Biochemical Pharmacology, Vol. 23, pp. 685 696. Pergamon Press, 1974. Printed in Great Britain.

RELATIONSHIP OF SERUM TRIGLYCERIDE LOWERING TO CHANGES IN HEPATIC COMPOSITION INDUCED BY DIFFERENT CLASSES OF DRUGS

COLIN DALTON, WILLIAM C. HOPE, HARRIET R. HOPE and HERBERT SHEPPARD

Department of Pharmacology, Research Division, Hoffmann-La Roche Inc., Nutley, N.J. 07110, U.S.A.

(*Received* 15 *March* 1973; *accepted* 29 *June* 1973)

Abstract—Dose-related serum trigylceride lowering has been demonstrated in the rat with the microsomal enzyme inducers, phenobarbital, 5,5'-diphenyl-2-thiohydantoin and chlorcyclizine and with the clinically tested anti-hyperlipidemic agents, clofibrate, 1-methyl-4-piperidyl bis(*p*-chlorophenoxy) acetate (SaH42-348) and 2-methyl-2-[*p*-(1,2,3,4-tetrahydro-1-naphthyl)-phenoxy] propionic acid (Su-13437). With both classes of drugs, serum lipid lowering was accompanied by hepatomegaly due to an increase in liver protein and phospholipid. Hepatomegaly was reversible upon cessation of drug treatment. The anti-hyperlipidemic drugs caused an increase in microsomal *N*-demethylase activity but this was slight when compared with the induction produced by known microsomal enzyme inducers. Examination of the subcellular compartments indicated that, even though both mitochondrial protein and phospholipid was increased with all the agents studied, the dominant change in the case of the microsomal enzyme inducers was in the microsomal compartment, whereas the anti-hyperlipidemic agents induced a larger increase in the mitochondrial fraction. The total accumulation of new membrane material, however, was similar for both classes of drugs. These data demonstrate that two classes of drugs which produce hepatomegaly through a proliferation of intracellular membranes also lower serum triglyceride levels.

ONE OF THE more obvious and consistent findings in experimental animals treated with clofibrate* and the somewhat related serum lipid lowering compounds, Su-13437 and SaH42-348, is hepatomegaly.[1-3] An increase in liver size is also seen with many drugs loosely classified as microsomal enzyme inducers. Our laboratory has recently shown that the microsomal enzyme inducer DPTH causes a related hypotriglyceridemia and hepatomegaly in the rat.[4,5] This frequent concurrence of both microsomal enzyme induction and hepatomegaly suggested to us that both phenomena might be related to serum lipid lowering mechanisms. The studies reported here demonstrate that two classes of drugs which produce hepatomegaly through a proliferation of intracellular membranes also cause serum triglyceride lowering.

EXPERIMENTAL PROCEDURES

Treatment of animals. Male albino rats (Charles River Breeding Laboratories, Inc.), weighing 180–200 g, were housed in individual stainless steel cages with wire screen

* Abbreviations: clofibrate, ethyl α-(*p*-chlorophenoxy) isobutyrate; Su-13437, 2-methyl-2-[*p*-(1,2,3,4-tetrahydro-1-naphthyl)-phenoxyl] propionic acid; SaH42-348, 1-methyl-4-piperidyl bis(*p*-chlorophenoxy) acetate; DPTH, 5,5'-diphenyl-2-thiohydantoin; CLZ, chlorcyclizine hydrochloride; PB, phenobarbital sodium.

bottoms and kept at 23°. All animals were fed *ad lib.* with Purina rat chow and had free access to tap water. Drugs were suspended or dissolved in aqueous suspending vehicle (NaCl, 9 g/l.; carboxymethyl cellulose, 5 g/l.; Tween 80, 4 g/l. and benzyl alcohol 8·6 ml/l. of distilled water) and administered at the doses indicated in 5 ml/kg of vehicle. Control rats received an equal volume of the vehicle. Routes and time of drug administration are indicated in the Results section. The animals were fasted overnight and sacrificed by decapitation. Body and liver weights were recorded. Separate portions of the liver were used for the preparation of subcellular fractions, analysis of lipids and the determination of *N*-demethylase activity.

Procedure for preparation of subcellular fractions. A 10 per cent liver homogenate in 0·25 M sucrose was prepared using six strokes of a motor-driven glass-Teflon homogenizer at 4°.

Mitochondria were isolated according to the method of Schneider.[6] Samples were centrifuged at 800 *g* at 2° for 10 min in an International Refrigerated Centrifuge, model PR-6, to remove nuclei and unbroken cells. The supernatant was collected and the residue washed twice with isotonic sucrose. Washes were combined with the supernatant and the residue was discarded. The combined supernatants were centrifuged at 8500 *g* at 2° for 10 min in a Sorvall Automatic Refrigerated Centrifuge, model RC2-B, to sediment the mitochondria. The supernatant was collected and the mitochondrial pellet was washed twice with isotonic sucrose.

Microsomes were prepared by centrifuging the combined supernatants from the mitochondrial fraction at 105,000 *g* at 2° for 75 min in a Beckman model L-2 Ultracentrifuge. The mitochondrial and microsomal pellets were resuspended in isotonic sucrose and rehomogenized gently with a glass–Teflon homogenizer to a final tissue equivalent of 150 mg/ml. The purity of the mitochondrial and microsomal fractions was verified by electron microscopy.[7]

Analytical procedures. Serum was prepared by centrifugation and analyzed for cholesterol,[8] triglycerides[9] and phospholipids.[10]

Protein content of the subcellular fractions was determined by the biuret reaction conducted on an AutoAnalyzer (Technicon Corp. methodology N-14-b). Lipids were extracted from the subcellular fractions with chloroform–methanol (2:1, v/v) using a modification of the Folch procedure[11] determined gravimetrically and analyzed for phospholipid content by the method of Whitley and Alburn.[10]

Aliquots of the 0·25 M sucrose homogenate were used for total liver protein determination and for separation of DNA and RNA.[12] DNA was assayed using the diphenylamine reaction of Burton[13] and RNA was determined by u.v. absorption at 260 nm.

Determination of hepatic N-*demethylase activity.* A 20 per cent liver homogenate in 0·01 M phosphate buffer containing 1·15% KCl was prepared by homogenizing in a high speed Waring blender three times for 10 sec. The homogenates were centrifuged in 50-ml polyethylene tubes in a Sorvall centrifuge at 10,000 *g* (SS-34 head) for 30 min at 2°. The supernatant was removed with a syringe and analyzed immediately for *N*-demethylase activity using an automated adaptation[14] of the method of Axelrod[15] utilizing ethyl morphine as the substrate.

Materials. The substances used in this study were 5,5'-diphenyl-2-thiohydantoin, Chemical Procurement Laboratories, Inc., College Point, N.Y. 11356; sodium phenobarbital, Merck & Co., Inc., Rahway, N.J.; chlorcyclizine hydrochloride, Bur-

roughs Wellcome & Co., Research Triangle Park, N.C. 27709; ethyl α-(*p*-chlorophenoxy) isobutyrate (clofibrate), Ayerst Laboratories, Inc., New York, N.Y.; 1-methyl-4-piperidyl bis(*p*-chlorophenoxy) acetate (SaH42-348), Sandoz Pharmaceutical, Hanover, N.J.; 2-methyl-2-[*p*-(1,2,3,4-tetrahydro-1-naphthyl)-phenoxy] propionic acid (Su-13437), Ciba Pharmaceutical Co., Summit, N.J. and antinomycin-D, Nutritional Biochemical Corp., Cleveland, Ohio 44128.

The statistical significance of the data was determined by the Student's *t*-test.

RESULTS

Administration of the anti-hyperlipidemic drugs, clofibrate, Su-13437 or SaH42-348 by oral intubation for 9 days to normal rats caused an expected decrease in serum cholesterol, triglycerides and phospholipids and an increase in liver weight and the ratio of liver to body weight (Table 1). In contrast to the anti-hyperlipidemic agents, the microsomal enzyme inducers did not have a depleting effect on the entire lipid spectrum. DPTH reduced only serum triglyceride concentration and increased serum cholesterol and phospholipid levels (Table 2). CLZ caused a great reduction in serum triglyceride concentration and also lowered serum phospholipid levels

TABLE 1. EFFECT OF ANTI-HYPERLIPIDEMIC DRUGS ON RAT LIVER WEIGHT AND SERUM LIPID CONTENT*

	Liver wt (g)	Liver/body wt (g/100 g)	Cholesterol (mg/100 ml)	Triglyceride (mg/100 ml)	Phospholipid (mg/100 ml)
Control	7·50 ± 0·24	3·10 ± 0·08	66 ± 3	60 ± 4	118 ± 4
Clofibrate	10·88 ± 0·26†	4·60 ± 0·12†	40 ± 1†	47 ± 3‡	89 ± 3†
Su-13437	13·05 ± 0·45†	5·51 ± 0·13†	52 ± 3§	41 ± 3§	94 ± 5§
SaH42-348	11·95 ± 0·34†	5·01 ± 0·09†	38 ± 1†	31 ± 2†	53 ± 3†

* Drugs were suspended in aqueous suspending vehicle and administered by oral intubation for 9 days at the following doses: clofibrate, 250 mg/kg, Su-13437 and SaH42-348, 50 mg/kg. Control rats were administered aqueous suspending vehicle. Data represent the mean of twelve rats per group ± standard error of the mean. The significance of the difference between the treated and the control groups is designated by the P values.

† $P < 0.001$.

‡ $P < 0.05$.

§ $P < 0.01$.

TABLE 2. EFFECT OF MICROSOMAL ENZYME INDUCERS ON RAT LIVER WEIGHT AND SERUM LIPID CONTENT*

	Liver wt (g)	Liver/body wt (g/100 g)	Cholesterol (mg/100 ml)	Triglyceride (mg/100 ml)	Phospholipid (mg/100 ml)
Control	8·9 ± 0·25	3·6 ± 0·06	62 ± 4·1	60 ± 3·7	89 ± 4·7
DPTH	10·6 ± 0·76	4·8 ± 0·19†	90 ± 1·5†	30 ± 3·9†	110 ± 2·1‡
Phenobarbital	10·1 ± 0·17‡	4·3 ± 0·12†	60 ± 5·9	44 ± 8·8	81 ± 4·5
Chlorcyclizine	9·6 ± 0·50	4·3 ± 0·13†	50 ± 4·3	24 ± 3·6†	72 ± 3·3§

* Drugs were suspended in aqueous suspending vehicle and administered by oral intubation at 50 mg/kg/day for 9 days. Control rats were administered aqueous suspending vehicle. Data represent the mean of six rats per group ± standard error. The significance of the difference between the treated and the control groups is designated by the P values.

† $P < 0.001$.

‡ $P < 0.01$.

§ $P < 0.05$.

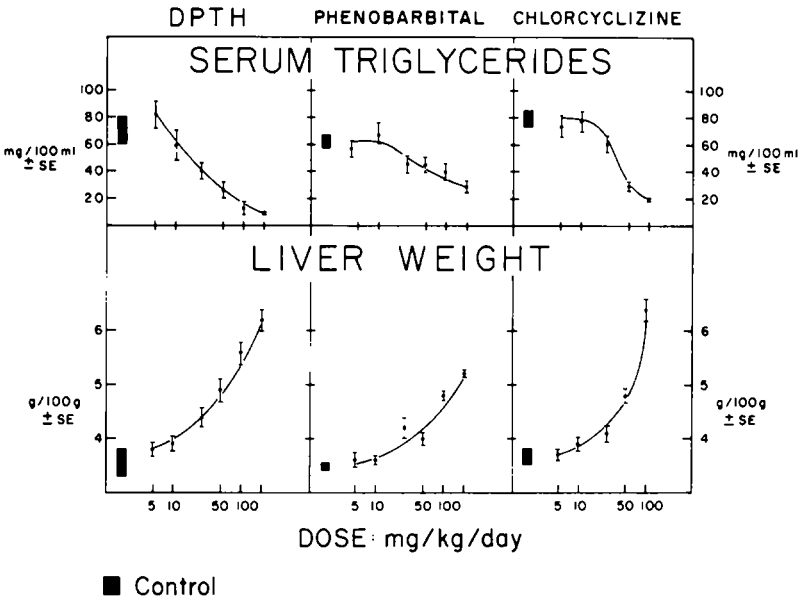

FIG. 1. Effect of chronic administration of microsomal enzyme inducers on liver weight and serum triglyceride concentration. Male Charles River rats (six per group) initially weighing between 170 and 180 g were administered the drugs by oral intubation at the doses indicated for 9 days. The animals were fasted overnight and sacrificed for liver weight determination and serum lipid analysis. Control represents the mean ± S. E. M. for six rats. The volume of the vehicle was kept consistent for control and treated groups. Final body weight of the control group and the highest dose groups of DPTH, phenobarbital and chlorcyclizine were 228 ± 4, 173 ± 5·9, 203 ± 10 and 171 ± 3·7 respectively.

(Table 2). PB caused a slight but not statistically significant lowering of serum triglycerides. These agents also increased the ratio of liver to body weight (Table 2) and at higher doses caused an actual increase in liver weight (Fig. 1). All of the agents studied shared the common property of lowering serum triglycerides and increasing liver weight. The nature of the hepatomegaly and its relationship to serum triglyceride lowering were explored further.

Examination of the composition of the liver obtained from rats treated with antihyperlipidemic agents revealed no change in DNA content of per cent water, indicating that neither hyperplasia nor edema was an explanation of the liver enlargement. However, significant increases in protein and lipid and some changes in liver RNA content were found (Table 3). Oral administration of DPTH has been reported to produce similar gross changes in rat liver composition.[7]

Oral administration of the microsomal enzyme inducers, DPTH, PB or CLZ, to rats for 9 days caused a dose-related decrease in serum triglyceride levels (Fig. 1). Rats treated with conventional hypolipidemic doses of clofibrate, SaH42-348 or Su-13437 also showed a dose-related parallel increase in liver weight and liver total lipid and protein content (Fig. 2).

Quantitation of the different lipid classes in the hepatomegalic livers, induced by representatives of the two groups of drugs under investigation, revealed an apparent decrease in cholesterol concentration when expressed on a mg/g basis, but no change in the total cholesterol content of the liver (Table 4). Su-13437 caused a decrease in

TABLE 3. EFFECT OF HYPOLIPIDEMIC DRUGS ON LIVER COMPOSITION*

	Protein (g/liver)	Lipid (mg/liver)	RNA (mg/liver)	DNA (mg/liver)	Water (%)
Control	1·5 ± 0·11	349 ± 27	57·9 ± 3·6	23·8 ± 1·9	72
Clofibrate	2·6 ± 0·10†	729 ± 57†	81·4 ± 3·6‡	26·3 ± 1·9	70
Su-13437	2·6 ± 0·07†	852 ± 60†	80·6 ± 5·7§	26·2 ± 2·0	70
SaH42-348	2·2 ± 0·09‡	704 ± 23†	69·0 ± 3·9	23·1 ± 2·6	70

* Drugs were suspended in aqueous suspending vehicle and administered by oral intubation for 9 days at the following doses: clofibrate, 250 mg/kg, Su-13437 and SaH42-384, 50 mg/kg. Control rats were administered aqueous suspending vehicle. Data represent the mean of six rats per group ± standard error. The significance of the difference between the treated and the control groups is designated by the P values.
† $P < 0.001$.
‡ $P < 0.01$.
§ $P < 0.05$.

liver triglyceride concentration but no change in total liver triglyceride content and the other agents did not significantly change liver triglyceride content (Table 4).

All of the agents studied caused an induction of the liver drug-metabolizing enzymes as shown by the ability of the microsomes to *N*-demethylate ethyl morphine (Table 5). The effect of the anti-hyperlipidemic agents, however, was less than that resulting from administration of the known microsomal enzyme inducers, DPTH, PB or CLZ.

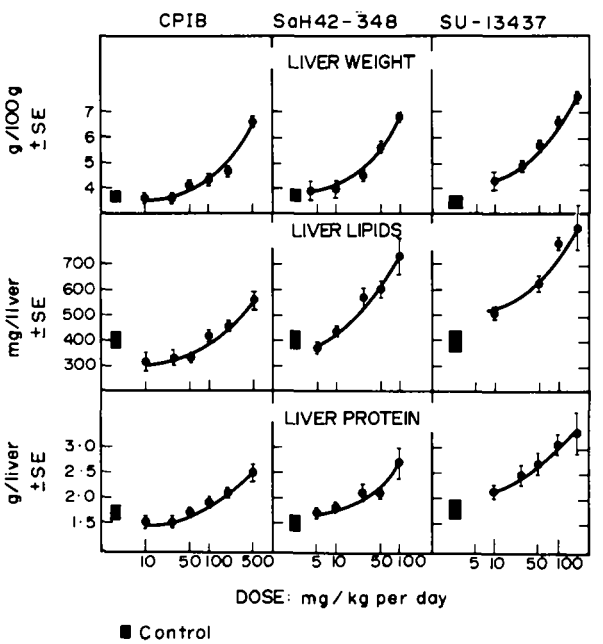

FIG. 2. Effect of hypolipidemic agents on rat liver weight and liver lipid and protein content. Male Charles River rats (six per group) initially weighing between 170 and 180 g were administered the drugs by oral intubation at the doses indicated for 9 days. The animals were fasted overnight before liver weight determination and composition analysis. Control represents mean ± S. E. M. for six rats. The volume of the vehicle was kept consistent for control and treated groups. Final body weight of the control groups and the highest dose groups of clofibrate (CPIB), SaH42-348 and Su-13437 were 223 ± 11, 185 ± 3·3, 199 ± 5·8 and 223 ± 15 respectively.

TABLE 4. EFFECT OF HYPOLIPIDEMIC DRUGS ON LIVER LIPID CONTENT*

	Cholesterol		Triglycerides		Phospholipids	
	(mg/g)	(mg/liver)	(mg/g)	(mg/liver)	(mg/g)	(mg/liver)
Control	4·1 ± 0·40	36 ± 1·4	5·9 ± 0·84	52 ± 7·8	31 ± 0·76	278 ± 12
Clofibrate	2·9 ± 0·12†	34 ± 2·0	5·7 ± 1·41	68 ± 17·1	35 ± 0·57‡	406 ± 28‡
Su-13437	2·5 ± 0·06†	36 ± 1·2	2·9 ± 0·37§	43 ± 5·3	30 ± 0·79	433 ± 12†
SaH42-348	3·1 ± 0·09†	34 ± 3·8	9·0 ± 1·70	102 ± 22·8	32 ± 0·46	357 ± 37
DPTH	3·3 ± 0·14‡	35 ± 2·1	4·2 ± 0·53	46 ± 7·7	38 ± 1·65‡	396 ± 24‡
Phenobarbital	3·9 ± 0·09	39 ± 0·7	6·2 ± 0·83	63 ± 8·6	36 ± 1·98	367 ± 17‡
Chlorcyclizine	3·8 ± 0·20	37 ± 2·2	7·6 ± 1·28	71 ± 10·9	35 ± 1·09§	339 ± 22

* Drugs were suspended in aqueous suspending vehicle and administered by oral intubation 5 days/week for 2 weeks at the following doses: clofibrate, 250 mg/kg, Su-13437, SaH42-348, DPTH, phenobarbital and chlorcyclizine, 50 mg/kg. Control rats were administered aqueous suspending vehicle. Data represent the mean of six rats per group ± standard error. The significance of the difference between the treated and the control groups is designated by the P values.

† $P < 0.001$.

‡ $P < 0.01$.

§ $P < 0.05$.

TABLE 5. EFFECT OF HYPOLIPIDEMIC DRUGS ON RAT LIVER *N*-DEMETHYLASE ACTIVITY*

Drug	(μmoles CH_2O formed/g/hr $\pm$ S. E.)
Control	13·6 $\pm$ 1·2
Clofibrate	36·8 $\pm$ 1·8†
Su-13437	30·6 $\pm$ 2·1†
SaH42-348	23·4 $\pm$ 2·7‡
DPTH	59·5 $\pm$ 9·1†
Phenobarbital	55·8 $\pm$ 6·8†
Chlorcyclizine	62·7 $\pm$ 2·7†

* Drugs were suspended in aqueous suspending vehicle and administered by oral intubation 5 days/week for 2 weeks at the following doses: clofibrate, 250 mg/kg, Su-13437, SaH42-348, DPTH, phenobarbital and chlorcyclizine, 50 mg/kg. Control rats were administered aqueous suspending vehicle. Data represent the mean of six rats per group $\pm$ standard error. The significance of the difference between the treated and the control groups is designated by the P values.

† $P < 0.001$.

‡ $P < 0.05$.

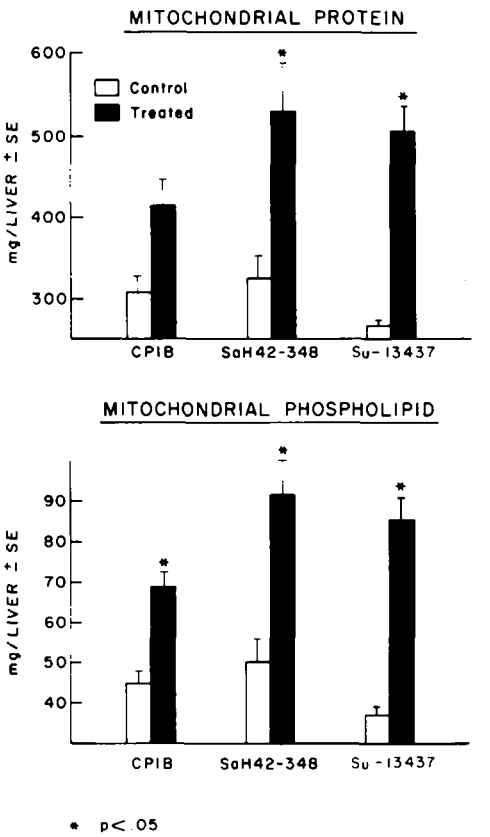

FIG. 3. Effect of chronic administration of anti-hyperlipidemic agents on liver mitochondrial protein and phospholipid content. Drugs were suspended in aqueous suspending vehicle and administered by oral intubation for 9 days at the following doses: clofibrate, 250 mg/kg, Su-13437 and SaH42-348, 50 mg/kg. Control rats were administered aqueous suspending vehicle. There were six rats per group.

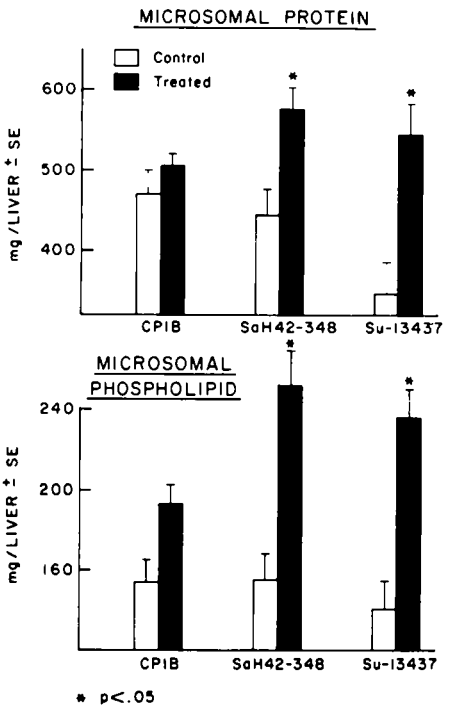

FIG. 4. Effect of chronic administration of anti-hyperlipidemic drugs on liver microsomal protein and phospholipid content. Drugs were suspended in aqueous suspending vehicle and administered by oral intubation for 9 days at the following doses: clofibrate, 250 mg/kg, Su-13437 and SaH42-348, 50 mg/kg. Control rats were administered aqueous suspending vehicle. There were six rats per group.

It was of interest to determine the subcellular compartmentation of the changes in liver composition. As shown in Fig. 3, the oral administration of 250 mg/kg of clofibrate, or 50 mg/kg of SaH42-348 and Su-13437 by intubation for 9 days increased rat liver mitochondrial phospholipid, and the latter two agents caused an increase in mitochondrial protein content. A slight but not statistically significant increase in liver microsomal protein and phospholipid was also obtained in rats treated with clofibrate and very significant increases in microsomal protein and phospholipid content were found upon treatment with SaH42-348 and Su-13437 (Fig. 4). Administration of DPTH, PB or CLZ by oral intubation caused a large increase in microsomal (Fig. 5) and mitochondrial (Fig. 6) protein and phospholipid content.

At conventional transcription blocking doses, actinomycin-D prevented the hepatomegaly, induction of microsomal *N*-demethylase activity and serum lipid lowering action of DPTH (Table 6). This experiment was possible with DPTH because this agent has a rapid onset of activity,[5] significant lowering of serum triglyceride being manifest within 2 days of the start of drug treatment. This time sequence was compatible with simultaneous actinomycin-D administration. A similar experimental approach was less satisfactory with the other agents because they required a longer period of drug administration to affect serum lipid levels and chronic treatment of actinomycin-D was not tolerated by the rats.

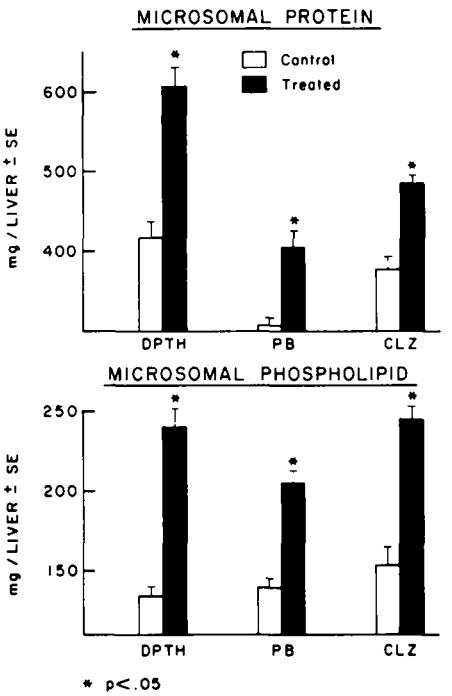

FIG. 5. Effect of chronic administration of microsomal enzyme inducers on liver microsomal protein and phospholipid content. Drugs were suspended in aqueous suspending vehicle and administered by oral intubation at 50 mg/kg/day for 9 days. Control rats were administered aqueous suspending vehicle. There were six rats per group.

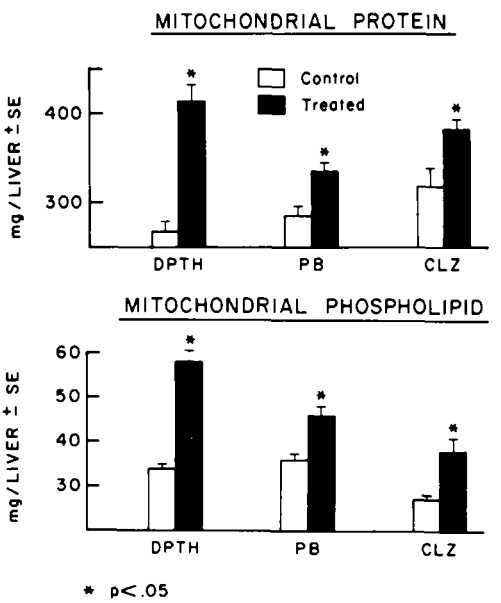

FIG. 6. Effect of chronic administration of microsomal enzyme inducers on liver mitochondrial protein and phospholipid content. Drugs were suspended in aqueous suspending vehicle and administered by oral intubation at 50 mg/kg/day for 9 days. Control rats were administered aqueous suspending vehicle. There were six rats per group.

TABLE 6. EFFECTS OF ACTINOMYCIN-D ON DPTH-INDUCED CHANGES IN THE RAT*

Treatment	Serum triglycerides (mg%)	Liver wt (g/100 g)	Liver phospholipids (mg/liver)	Liver *N*-demethylase activity (μmoles of CH_2O formed/g/hr)
Control	107 ± 10	3·6 ± 0·13	201 ± 8	18·3 ± 1·14
DPTH	30 ± 2†	5·1 ± 0·28‡	299 ± 33§	63·9 ± 3·90†
DPTH + actinomycin	137 ± 15	4·1 ± 0·16	240 ± 16	28·6 ± 10·3
Actinomycin	84 ± 6	3·7 ± 0·11	202 ± 15	7·8 ± 0·84

* Male rats (five per group) weighing between 150 and 170 g were given 50 mg/kg of DPTH by oral intubation twice a day for 2 days. Actinomycin-D (80 μg/kg) was injected i.p. at the same time. Data represent the mean of five rats per group ± standard error. Control rats received an equal volume of vehicle. The significance of the difference between the treated and the control groups is designated by the P values.
† $P < 0.001$.
‡ $P < 0.01$.
§ $P < 0.05$.

DISCUSSION

The data presented here demonstrate that the chronic administration of microsomal enzyme inducers or the anti-hyperlipidemic agents, clofibrate, SaH42-348 or Su-13437, caused an accumulation of phospholipids, protein and some RNA but no triglyceride or cholesterol in the liver. This is in agreement with the findings of others[1,16–19] and clearly removes the hepatomegalic condition from the class of fatty livers which are pathological and have high levels of triglyceride and cholesterol esters associated with fat droplets. The increased phospholipid and protein are associated with sub-cellular organelles and are undoubtedly membrane bound. Upon cessation of drug administration, the hepatomegaly and ultrastructural changes induced by clofibrate,[20–22] DPTH[5] or PB[23] were readily reversible.

We would like to suggest that the primary event, after administration of the microsomal enzyme inducers or the anti-hyperlipidemic agents, clofibrate, SaH42-348 or Su-13437, is an induction of enzyme protein resulting in structural changes associated with a functional enlargement of the liver membranes. The structural changes in the liver after injection of PB are readily interpreted in terms of the induction of microsomal enzyme and electron transport systems which are involved in the metabolism of that substance. It is well established that induction of drug-metabolizing enzymes may be non-specific with regard to both the substrate requirement and the non-obligatory structural changes in the endoplasmic reticulum. In the case of DPTH, there is some evidence that even greater non-specificity is involved, since DPTH is equipotent to PB as an inducer of the mixed function oxidase system but causes a greater accumulation of phospholipid and protein in the liver[7] and is more potent as a hypotriglyceridemic agent (Fig. 1).

The induction of microsomal enzymes reported here for the anti-hyperlipidemic agents (Table 5) is in agreement with the observations of Salvador *et al.*,[24] in rats treated with clofibrate or SaH42-348. Little is known about the metabolism of clofibrate other than the fact that it is excreted in the urine as the glucuronide.[25] Su-13437 is almost completely metabolized to more polar metabolites[26] presumably by microsomal enzymes. It is possible that the increase in microsomal protein and phospholipid is associated with the induction of microsomal enzymes responsible for the metabolism of these anti-hyperlipidemic agents. However, these drugs cause an even

larger increase in mitochondrial protein and phospholipid, an effect which is difficult to link with drug metabolism. Our observation is in conformance with the increase in size and number[27] as well as protein content of the mitochondria[28] seen after the administration of clofibrate. These intracellular changes are unexplained at the moment but may be related to the protein binding capacity of the drugs.[25]

An affinity for repressor substances could result in de-repression and thereby unleash new protein synthesis. Clofibrate has a broad unexplained influence on mitochondrial enzyme activity.[29-31] A non-specific binding with enzyme protein may explain some of these observations and may be unrelated to hypolipidemic mechanisms.

Many explanations have been suggested for the serum lipid lowering mechanism of clofibrate. These include an enhancement of thyroid hormone activity secondary to competition for binding sites on plasma proteins,[32] inhibition of cholesterol[33] and fatty acid synthesis,[34] increased hepatic cholesterol oxidation,[35] decreased rate of release of hepatic lipoproteins,[17] increased peripheral removal of lipoproteins[36] and increased neutral sterol excretion.[37] In view of our observation of an increase in liver membrane lipid and protein content in rats treated with clofibrate and five other lipid lowering drugs, we would like to suggest an alternative mechanism of action of these agents. It is proposed that the demand for new membrane material restricts the production of triglycerides and phospholipids available for the formation of secretory lipoproteins and hence their transport into the circulation with the resultant reduction in circulating triglyceride-rich lipoprotein. This conclusion appears more reasonable when it is recognized that both triglyceride and phospholipid synthesis share the common precursor, phosphatidic acid. Under such circumstances, the demand for one product could result in a decrease in the production of the other.

The validity of this proposed mechanism will ultimately be judged by the findings with other mammals and humans. It is felt, however, that this hypothesis should provide new experimental designs aimed at elucidating the mechanism of drug-induced hypolipidemia.

Acknowledgements—The authors would like to express their appreciation to Robert Scism, Diane DiSalvo, Herman Crowley, Virginia Cubberley, Loraine Martikes and John P. Mallon for technical contributions, and to Magdalena Johnson for typing the manuscript.

REFERENCES

1. M. M. Best and C. H. Duncan, *J. Lab. clin. Med.* **64,** 634 (1964).
2. R. Hess and W. L. Bencze, *Experientia* **24,** 418 (1968).
3. A. R. Timms, L. A. Kelly, R. S. Ho and J. H. Trapold, *Biochem. Pharmac.* **18,** 1861 (1969).
4. C. Dalton, V. Cubberley, H. Kahan and H. Crowley, *Pharmacologist* **13,** 277 (1971).
5. C. Dalton and K. Verebely, *J. Pharmac. exp. Ther.* **180,** 484 (1972).
6. W. Schneider, *J. biol. Chem.* **176,** 259 (1948).
7. C. Dalton, J. J. Kamm and P. Bartl, *J. Pharmac. exp. Ther.* **182,** 491 (1972).
8. W. D. Block, K. J. Jarrett and J. B. Levine, in *Automation in Analytical Chemistry* (Ed. L. T. Skeggs, Jr.), pp. 345–7. Mediad, New York (1966).
9. G. Kessler and H. Lederer, in *Automation in Analytical Chemistry* (Ed. L. T. Skeggs, Jr.), pp. 341–344. Mediad, New York (1966).
10. R. Whitley and H. Alburn, *Ann. N.Y. Acad. Sci.* **130,** 634 (1965).
11. J. Folch, M. Lees and G. H. Sloane Stanley, *J. biol. Chem.* **226,** 497 (1957).
12. S. Shibko, P. Koivistoinen, C. A. Tratnyek, A. R. Newhall and L. Friedman, *Analyt. Biochem.* **19,** 514 (1967).
13. K. Burton, *Biochem. J.* **62,** 315 (1956).
14. C. Dalton and D. DiSalvo, *Technicon Q.* **4** (3), 20 (1972).

15. J. AXELROD, *J. Pharmac. exp. Ther.* **117,** 322 (1956).
16. J. M. THORP and W. S. WARING, *Nature, Lond.* **194,** 948 (1962).
17. R. G. GOULD, E. A. SWYRYD, B. J. COAN and D. R. AVOY, *J. Atheroscler. Res.* **6,** 555 (1966).
18. Y. KURIYAMA, T. OMURA, P. SIEKEVITZ and G. E. PALADE, *J. biol. Chem.* **244,** 2017 (1969).
19. J. L. HOLTZMAN and J. R. GILLETTE, *J. biol. Chem.* **243,** 3020 (1968).
20. R. HESS, W. STRAUBLI and W. REISS, *Nature, Lond.* **208,** 856 (1965).
21. D. SVOBODA and D. AZARNOFF, *J. Cell Biol.* **30,** 442 (1966).
22. D. SVOBODA, H. GRADY and D. AZARNOFF, *J. Cell Biol.* **35,** 127 (1967).
23. S. ORRENIUS and L. ERNSTER, *Biochem. biophys. Res. Commun.* **16,** 60 (1964).
24. R. A. SALVADOR, S. A. HABER, B. W. GOMMI and R. M. WELCH, *Life Sci.* **9,** 397 (1970).
25. J. M. THORP, *Lancet* **I,** 1323 (1962).
26. J. R. BIANCHINE, P. WEISS, M. J. T. PEASTON, R. M. HERSEY and L. LASAGNA, *Clin. Pharmac. Ther.* **11,** 97 (1970).
27. G. E. PAGET, *J. Atheroscler. Res.* **3,** 729 (1963).
28. C. K. R. KURUP, H. N. AITHAL and T. RAMASARMA, *Biochem. J.* **116,** 773 (1970).
29. D. S. PLATT and B. L. COCKRILL, *Biochem. Pharmac.* **15,** 927 (1966).
30. D. S. PLATT and B. L. COCKRILL, *Biochem. Pharmac.* **16,** 2257 (1967).
31. U. SCHACHT and E. GRANZER, *Biochem. Pharmac.* **19,** 2963 (1970).
32. J. M. THORP, *J. Atheroscler. Res.* **3,** 351 (1963).
33. D. R. AVOY, E. A. SWYRYD and R. G. GOULD, *J. Lipid. Res.* **6,** 369 (1965).
34. M. E. MARAGOUDAKIS, *J. biol. Chem.* **244,** 5005 (1969).
35. D. KRITCHEVSKY and S. A. TEPPER, *Fedn Proc.* **27,** 822 (1968).
36. W. G. RYAN and T. B. SCHWARTZ, *J. Lab. clin. Med.* **64,** 1001 (1964).
37. S. M. GRUNDY, E. A. AHRENS, G. SALEN and E. QUINTAO, *J. clin. Invest.* **48,** 33a (1969).