB, however, from one species to the next remains the same. The apparent "loss" of effect with age is also understandable, when it is realised that the normal pattern of these factors may change with age; for example, the diminution in thyroid function with age.

It must be stressed that the involvement of other hormones, particularly the steroids, cannot be ruled out of the overall picture but the weight of evidence accumulated favours thyroxine as the prime factor in establishing a change in liver weight, protein and glycogen content. We cannot agree, therefore, with Best and Duncan,[4] who have postulated that the effect of CPIB in producing hypertrophy of the liver in the rat is due to a non-specific response of the rat to lipid-soluble compounds in general.

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