FATTY ACID COMPOSITION OF CHOLESTERYL ESTERS
IN CHOLESTEROL-FED RABBITS TREATED WITH
LIPID-LOWERING AGENT
(ETHYL 2-(*p*-CHLOROPHENOXY) ISOBUTYRATE)*

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Abstract—Regression mechanism of Ethyl 2-(*p*-chlorophenoxy) isobutyrate (CPIB) in the arterial wall was studied for elucidation in terms of fatty acid-compositional change in cholesterol-fed rabbits. The following evidence was obtained: (1) The amount of cholesterol and cholesteryl ester in the atheromatous aorta were reduced after CPIB treatment. (2) CPIB resulted in a significant increase in the ratio of linoleate to oleate in both the arterial wall and serum after withdrawal of the cholesterol diet. It is suggested that there is a preferential hydrolysis of linoleate-rich cholesteryl ester in both the arterial wall and the serum.

The lipid-lowering activity of ethyl 2-(*p*-chlorophenoxy) isobutyrate (Clofibrate, CPIB) has been well documented regarding rats (1-3), dogs, monkeys (4-5), and men (6-10). The reduction mechanisms of the compound has been proposed to be: 1) enhancement of endogenous hormone activity, secondary to competition for binding sites on plasma proteins (2), 2) decrease in rate of fatty acid (FA) mobilization (11), 3) decrease in rate of hepatic cholesterol biosynthesis (11, 12), 4) increase in rate of hepatic cholesterol oxidation (13), 5) decrease in rate of hepatic release of lipoproteins (14), 6) increase in rate of peripheral uptake of lipoproteins (15), 7) decrease in rate of fatty acid biosynthesis (16). These mechanisms in the atheromatous aorta remain to be clarified. In cholesterol-fed rabbits, Kritchevsky *et al.* (17) and Feher *et al.* (18) also pointed out that CPIB produced significant reduction of cholesterol in arch atheroma but not so much in serum.

We have shown in previous papers (19, 20) that polyunsaturated FAs of cholesteryl ester (CE) might be preferentially hydrolyzed by CE-hydrolytic enzymes, and subsequently cholesterol might be eliminated from the tissues. FA-modification, such as increase in ratio of linoleate to oleate after CPIB treatment, may explain the reduction of cholesterol in the arterial wall.

The purpose of this study was an attempt to elucidate the mechanism of reduction of cholesterol in atheroma in terms of FA compositional improvement after CPIB treatment. This is the first paper to describe an increase in ratio of linoleate to oleate in cholesterol-fed rabbits treated with CPIB.

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MATERIALS AND METHODS

Experimental Protocol: Unless otherwise specified, all animals were female albino rabbits purchased from Miyamoto Rabbitry. The initial weight of the animals was about 2.4 kg. When not on a special diet, the animals were maintained on regular rabbit chow (RC-4 Oriental Kobo Kogyo). For the cholesterol feeding experiments, to the rabbit chow was added with 1% cholesterol according to the method of Wang *et al.* (21). The diet was fed *ad libitum* to animals for a period of 150 days. The cholesterol diet was withdrawn, thereafter, and the animals were maintained on regular rabbit chow for an additional 30 days. Daily consumption of rabbit chow of each animal was 100 g, which is equivalent to 1 g cholesterol per day during administration. Rabbits on the 1% cholesterol added diet were pre-treated with CPIB, for a period of 30 days. It was confirmed that this amount of CPIB produced a significant reduction in serum cholesterol in rats fed a high-fat cholesterol diet. For an additional 30 days, 0.4% CPIB, incorporated regular rabbit chow, was given after withdrawal of the cholesterol diet. In each group, 8 animals were examined. This dose of CPIB did not cause a significant change in body weight gain: (control group; 2.40 ± 0.10 – 3.30 ± 0.30 kg, CPIB group; 2.46 ± 0.12 – 3.30 ± 0.20 kg). Slight hepatomegaly was observed in the CPIB group as compared with the control group: (control group; 140.8 ± 1.61 g, CPIB group; 148.9 ± 2.61 g). Blood samples were collected from an ear vein at 0, 15, and 30 days after withdrawal of the cholesterol diet. As a normal control, the samples were collected from rabbits before the cholesterol feeding. Thirty days after ingestion of the cholesterol diet, these animals were sacrificed and the aorta from the heart to the iliac bifurcation was removed. Preparation of homogenate from arterial intima was carried out according to the method described previously (20).

Analytical Techniques: Serum cholesterol and CE were measured by a slightly modified method of *o*-phthalaldehyde (22) and arterial counterparts were measured by means of gas liquid chromatography (GLC). Extraction of lipid fraction was carried out by the modified method of Folch *et al.* (23), and separation of cholesterol and CE from other lipids was performed by silicic acid thin layer chromatography (TLC) plate (20 cm $\times$ 20 cm, Eastman Kodak, 6060, plastic plate with fluorescent indicator). For microassay of cholesterol and CE by means of GLC, the instruments used were Hitachi gas chromatograph (type 163) with Hewlett-Packard integrator (type 3380A). Chromatography carried out on 1 m glass column filled with 3% OV-17 on Gas Chrom Q 100/120 mesh (Shimadzu Column Packing). Temperature program was set from 250°C at the rate of 1°C per min. As an internal standard, 50 μ g of campesterol pentadecanoate, which was synthesized by the reaction of campesterol (Applied Sci.) and pentadecanoylchloride (Sigma), according to the slightly modified method of Goodman (24), were added before extraction. Recovery of free and esterified cholesterol was 91.7% and 94.1% respectively. In several dilutions of cholesterol and cholesteryl pentadecanoate, there were linear relationships in both free and esterified cholesterol. Quantitative analysis for FAs of CE were carried out by the method described previously (20).

RESULTS

Effect of CPIB on amount of Cholesterol, CE and FA-composition of CE in atheromatous aorta: There is circumstantial evidence that the disease affects first the origin of the large vessels in the aortic arch, spreads therefrom to more extensive areas of the arch, later invades the thoracic aorta, and finally extends down to the abdominal aorta (25). In this respect, repressive effect of CPIB on these three portions; arch, thoracic and abdominal aortas was examined. Table 1 shows that both cholesterol and CE decreased remarkably after CPIB administration particularly in the arch portions; about 31.3% reduction in free and about 40.9% in CE were observed. In both thoracic and abdominal aorta, however, no regression was observed. In these portions, adventitia tissues were removed from intima only with great difficulty. These results suggested that, at least in the arch portion, CPIB induced a significant regression of cholesterol and CE after CPIB treatment.

To elucidate one of the mechanisms involved, FA-compositional change of CE in the

TABLE 1. Regression in levels of cholesterol and CE after CPIB administration

AORTA		CONTROL		CPIB		Regression	Student's <i>t</i> -test
		n	mg/g*	n	mg/g*	%	
ARCH	CE	8	80.5 $\pm$ 2.6	8	47.6 $\pm$ 2.1	40.9	p<0.005
	cholesterol	8	38.6 $\pm$ 2.8	8	26.5 $\pm$ 0.9	31.3	p<0.005
THORACIC	CE	8	25.8 $\pm$ 3.8	8	24.9 $\pm$ 5.4	3.5	p>0.1
	cholesterol	8	7.6 $\pm$ 0.8	8	8.3 $\pm$ 1.4	-9.2	p>0.1
ABDOMINAL	CE	8	16.1 $\pm$ 0.6	8	17.0 $\pm$ 1.0	-5.5	p>0.1
	cholesterol	8	7.8 $\pm$ 0.5	8	7.2 $\pm$ 0.6	7.6	p>0.1

*means $\pm$ SE mg/g wet wt. tissue. Amount indicated in the table represents that of free cholesterol in both cholesterol and CE.

TABLE 2. FA-composition of CE in atheromatous aorta with and without CPIB administration

	CONTROL		CPIB		Amount reduced**	Student's <i>t</i> -test
	n	mg/g*	n	mg/g*	mg/g	
C16:0***	8	2.42 $\pm$ 0.13	8	1.79 $\pm$ 0.09	0.63	p<0.005
C16:1	8	0.77 $\pm$ 0.06	8	0.39 $\pm$ 0.05	0.38	p<0.005
C18:0	8	0.63 $\pm$ 0.03	8	0.54 $\pm$ 0.05	0.09	p<0.1
C18:1	8	10.75 $\pm$ 0.49	8	5.73 $\pm$ 0.41	5.02	p<0.005
C18:2	8	3.33 $\pm$ 0.17	8	2.38 $\pm$ 0.22	0.95	p<0.005
C18:3	8	1.02 $\pm$ 0.06	8	0.78 $\pm$ 0.06	0.24	p<0.01
C20:4	8	0.57 $\pm$ 0.02	8	0.32 $\pm$ 0.07	0.25	p>0.1
C18:2/C18:1	8	0.31 $\pm$ 0.01	8	0.41 $\pm$ 0.02	---	p<0.05

* means $\pm$ SE mg/g wet wt. tissue.

** means difference between CPIB and control group.

*** Palmitate (C16:0), Palmitoleate (C16:1), Stearate (C18:0), Oleate (C18:1), Linoleate (C18:2), Linolenate (C18:3), Arachidonate (C20:4).

FIG. 1. FA-frequency (%) of CE in atheromatous aorta was arranged according to FA-specificity of CE-hydrolytic enzymes from right of left: arachidonate (C20:4), palmitoleate (C16:1), linolenate (C18:3), linoleate (C18:2), oleate (C18:1), palmitate (C16:0) and stearate (C18:0). Solid line with shadow and broken line represent CPIB and control group, respectively.

arch was observed. Table 2 clearly shows that the major component which decreased after CPIB treatment, was cholesteryl oleate. Consequently, the ratio of linoleate to oleate increased from 0.31 to 0.41 with this treatment. Since linoleate is considered to be more easily hydrolyzed by CE-hydrolytic enzymes than oleate, CE in CPIB group, therefore, might be more rapidly degraded. To demonstrate the participation of these hydrolytic enzymes on hydrolysis of CE in atheromatous aorta, FAs of CE was arranged according to the FA-specificity of CE-hydrolytic enzymes from right to left. Fig. 1 shows that the proportion of FAs tends to shift from right to left slightly after CPIB treatment. This suggests that FAs, which have a higher affinity to the enzymes increased after the treatment.

Effect of CPIB on FA-composition of CE in Serum: CPIB effect on FA-composition in arterial wall might be also explained from linoleate-rich lipoprotein, which penetrates into the arterial wall from the blood stream, as well as FA-specificity of arterial CE-hydrolytic enzymes. In this respect, FA-composition of CE in serum was measured. Regarding the amount of cholesterol and CE in serum, such increased progressively throughout the term that the animals were on the cholesterol diet. No significant difference, however, could be observed between control and CPIB groups. Little difference was observed in FA-composition of CE in these periods.

After discontinuation of the cholesterol diet, cholesterol and CE levels in the serum decreased at the almost same rate in both control and CPIB groups; there was a 70–80%

TABLE 3. Cholesterol and CE in serum after withdrawal of cholesterol diet

days		CONTROL		CPIB		Student's <i>t</i> -test
		n	mg/ml*	n	mg/ml*	
0	CE	8	27.3 ± 2.7	8	23.1 ± 1.2	p < 0.1
	cholesterol	8	5.9 ± 0.7	8	5.5 ± 0.3	p > 0.1
15	CE	8	16.2 ± 1.7	8	13.2 ± 2.0	p > 0.1
	cholesterol	8	3.0 ± 0.3	8	3.3 ± 0.5	p > 0.1
30	CE	8	8.9 ± 1.3	8	5.2 ± 0.3	p < 0.01
	cholesterol	8	1.1 ± 0.3	8	1.4 ± 0.1	p > 0.1

*means ± SE mg/ml serum. Amount indicated in the table represents that of free cholesterol in both cholesterol and CE.

TABLE 4. FA-composition of CE in serum after withdrawal of cholesterol diet

days	FA	CONTROL		CPIB	
		n	mg/ml*	n	mg/ml*
0 days	C16:0	8	1.93 $\pm$ 0.11	8	1.96 $\pm$ 0.21
	C16:1	8	0.56 $\pm$ 0.04	8	0.52 $\pm$ 0.06
	C18:0	8	0.42 $\pm$ 0.03	8	0.51 $\pm$ 0.05
	C18:1	8	3.67 $\pm$ 0.17	8	3.03 $\pm$ 0.37
	C18:2	8	2.97 $\pm$ 0.13	8	2.76 $\pm$ 0.32
	C18:3	8	1.55 $\pm$ 0.25	8	1.01 $\pm$ 0.12
	C20:4	8	0.06 $\pm$ 0.00	8	0.06 $\pm$ 0.01
15 days	C16:0	8	1.07 $\pm$ 0.14	8	1.45 $\pm$ 0.32
	C16:1	8	0.37 $\pm$ 0.03	8	0.38 $\pm$ 0.08
	C18:0	8	0.17 $\pm$ 0.05	8	0.33 $\pm$ 0.08
	C18:1	8	3.12 $\pm$ 0.34	8	2.12 $\pm$ 0.46
	C18:2	8	1.67 $\pm$ 0.15	8	1.94 $\pm$ 0.42
	C18:3	8	0.67 $\pm$ 0.10	8	0.55 $\pm$ 0.10
	C20:4	8	0.00 $\pm$ 0.00	8	0.04 $\pm$ 0.01
30 days	C16:0	8	0.66 $\pm$ 0.07	8	0.40 $\pm$ 0.09
	C16:1	8	0.21 $\pm$ 0.02	8	0.11 $\pm$ 0.02
	C18:0	8	0.11 $\pm$ 0.01	8	0.08 $\pm$ 0.02
	C18:1	8	1.54 $\pm$ 0.16	8	0.76 $\pm$ 0.20
	C18:2	8	0.59 $\pm$ 0.06	8	0.78 $\pm$ 0.19
	C18:3	8	0.41 $\pm$ 0.04	8	0.24 $\pm$ 0.05
	C20:4	8	0.01 $\pm$ 0.00	8	0.00 $\pm$ 0.00

*means $\pm$ SE respective FA mg/ml serum.

FIG. 2. FA-frequency (%) of CE in serum after withdrawal of cholesterol diet was arranged according to FA-specificity of CE-hydrolytic enzymes. Solid line with shadow represents FA-frequency of CE remaining in serum with and without CPIB treatment. Broken line indicates FA-frequency from normal rabbits.

FIG. 3. Ratio of linoleate (C18:2) to oleate (C18:1) after withdrawal of cholesterol diet. Broken line and solid line represent CPIB and control group.

FIG. 4. Elimination of linoleate and oleate of CE in serum after withdrawal of cholesterol diet with and without CPIB treatment. Broken line and solid line represent oleate and linoleate of cholesterol ester.

reduction in cholesterol and CE over a 30 day period in both groups (Table 3). The striking difference in FA-composition, however, was observed between the control and CPIB group. As is shown in Table 4 and Fig. 2, oleate was reduced more rapidly in the CPIB group than in the control (in CPIB group, 71.4% of oleate and 74.9% linoleate and in control group, 58.3% of oleate and 80.1% of linoleate was reduced for 30 days after withdrawal of cholesterol diet). Consequently, the ratio of linoleate to oleate in the CPIB group gradually increased toward normal values. On the contrary, the ratio in the control group tended to decrease after discontinuation of the cholesterol diet (Fig. 3). The reason why the ratio increased in the CPIB group may be due to the fact that the elimination rate of oleate was almost the same as that of linoleate, though, generally, linoleate is eliminated more rapidly than oleate in non-treated samples as was seen in the control group (Fig. 4). These results indicate that the distribution of FA from the CPIB group appears to have a similar profile to that seen in normal serum.

DISCUSSION

The present study indicates that ratio of linoleate to oleate of CE increased remarkably after the CPIB treatment in both serum and arterial wall of cholesterol-fed rabbits (Table 2, Fig. 3). It is well known that linoleate-rich CE can be hydrolyzed more preferentially by CE-hydrolytic enzymes than oleate (20, 26). Therefore, linoleate may be more easily eliminated from serum, arterial wall and other tissues. We suggested, herewith, that the improvement of FA-composition of CE might be one of the mechanisms of reduction of CPIB in addition to other functions of the compound.

Increase in the ratio of linolenate to oleate in serum after CPIB treatment may depend on the elimination rate of oleate as such was almost the same as that of linoleate in the CPIB group, though, generally, linoleate is more easily eliminated than oleate (20, 26), as was shown in control groups (Fig. 4). Distribution of FA-composition after CPIB

treatment tended to have a similar profile with that of normal serum (Table 4, Fig. 2).

Some investigators (26-28) pointed out that the ratio decreased in serum of human subjects after CPIB treatment. Bowyer *et al.* (30) stated that oleate may increase because of a preferential resistance of cholesteryl oleate to hydrolysis. Such indeed may be the case as the amount of cholesterol and CE in the serum decreased after the treatment in this particular case. These results, however, are contrary to our observations, obtained in cholesterol-fed rabbits. CPIB does not induce a significant reduction in cholesterol and CE of serum in rabbits on a cholesterol diet (31, 32), though it does in human subjects. Different effects of CPIB on the ratio in rabbit and human subjects may be explained by this reduction of cholesterol and CE of serum. Thus, CPIB may increase the ratio of linoleate to oleate. In the case of cholesterol-fed rabbits, linoleate-rich CE would be detected in this fraction, because the amount of cholesterol and CE of serum did not decrease even after CPIB treatment. On the contrary, in the case of human subjects, a significant decrease in cholesterol and CE occurs and oleate-rich CE which is resistant to hydrolysis, therefore, would remain in the serum.

Considering the increase in the ratio in CE of arterial wall after CPIB treatment, it appears that 1) linoleate-rich lipoprotein, which increased in serum, may penetrate into the arterial wall, 2) in the arterial wall also, the elimination of oleate occurs at almost the same rate as that of linoleate. Consequently, ratio of linoleate to oleate might increase in atheromatous aorta after the treatment.

If one compares the increase of ratio between serum and atheromatous aorta, the increase in arterial wall was much less than that in the serum (Table 2, Fig. 3). In the case of atheromatous aorta, CE was apparently accumulated as lipid crystals rich in oleate (ratio of linoleate to oleate is about 0.12) (20, 33). Thus, oleate-rich lipid crystal (as solid phase) may not be so readily hydrolyzed and the decrease of oleate was faster in the serum than in the arterial wall.

The improvement in terms of FA-composition, therefore, may be one plausible explanation for this reduction mechanism of CPIB in atheroma: 1) linoleate-rich CE may be hydrolyzed more rapidly than oleate and free cholesterol might be excreted out from the arterial wall, 2) linoleate-rich CE from the arterial wall may be easily mobilized. Further studies are required to elucidate how the hydrolysis of oleate in serum occurred at almost the same rate as that of linoleate after CPIB treatment.

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REFERENCES

- 1) THORP, J.M. AND WARING, W.S.: Modification of metabolism and distribution of lipids by ethyl chlorophenoxyisobutyrate. *Nature* **194**, 948-949 (1962)
- 2) THORP, J.M.: An experimental approach to the problem of disordered lipid metabolism. *J. Atheroscl. Res.* **3**, 351-360 (1963)

- 3) WESTFELT, W.W., RICHERT, D.A. AND RUEGAMER, W.R.: The role of the thyroid hormone in the effect of *p*-chlorophenoxyisobutyrate in rats. *Biochem. Pharmacol.* **17**, 1003–1016 (1968)
- 4) THORP, J.M.: Experimental evaluation of an orally active combination of androsterone with ethyl chlorophenoxyisobutyrate. *Lancet* **i**, 1323–1326 (1962)
- 5) PLATT, D.S. AND THORP, J.M.: Changes in the weight and composition of the liver in the rat, dog and monkey treated with chlorophenoxyisobutyrate. *Biochem. Pharmacol.* **15**, 915–925 (1966)
- 6) GREEN, K.G., INMAN, W.H.W. AND THORP, J.M.: Multicentre trial in the United Kingdom and Ireland of a mixture of ethyl chlorophenoxyisobutyrate and androsterone (Atromid). A preliminary report. *J. Atheroscl. Res.* **3**, 593–616 (1963)
- 7) OLIVER, M.F.: Further observations on the effects of atromid and of ethyl chlorophenoxyisobutyrate on serum lipid levels. *J. Atheroscl. Res.* **3**, 427–444 (1963)
- 8) HELLMAN, L., ZUMOFF, B., KESSLER, G., KARA, E., RUBIN, I.L. AND ROSENFELD, R.S.: Reduction of cholesterol and lipids in man by ethyl *p*-chlorophenoxyisobutyrate. *Ann. Int. Med.* **59**, 477–494 (1963)
- 9) HOWARD, R.P., ALAUPOVIC, P., BRUSCO, O.J. AND FURMAN, R.H.: Effects of ethyl chlorophenoxyisobutyrate, alone or with androsterone (Atromid) on serum lipids, lipoproteins and related metabolic parameters in normal and hyperlipidemic subjects. *J. Atheroscl. Res.* **3**, 482–499 (1963)
- 10) GRUNDY, S.M., AHRENS, JR., E.H., SALEU, G., SCHREIBMAN, P.H. AND NESTEL, P.J.: Mechanisms of action of clofibrate on cholesterol metabolism in patients with hyperlipidemia. *J. Lipid Res.* **13**, 531–551 (1972)
- 11) STEINBERG, D.: Drugs inhibiting cholesterol biosynthesis, with special reference to clofibrate. Proc. of 2nd Intern. Symp. *Atherosclerosis*, Edited by JONES, R.J., p. 500–508, Springer-Verlag, New York, Heidelberg and Berlin (1970)
- 12) AVOY, D.R., SWYRYD, E.A. AND GOULD, R.G.: Effect of α -*p*-chlorophenoxyisobutyryl ethyl ester (CPIB) with and without androsterone on cholesterol biosynthesis in rat liver. *J. Lipid Res.* **6**, 369–376 (1965)
- 13) KRITCHEVSKY, D., TEPPER, S.A., SALLATA, P., KABAKJIAN, J.R. AND CRISTOFALO, V.J.: Effect of the ethyl ester and sodium salt of α -*p*-chlorophenoxyisobutyric acid on cholesterol oxidation by rat liver mitochondria. *Proc. Soc. exp. Biol. Med.* **132**, 76–82 (1969)
- 14) GOULD, R.G., SWYRYD, E.A., AVOY, D. AND COAN, B.: The effect of α -*p*-chlorophenoxyisobutyrate on the synthesis and release into plasma of lipoproteins in rat. *Prog. biochem. Pharmacol.* **2**, 345–359 (1967)
- 15) NESTEL, P.J. AND AUSTEN, W.: The effect of ethyl chlorophenoxyisobutyrate (CPIB) on the uptake of triglyceride fatty acids, activity of lipoprotein lipase and lipogenesis from glucose in fat tissue of rats. *J. Atheroscl. Res.* **3**, 827–841 (1968)
- 16) MARAGODAKIS, M.E.: Inhibition of hepatic acetyl-CoA carboxylase by hypolipidemic agents. *J. biol. Chem.* **244**, 5005–5013 (1969)
- 17) KRITCHEVSKY, D., SALLATA, P. AND TEPPER, S.A.: Influence of ethyl *p*-chlorophenoxyisobutyrate (CPIB) upon establishment and progression of experimental atherosclerosis in rabbits. *J. Atheroscl. Res.* **8**, 755–761 (1968)
- 18) FEHÉR, J., BIHARI-VARGA, M., VARSÁNYI, M. AND GERÖ, S.: The effect of clofibrate on cholesterol induced aortic lesions. *Brit. J. exp. Pathol.* **53**, 509–517 (1972)
- 19) TAKANO, T., BLACK, W.J., PETERS, T.J. AND DE DUVE, C.: Assay, kinetics, and lysosomal localization of an acid cholesteryl esterase in rabbit aortic smooth muscle cells. *J. biol. Chem.* **249**, 6732–6737 (1974)
- 20) TAKANO, T. AND SATO, M.: Enhancement of oleate-rich cholesteryl ester after removal of cholesterol diet from cholesterol-fed rabbits. *Chem. Pharm. Bull.* **25**, 2669–2675 (1977)
- 21) WANG, C.I., SCHAEFFER, L.E. AND HOLERSBERG, D.: Plasma lipid partition of the normal and cholesterol-fed rabbit. *J. Mt. Sinai Hosp. N. Y.* **21**, 19–25 (1954)
- 22) RUDEL, L.L. AND MORRIS, M.D.: Determination of cholesterol using *o*-phthalaldehyde. *J. Lipid Res.* **14**, 364–366 (1973)
- 23) FOLCH, J., LEES, M. AND STANLEY, G.H.S.: A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. Chem.* **226**, 497–509 (1957)

- 24) GOODMAN, D.S.: Hydrolysis and formation of cholesterol esters in rat liver. *Methods in Enzymology*, Steroid and Terpenoids, Edited by CLAYTON, R.B., Vol. **15**, p. 522-537, Academic Press, New York and London (1969)
- 25) PARKER, F. AND ODLAND, G.F.: A correlative, histochemical, biochemical and electron-microscopic study of experimental in the rabbit aorta with special reference to the myointimal cell. *Am. J. Pathol.* **48**, 197-239 (1966)
- 26) PORTMAN, O.W.: Arterial composition and metabolism: Esterified fatty acids and cholesterol. *Adv. in Lipid Res.*, Edited by PAOLETTI, R. AND KRITCHEVSKY, D., Vol. **8**, p. 41-114, Academic Press, New York and London (1970)
- 27) BERRY, C., MAXHAM, A., SMITH, E., KELLIE, A.E. AND NABBARO, J.D.N.: The effects of Atromid on the metabolism of adrenal steroids and on plasma lipid fractions. *J. Atheroscl. Res.* **3**, 380-395 (1963)
- 28) JURAND, J. AND OLIVER, M.F.: The effect of ethylchlorophenoxyisobutyrate on serum cholesteryl, triglyceride and phospholipid fatty acid. *J. Atheroscl. Res.* **3**, 547-553 (1963)
- 29) HAGOPIAN, M. AND ROBINSON, R.W.: The effect of chlorophenoxyisobutyrate on plasma composition of cholesteryl esters and on levels of neutral lipids. *J. Atheroscl. Res.* **8**, 21-27 (1968)
- 30) BOWYER, D.E., PATELSKI, J., HOWARD, A.N. AND GRESHAM, G.A.: Cholesterol esters and hypocholesterolaemic drugs. *J. Atheroscl. Res.* **8**, 864-867 (1968)
- 31) IMAI, Y., MATSUMURA, H., KANAMARU, K. AND SHIMAMOTO, K.: Biological studies of AF-308. III. Effect of AF-308 a new derivative of 1,2,4-oxadiazole on atherosclerosis in cholesterol-fed rabbits. *J. Takeda Res. Lab.* **32**, 45-55 (1973)
- 32) BRATISAND, R.: The effect of niceritol (pentaerythritol tetra nicotinate) and clofibrate upon hyperlipemia and atherosclerosis induced in rabbits by cholesterol free semisynthetic diets. *Atheroscl.* **20**, 453-467 (1974)
- 33) HATA, Y., HOWER, J. AND INSULL JR., W.: Cholesteryl ester-rich inclusions from human aortic fatty streak and fibrous plaque lesions of atherosclerosis. *Am. J. Pathol.* **75**, 423-456 (1974)