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LABORATORY STUDIES OF 1-METHYL-4-PIPERIDYL *BIS*(*p*-CHLOROPHENOXY) ACETATE (SaH 42-348)— A NEW HYPOLIPIDEMIC AGENT

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Abstract—The hypolipidemic properties of 1-methyl-4-piperidyl *bis*(*p*-chlorophenoxy) acetate (SaH 42-348) in the male Wistar rat have been described. This agent is eight to nine times more active on a weight for weight basis than chlorphenoxy isobutyric acid ethyl ester (CPIB) (Atromid-S), and in common with CPIB lowers the levels of all major classes of serum lipid. No increase in liver lipid content was seen in animals treated with SaH 42-348, although hepatomegaly was seen after treatment with SaH 42-348 or with CPIB. Evidence is presented suggesting that the mechanism of action of SaH 42-348 in lowering serum cholesterol in the rat differs from that of CPIB. Thus, it was not possible to demonstrate that SaH 42-348 inhibited cholesterol biosynthesis *in vivo* from 1-^{14}C acetate, while the inhibitory effect of CPIB was readily demonstrated. In addition, *n*-propylthiouracil (PTU) inhibited the hypocholesterolemic activity of CPIB in both normolipemic and hyperlipemic rats, whereas the activity of SaH 42-348 was unaffected by PTU.

ONE OF a series of *bis*(*p*-chlorphenoxy) compounds synthesized as potential hypolipidemic agents is SaH 42-348 (42-348).* Chemically, 42-348 is 1-methyl-4-piperidyl *bis*(*p*-chlorophenoxy) acetate, and the structural formula is shown below:

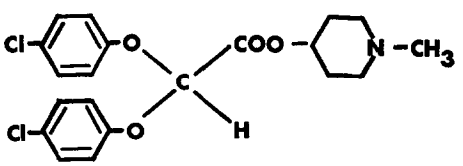

FIG. 1. Structural formula of 1-methyl-4-piperidyl *bis*(*p*-chlorophenoxy) acetate (42-348).

On a weight for weight basis, the hypolipidemic activity of 42-348 is eight to nine times greater than that of CPIB† in the male Wistar rat. Furthermore, the mode of action of 42-348 in decreasing serum cholesterol levels seems to differ in certain fundamental respects from that of CPIB. For example, the hypocholesterolemic activity of 42-348 in the rat is not blocked by pretreatment of the animals with PTU, whereas PTU has marked inhibitory activity on the hypocholesterolemic activity of CPIB, as described for thiouracil by Best and Duncan.[1] In addition, the incorporation of ^{14}C from 1-^{14}C acetate into cholesterol is not inhibited by effective hypocholestero-

* All syntheses were performed by Dr. Rudolf G. Griot, Sandoz Research Department, Hanover, N.J.

† Abbreviations: CPIB, Chlorphenoxy isobutyric acid ethyl ester; PTU, *N*-propylthiouracil.

lemic doses of 42-348, while CPIB did show inhibitory activity under these conditions, both in our experiments and in those reported previously by Avoy *et al.*[2] Experiments which illustrate these points are the subject of this communication.

METHODS

Hypolipidemic activity—"standard test conditions". Male Wistar rats, purchased from Royal Hart Breeders, N.Y., were placed randomly in pairs in metal cages and fed powdered Purina laboratory chow *ad lib.* for a period of 5–7 days to accommodate the animals to a powdered diet. At this time they were randomly grouped, and their individual initial body weights were recorded. During the 6-day experimental period which followed, the animals were fed *ad lib.* medicated (drug containing) or control diets prepared in the following manner.

42-348 was incorporated mechanically as a dry powder into the diet. Weighed amounts of 42-348 were triturated with a 20-30 g aliquot of the powdered diet in a mortar. When thoroughly mixed, a further 30 g of powdered diet was added and trituration continued. This mixture was then transferred to a motor-driven mixer and the remainder of the calculated quantity of powdered diet was incorporated gradually. CPIB (Atromid-S, Clofibrate) was dissolved in 5 vols. of acetone and incorporated into an aliquot of the food in a mortar. When the acetone had evaporated, the remainder of the powdered food was incorporated using a motor-driven mixer. Diets containing drugs were prepared fresh for every experiment.

Body weight increases were determined throughout the experimental period.

Serum and liver samples were obtained at the end of each experiment in the following way. The unfasted animals were anaesthetised by intraperitoneal injection of sodium hexobarbital (120 mg/kg) and secured in the supine position. Blood was obtained by incision of the jugular veins and collected in acid-washed tubes. The serum was separated by centrifugation and extracted by the method described below. These conditions were used in the majority of these studies. They will be referred to as the "standard test conditions", and any deviation from them will be indicated.

Hyperlipemic animals. In some experiments, hyperlipemic rather than normolipemic rats were used. The animals were placed on a thrombogenic diet* for 2 weeks. During the third week, the animals received the same diet in which 42-348 or CPIB had been incorporated mechanically. In these experiments, however, the CPIB was not dissolved in acetone, but was incorporated directly as a liquid into the diet. The control animals continued on the untreated thrombogenic diet for the third week. Food consumption was measured at 2-day intervals throughout the third week and the body weights of the animals were measured at the beginning and the end of the third week. Some experiments were performed using the same thrombogenic diet but without PTU.

Extraction of serum for routine cholesterol and triglyceride analysis. All solvents used in lipid analyses were redistilled prior to use. One ml of serum was added to 9·0 ml of redistilled isopropanol in a stoppered centrifuge tube and mixed. Two g of the following mixture[3] were added to the isopropanol extracts to remove the phospholipids, carbohydrates and traces of bilirubin: 200 g Zeolite (100 mesh,

* Nutritional Biochemicals Inc., Cleveland, Ohio. The composition is (% w/w) alphacel 6·0 butter fat 40·0, cholesterol 5·0, choline chloride 0·2, PTU 0·3, salt mixture 4·0, sodium cholate 2·0, sucrose 20·5, vitamin fortification mixture 2·0, vitamin-free casein 20·0.

activated at 100° for 10 hr), 10 g $CuSO_4$: 10, H_2O (powdered), 20 g $Ca(OH)_2$, 20 g Lloyd's reagent.* The tubes were either shaken mechanically for 2 hr or shaken briefly and allowed to stand at 0–4° overnight. Either of these procedures achieved complete removal of phospholipids, glucose and bilirubin. The tubes were then centrifuged at 3000 rpm for 10 min at room temperature and aliquots of the clear supernatants were taken for cholesterol and triglyceride analysis. Cholesterol was determined on the AutoAnalyzer† by the method of Lofland[4] and triglycerides were determined by either the enzymatic or semiautomated colorimetric method of Timms *et al.*[5]

Lipid class analysis. In some experiments the serum and liver lipids were fractionated for analysis of free and esterified cholesterol, triglycerides and phospholipids. For this purpose serum was extracted and purified by the method of Folch *et al.*[6] Phospholipids were determined by a phosphorus analysis[7] on aliquots of the purified extract. A factor of 25 times the phospholipid phosphorus was used to calculate the phospholipid content. The remainder of the extract was used for determination of triglycerides and free and esterified cholesterol. The neutral lipid fraction was isolated with silicic acid-supercel columns as described by Van Handel.[8] Triglycerides were determined on aliquots of the neutral lipid fraction by the method of Van Handel and Zilversmit[9] as modified by Newman *et al.*[10] Free and esterified cholesterol were isolated from the remainder of the neutral lipid fraction by a further passage through a silicic acid-supercel column. The free and esterified cholesterol fractions were saponified by the method of Abel *et al.*[11] and cholesterol was determined as described by Zlatkis *et al.*[12]

The lipid fractions of liver were analysed in the same manner by extraction[6] of 5-g samples of liver.

Incorporation of 1-^{14}*C-acetate into lipid fractions of serum and liver.* To assess the effect of 42-348 on the biosynthesis of cholesterol, phospholipids and triglycerides, the incorporation of ^{14}C from 1-^{14}C-acetate (Calbiochem) into these fractions was measured. Rats treated with 42-348 under the standard test conditions received an intraperitoneal injection of Na-1-^{14}C acetate (30 μc/100 g body weight) after 6 days of drug administration. Control animals were treated in the same way after 6 days *ad lib.* feeding of a drug-free diet. Serum and liver samples were obtained for analysis 3 hr after administration of ^{14}C-acetate. Aliquots of the phospholipid fraction, separated from the neutral lipid fraction as described above, were counted in 10 ml of 1% PPO‡ and 0·06% POPOP‡ in toluene. Phospholipid phosphorus was determined by the method of Bartlett.[7] The neutral lipid fraction eluted from the silicic acid:supercel columns was further fractionated by thin-layer chromotography on silica gel H§ using a solvent system of Skelly B‖:ethyl ether:acetic acid (80:20:1, v/v).[13] Free cholesterol, cholesterol esters and triglycerides were identified on the thin-layer plates by comparison with standards after a brief exposure to iodine vapor. The free and esterified cholesterol bands were scraped off and eluted with 2:1 chloroform:methanol and were saponified by the method of Abel *et al.*[11] Aliquots of the nonsaponifiable fraction were taken for counting in 19 ml 0·5% PPO and 0·3%

* Hydrated aluminum silicate, Arthur H. Thomas Co., Philadelphia, Pa.
† Technicon Control, Inc., New York, N.Y.
‡ PPO, 2·5 diphenyloxazole; POPOP, *bis*-2-(5-phenyloxazolyl)-benzene.
§ Silica gel H, E. Merck, Darmstedt, Germany; distributors, Brinkman Inst., Inc., Great Neck, N.Y.
‖ Skelly Oil Co., Kansas City, Mo.

POPOP in toluene. Colorimetric determinations of cholesterol were made by the method of Zlatkis *et al.*[12] The triglyceride band was scraped into a counting vial, 19 ml 0·5% PPO and 0·3% POPOP in toluene were added and the samples were counted in a Packard Tri-Carb liquid scintillation spectrometer. Quenching corrections were made using an internal standard. Triglycerides were determined colorimetrically in the neutral lipid fraction by the method of Van Handel and Zilversmit[9] as modified by Newman *et al.*[10]

Desmosterol determinations. To determine whether desmosterol accumulation occurred as the result of treatment with 42-348, aliquots of the nonsaponifiable fraction from isopropanol extracts of serum (see above) were evaporated in a stream of nitrogen and redissolved in a small quantity of Skelly B (petroleum ether b.p. 60–80°). Cholesterol and desmosterol were separated with an F & M 810 gas chromotograph, using glass columns containing 2 per cent Epon resin 1001 on 80–100 Diatoport-S at a column temperature of 260° with a hydrogen flame detector. The carrier gas was helium, at a flow rate of 55–60 ml/min. Quantities of desmosterol equivalent to 0·1 per cent or less of the total cholesterol content were easily detected by this method in mixtures of authentic desmosterol and cholesterol.

Glassware. All glassware was washed with chromic acid to avoid hydrolysis of triglycerides by traces of alkaline detergents which sometimes adhered to the glassware.

Statistical methods. The significance of differences was calculated using the Student *t*-test, as described by Bernstein and Weatherall.[14]

RESULTS

Influence of 42-348 *on food consumption, body weight gain and liver weight of rats under standard test conditions.* Food consumption, body weight gain and liver weights were monitored in all experiments. Neither body weight nor food consumption was affected by concentrations of 42-348 below 0·12 per cent in the diet, compared with controls. CPIB, under comparable conditions, did not affect body weight or food

TABLE 1. EFFECT OF 42-348 AND OF CPIB ON ABSOLUTE AND RELATIVE LIVER WEIGHTS OF RATS UNDER STANDARD TEST CONDITIONS*

		42-348	CPIB
	Control	0·03% in diet	0·3% in diet
Mean Liver weight (g)	10·9 ± 0·2 (44)	13·6 ± 0·2 (45)	14·4 ± 0·3 (43)
Changes in liver weight as % of control		+ 24·7 P < 0·01	+ 32·2 P < 0·01
Mean liver to body weight ratio (%)	5·1 ± 0·08	6·3 ± 0·11	7·0 ± 0·11
Change in liver weight to body weight ratio as % of control		+ 23·5 P < 0·01	+ 3·72 P < 0·01

* Mean values ± S.E.M. are calculated from the number of animals shown in parentheses.

consumption in concentrations up to 0·3 per cent in the diet. Both agents, however, caused hepatomegaly (see Table 1), an effect previously reported for CPIB by Best and Duncan[1] and later by Avoy *et al.*[2] It should be noted that in the experiments reported in Table 1 the concentration of 42-348 was only one-tenth that of CPIB (0·03% vs. 0·3%). These concentrations were chosen on the basis of the doses necessary to produce approximately the same degree of hypocholesterolemic and hypotriglyceridemic activity with both compounds (see Fig. 2). On the basis of average food

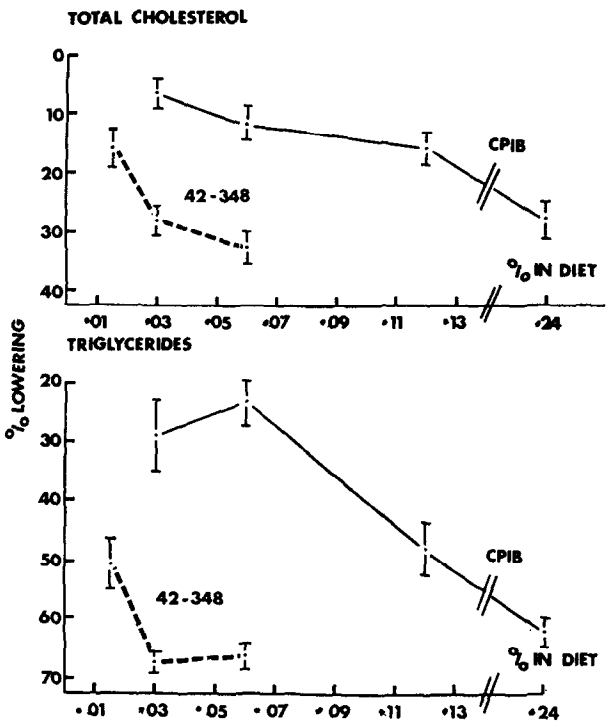

FIG. 2. Dose–effect curves for 42-348 and CPIB on serum total cholesterol and triglyceride levels of normal male Wistar rats, tested under the standard test conditions (see Methods).

consumption, it was calculated that 0·03 per cent of 42-348 in the diet corresponded to a daily drug intake of 28·5 mg/kg under standard test conditions.

Influence of 42-348 on serum and liver lipid levels of normal rats. The hypolipidemic activity of 42-348 in the male Wistar rat is related to the concentration of drug in the diet in the manner indicated in Fig. 2. The hypocholesterolemic activity ranged from 15 per cent at 0·015 per cent in the diet (15 mg/kg/day) to approximately 33 per cent at 0·06 per cent in the diet (60 mg/kg/day). CPIB, on the other hand, was significantly less active causing only a 15 per cent decrease in serum cholesterol even at 0·12 per cent in the diet, and approximately 28 per cent decrease at 0·24 per cent in the diet.

Similarly, 42-348 was significantly more active than CPIB in lowering serum triglyceride levels. The maximum hypotriglyceridemic effect was achieved with 0·03 per cent of the drug in the diet, whereas CPIB failed to produce a significant

hypotriglyceridemic effect at this dose. In fact, to achieve a comparable effect to that seen with 0·03 per cent of 42-348 it was necessary to use at least 0·24 per cent of CPIB in the diet.

Under these conditions, therefore, it is estimated that 42-348 is between eight to nine times more potent than CPIB in lowering serum cholesterol and triglyceride levels. On the basis of these observations, subsequent experiments comparing 42-348 and CPIB have generally employed 0·03 per cent of 42-348 and 0·3 per cent of CPIB in the diet in order to achieve approximately equal hypolipidemic activity.

The serum and liver lipids of male Wistar rats treated with 42-348 (0·03 per cent under the standard test conditions) were fractionated into free and esterified cholesterol, triglycerides and phospholipids and the values were compared with those obtained with a control group which received an unmedicated diet. The results are shown in Table 2. In serum, 42-348 caused statistically significant lowering of all

TABLE 2. EFFECT OF 42-348 ON SERUM AND LIVER LIPIDS OF MALE WISTAR RATS

Lipid fraction	Serum lipids (mg/100 ml ± S.E.M.)		% Change from control	Liver lipids (mg/g wet wt. ± S.E.M.)		% Change from control
	Control	42–348*		Control	42–348*	
Esterified chol.	34 ± 2‡ (10)	26 ± 2† (10)	−24	0·37 ± 0·02 (10)	0·29 ± 0·02† (10)	−22
Free chol.	16 ± 1 (10)	13 ± 1† (10)	−19	1·67 ± 0·15 (10)	1·61 ± 0·07 (9)	− 3
Triglycerides	67 ± 5 (10)	30 ± 4† (10)	−55	3·97 ± 0·23 (9)	4·31 ± 0·35 (10)	+ 4
Phospholipids	119 ± 2 (10)	94 ± 3† (10)	−21	27 ± 2 (10)	30 ± 2 (10)	+10
Total§	236 ± 12	163 ± 10	−30	33·0 ± 2·4	36·2 ± 2·4	+ 9

* 42-348 was administered under standard test conditions at 0·03% in the diet for 6 days.
† Denotes highly significant difference from appropriate control (P = 0·01).
‡ Figures in parenthesis are number of animals.
§ Arithmetic sum of individual fractions.

lipid fractions, ranging from 19 per cent depression of free cholesterol to 55 per cent depression of triglycerides. The esterified cholesterol: free cholesterol ratio was 2·12 in the control animals and 2·00 in the treated animals. The ratio of total cholesterol to phospholipid was 0·42 in the controls and 0·41 in the treated animals.

In the liver, the only significant change observed was a 22 per cent decrease in the esterified cholesterol fraction. Total liver lipids, taken as the sum of the fractions, were not significantly affected (33 mg/g for the controls and 36 mg/g for the 42-348 treated group). It should be pointed out that the fat-free residue of the control livers (23·7 ± 0·5 per cent by weight) was not significantly different from that of the treated livers (22·5 ± 0·7 per cent by weight). In view of this observation, it is felt that comparisons of lipid composition on a wet weight basis are valid.

The effect of 42-348 on serum desmosterol levels. To determine whether 42-348

causes a significant accumulation of desmosterol in serum, rats were fed 42-348 (0·03 per cent in the diet) for 6 days under the standard test conditions. Control animals were fed an unmedicated diet. Serum samples were obtained and saponified. Aliquots of the nonsaponifiable material were extracted into Skelly B and the sterols in this fraction were separated by gas chromatography. Chromatograms from these samples revealed that no significant accumulation of desmosterol occurred, compared with controls (Fig. 3).

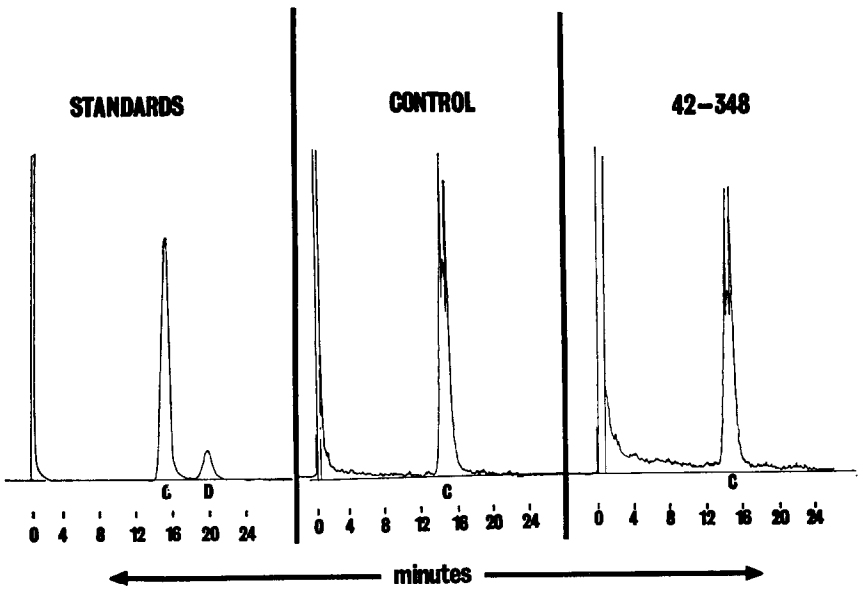

FIG. 3. Showing the absence of desmosterol accumulation in the serum of control or rats treated for 6 days with 42-348 (0·03% in the diet). The presence of desmosterol is clearly shown in the panel labelled "Standards" where a 7:1 mixture of authentic cholesterol (C):desmosterol (D) was applied to the column. Despite the significantly greater amounts of cholesterol applied in the Control and 42-348 panels, no significant levels of desmosterol were detectable.

The influence of 42-348 on the incorporation of 1-^{14}C-acetate into cholesterol of rat serum and liver. In view of the observation by Avoy *et al.*[2] that CPIB inhibited the incorporation of ^{14}C-acetate into cholesterol in the rat, experiments were conducted to determine whether 42-348 acted in a similar way. Rats fed a control diet, or the same diet containing 0·03 per cent of 42-348 or 0·3 per cent of CPIB received an i.p. injection of 1-^{14}C-acetate after 6 days of treatment. The incorporation of the label into the serum and liver cholesterol was determined, and the results are shown in Tables 3 and 4. In serum, both compounds produced the expected decrease in total cholesterol, but only in the CPIB-treated animals was a statistically significant reduction in ^{14}C incorporation observed. 42-348 did not cause a statistically significant reduction in ^{14}C-acetate incorporation into either serum or liver cholesterol under these conditions.

The effect of 42-348 and of CPIB on serum cholesterol levels of hyperlipemic (PTU-treated) rats. In the first experiment designed to compare 42-348 with CPIB in hyperlipemic rats, 60 male Wistar (Royal Hart) rats were divided randomly into 3

TABLE 3. EFFECT OF CPIB AND 42-348 ON THE INCORPORATION OF ACETATE-1-^{14}C INTO CHOLESTEROL OF SERUM OF RATS*

		Cholesterol (mg/100 ml)	Specific activity (dpm × 10^{-3}/mg cholesterol)	Total dpm × 10^{-3} in cholesterol (per ml serum)
	Control (5)	56 ± 5	9·24 ± 1·61	5·09 ± 0·69
Exp. 1	CPIB (0·3%) (9)	37 ± 4	4·62 ± 0·37 $P < 0·01$	1·66 ± 0·17 $P < 0·01$
	Control (10)	50 ± 2	3·13 ± 0·25	1·56 ± 0·14
Exp. 2	42–348 (0·03%) (10)	39 ± 2	3·81 ± 0·72 $P \simeq 0·4$	1·46 ± 0·28 $P \simeq 0·7$

* Figures are means ± S.E.M. from number of animals shown in parentheses.

TABLE 4. EFFECT OF CPIB AND OF 42-348 ON INCORPORATION OF ACETATE-1-^{14}C INTO CHOLESTEROL OF RAT LIVER*

		Cholesterol (mg/g)	Specific activity (dpm × 10^{-3}/mg cholesterol)	Total dpm × 10^{-3} in cholesterol (per g liver)
	Control (5)	1·77 ± 0·1	6·61 ± 1·0	11·80 ± 1·8
Exp. 1	CPIB (0·3%) (5)	1·55 ± 0·05 $P \simeq 0·3$	2·59 ± 0·18 $P < 0·01$	4·0 ± 0·27 $P < 0·01$
	Control (10)	2·03 ± 0·14	3·71 ± 0·68	7·54 ± 1·22
Exp. 2	42-348 (0·03%) (10)	1·81 ± 0·12 $P \simeq 0·3$	2·72 ± 0·47 $P \simeq 0·3$	5·06 ± 1·05 $P \simeq 0·2$

* Figures are means ± S.E.M. from the number of animals shown in parentheses.

groups of 20 each after 14 days of feeding the complete thrombogenic diet (i.e. containing PTU). The control group continued for a further 6 days on the same diet, while the other group received the same diet containing either 0·03 per cent of 42-348 or 0·3 per cent of CPIB. The total cholesterol levels, determined on sera from these animals, are shown on the left-half of Fig. 4. It can be seen that 42-348 produced a significant lowering of the serum cholesterol levels under these conditions, whereas CPIB was ineffective.

The effect of 42-348 and of CPIB on serum cholesterol levels of rats fed a thrombogenic diet free from PTU. A second experiment was conducted in which PTU was omitted from the thrombogenic diet. Both 42-348 and CPIB produced significant and approximately equal falls in the serum cholesterol levels (see right-half of Fig. 4). These results confirmed the observation of Best and Duncan[1] who showed an inhibitory activity of thiouracil on the hypocholesterolemic activity of CPIB in the rat. In

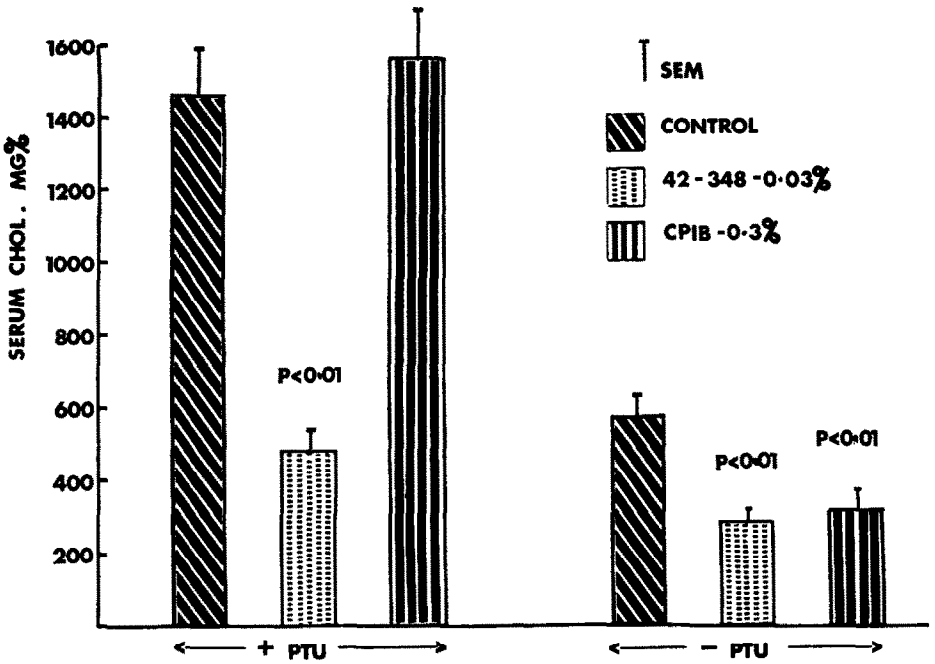

FIG. 4. Effect of 42-348 and CPIB on the serum total cholesterol levels of hyperlipemic male Wistar rats. 42-348 significantly reduced the cholesterol level in both the presence and the absence of PTU in the diet. CPIB was effective only in the absence of PTU.

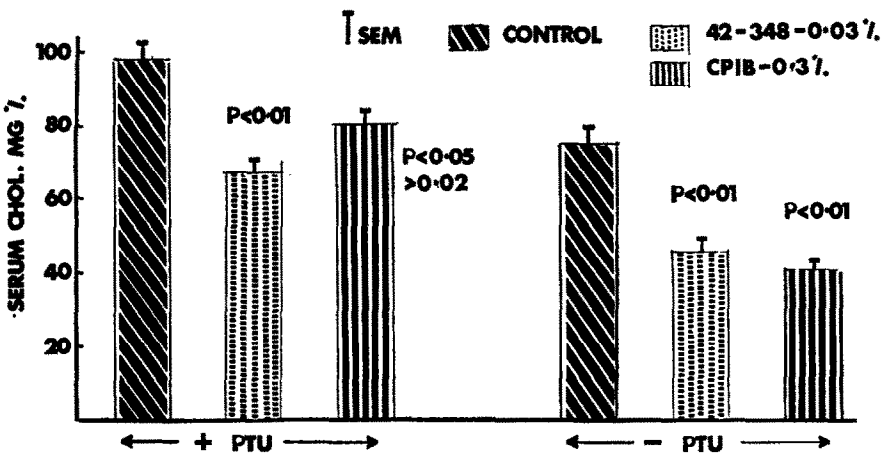

FIG. 5. Effect of PTU on the hypocholesterolemic activity of 42-348 and CPIB in male Wistar rats fed a normal powdered laboratory chow. Note that PTU significantly reduces the hypocholesterolemic activity of CPIB but not that of 42-348.

addition, the results suggest an important difference between 42-348 and CPIB in their mode of action as hypocholesterolemic agents.

Further evidence to substantiate this contention was obtained in rats fed normal diets with and without PTU. PTU was incorporated at a concentration of 0·3 per cent in normal laboratory chow and was fed *ad lib.* to male Wistar rats (Royal Hart) for 14 days. (Previous experiments under the same conditions had shown the animals' thyroid glands to be increased in size about 3- or 4-fold, compared with rats fed a PTU-free diet for the same period.) The control animals continued for a further 6 days on the PTU-containing diet, and the test groups received the PTU diet containing in addition 0·3% CPIB or 0·03% 42-348. Figure 5 shows the results obtained. On the left-hand side of the diagram the results from the PTU-treated animals are shown. On the right-hand side the results are shown from animals treated identically except that PTU was not included in the diet. 42-348 produced a significant hypocholesterolemic effect in both PTU-treated and in normal animals. The activity of CPIB was significantly reduced by PTU pretreatment, as seen by comparing the relatively small effect in the PTU-treated animals with the effect seen in the absence of PTU.

This may be interpreted as additional evidence that the hypocholesterolemic activity of 42-348 in the rat is mediated by a different mechanism from that of CPIB.

DISCUSSION

The results presented demonstrate the effectiveness of 42-348 as a hypolipidemic agent in the male Wistar rat. The data indicate that 42-348 is about nine times more potent than CPIB.

The hepatomegalic effect previously reported with CPIB in rats[1, 2] was confirmed in the present experiments. A similar effect was seen with 42-348, even though this agent was administered at only one-tenth of the dosage used with CPIB.

When the serum and liver lipids were fractionated it was observed that 42-348 treatment resulted in statistically significant reductions in all of the major lipid fractions of the serum. In the liver, the only statistically significant change from the controls was a 22 per cent reduction in the esterified cholesterol fraction. It should be pointed out, however, that because of the hepatomegaly produced by 42-348 treatment, the total amount of lipid in the whole liver of the treated animals is greater than in the controls (403 mg in the treated vs. 350 mg in the controls). It is conceivable, therefore, that the lipids lost from the serum pool are shifted to the enlarged liver, and this possibility is being investigated further.

In the remaining studies attention was focused on the mechanism of action of 42-348 in lowering serum cholesterol levels. It is clear from these studies that neither 42-348 nor CPIB caused significant accumulation of desmosterol in the serum of rats, under conditions where significant reductions of total serum cholesterol were observed with both agents. Apparently, neither CPIB nor 42-348 inhibit the Δ24 reductase step in the biosynthetic pathway for cholesterol, and in this respect both agents differ from MER-29 (Triparanol). In view of the observation of Avoy *et al.*[2] that CPIB inhibited ^{14}C incorporation from ^{14}C-acetate into cholesterol by rat liver, the effect of 42-348 on cholesterogenesis from acetate was examined. Although the present results confirm those of Avoy *et al.*[2] in showing significant reduction of cholesterogenesis from acetate in CPIB-treated animals, no conclusive evidence from such an effect was obtained with 42-348. In the serum there was no change in the specific

activity of the cholesterol fraction or in the total dpm in cholesterol per ml of serum compared with control animals (Table 3). In the liver also it was not possible to demonstrate a statistically significant decrease either in the specific activity or in the total dpm in cholesterol per gram of liver as a result of 42-348 treatment, at the indicated dose. Therefore, the present evidence indicates that 42-348 does not inhibit cholesterogenesis from acetate in the rat. In this respect, the compound appears to differ significantly from CPIB.

Further evidence illustrating the difference in mode of hypocholesterolemic activity between 42-348 and CPIB was obtained in experiments employing PTU. In rats fed a high fat thrombogenic diet, as well as rats fed a regular powdered Purina chow, the presence of PTU was inhibitory to the hypocholesterolemic activity of CPIB. The activity of 42-348, on the other hand, was unaffected by pretreatment of the animals with PTU with either the thrombogenic diet or a regular Purina diet. Best and Duncan[1] had previously reported inhibition of hypocholesterolemic activity of CPIB by thiouracil and our results would support their observation. However, as Duncan and Best[15] pointed out, such an inhibition by thiouracil does not imply that CPIB lowers cholesterol by an effect on the thyroid, because thiouracil is known to elevate cholesterol by a thyroid-independent mechanism.

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REFERENCES

1. M. M. Best and C. H. Duncan, *J. Lab. clin. Med.* **64**, 634 (1964).
2. D. R. Avoy, E. A. Swyryd and R. G. Gould, *J. Lipid Res.* **6**, 369 (1965).
3. G. Kessler and H. Lederer, *Technicon Symposium on Automation in Analytical Chemistry*, p. 345. Mediad Inc., New York (1965).
4. H. B. Lofland, *Analyt. Biochem.* **9**, 393 (1964).
5. A. R. Timms, L. A. Kelly, J. A. Spirito and R. G. Engstrom, *J. Lipid Res.* **9**, 675 (1968).
6. J. Folch, A. Lees and G. H. Sloane-Stanley, *J. biol. Chem.* **226**, 497 (1957).
7. C. R. Bartlett, *J. biol. Chem.* **234**, 466 (1959).
8. E. Van Handel, *J. Am. Oil Chem. Soc.* **36**, 294 (1959).
9. E. Van Handel and D. B. Zilversmit, *J. Lab. clin. Med.* **50**, 152 (1957).
10. H. A. I. Newman, C. Liu and D. B. Zilversmit, *J. Lipid Res.* **2**, 403 (1961).
11. L. L. Abel, B. B. Levy, B. B. Brody and F. E. Kendall, *J. biol. Chem.* **195**, 357 (1952).
12. A. Zlatkis, B. Zak and A. J. Boyle, *J. Lab. clin. Med.* **41**, 486 (1953).
13. P. Wood, K. Imaichi, J. Knowles, G. Michaels and L. Kinsell, *J. Lipid Res.* **5**, 225 (1964).
14. L. Bernstein and M. Weatherall, *Statistics for Medical and Other Biological Students*, p. 74. Livingstone, London (1952).
15. C. H. Duncan and M. M. Best, *Am. J. Physiol.* **194**, 351 (1958).