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HYPOLIPIDEMIC ACTIVITY OF 2,8-DIBENZYL-CYCLOOCTANONE IN RATS

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Abstract—Male rats were given orally 2,8-dibenzylcyclooctanone (DBCO) at doses ranging from 0.3 to 30 mg/kg/day for 1–6 weeks. The decrease in serum cholesterol after 1 week of treatment was dose dependent, and was due primarily to a lowering of cholesterol in the serum high density lipoprotein (HDL) fraction. Upon prolonged administration, the hypocholesterolemic effect of DBCO became smaller; thus, at 30 mg/kg/day, serum cholesterol in male rats decreased by 83 per cent after 1 week, and by 51 per cent after 4 weeks of treatment. The rate of hepatic cholesterol synthesis was suppressed and liver cholesterol was elevated only in rats given DBCO for 1 week. Similar changes were elicited in female rats. The effect of DBCO on serum triglycerides was sex dependent: in male rats, triglycerides were reduced on short-term treatment, but elevated when treatment was prolonged. In contrast, DBCO was hypertriglyceridemic in female rats, independent of duration of treatment. At doses which were hypocholesterolemic, DBCO produced dose-dependent decreases in accessory sex organ weights and increased adrenal weights in male rats; these findings were suggestive of estrogenic activity.

The synthesis and hypolipidemic activity of 2,8-dibenzylcyclooctanone (DBCO) have been reported by Piantadosi *et al.* [1] and Carlson *et al.* [2], who found that the weakly estrogenic DBCO lowered serum cholesterol levels in rats; in addition, DBCO was also reported to lower serum triglycerides.

In view of its potency and unusual structure (Fig. 1), we have investigated the effects of DBCO on rat lipid metabolism in greater detail. The results of these studies are presented in this report.

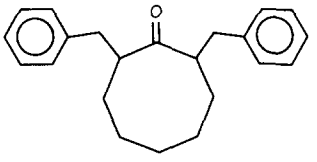

Fig. 1. Structure of 2,8-dibenzylcyclooctanone (DBCO).

MATERIALS AND METHODS

Materials. DBCO was kindly supplied through the courtesy of Dr. C. Piantadosi, University of North Carolina, Chapel Hill. The compound was suspended in either 5% gum acacia or in 2% Tween 80, and was administered to rats orally by gastric intubation. Cholesterol, used as carrier in studies of cholesterol biosynthesis and for feeding, was purified via its 5,6-dibromo derivative [3]. Sodium [2-^{14}C]acetate and [^{3}H]DL-mevalolactone were purchased from New England Nuclear Corp. and from Amersham–Searle respectively. The mevalolactone was hydrolyzed to mevalonic acid prior to use.

Animals. Male or female albino Charles River CD rats were kept under observation for 3–4 days prior to each experiment. Only animals with normal food intake and weight gain were used. Dietary regimen comprised Purina Laboratory chow.

Lipid analyses. Total cholesterol levels in nonsaponifiable lipid extracts were determined by the method of Zlatkis *et al.* [4] as modified for the Auto-analyzer (method Np-24). Phospholipid [5], triglyceride [6] and nitrogen [7] levels were measured according to previously described techniques. Serum lipoproteins were separated with dextran sulfate [8] into fractions of low density (LDL + VLDL) and high density (HDL).

Cholesterogenic activity in liver homogenates obtained from rats treated with DBCO was measured as described previously [9]: homogenates were incubated simultaneously with 5 μCi sodium [2-^{14}C]acetate and/or 0.67 μCi [^{3}H]mevalonate, together with appropriate cofactors; cholesterol was isolated, purified and counted as its 5,6-dibromo derivative [3].

Estrogenicity. Estrogenicity in male rats was assessed by measuring the weights of the adrenals, seminal vesicles, levator ani and dorsolateral prostate.

RESULTS

Effect of DBCO on body weight. Groups of ten male rats (initial body weight, 70–80 g) were treated for 1, 3 and 6 weeks with daily doses of 10 and 30 mg/kg of DBCO as follows: the animals treated for 1 week received the vehicle (5% gum acacia) daily for 5 weeks and DBCO in the sixth week, and the 3-week group received the vehicle for the first 3 weeks and DBCO for the last 3 weeks; the 6-week group received DBCO during the entire study. Body weights were measured each week. As shown in Fig. 2, body weights were lower in all the treated groups.

Effect of DBCO on serum and liver lipid levels in male rats. Male rats (weighing 140–150 g) were given DBCO by gavage (in 5% gum acacia), at doses ranging from 0.3 to 30 mg/kg/day for 1 week. Animals were decapitated, serum lipoproteins were fractionated, and levels of cholesterol and phospholipids were measured. Triglyceride levels were determined in whole serum. Liver lipids were also measured.

DBCO produced dose-dependent decreases in food intake and body weight gain. Liver cholesterol was

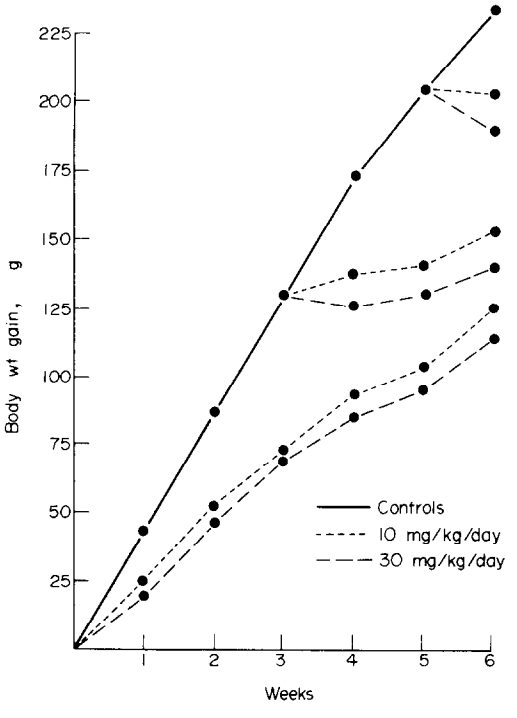

Fig. 2. Effect of 1-, 3- and 6-week treatment with DBCO on body weight gain in male rats.

elevated at the two higher doses (Table 1). Serum cholesterol was reduced at a dose as low as 1 mg/kg/day; the most pronounced decrease was in the HDL fraction (Table 2). At least 10 mg/kg/day was required to lower serum triglyceride and phospholipids.

Effect of DBCO on cholesterol biosynthesis. Male rats (weighing 140–150 g) were treated for 1 or 3 weeks with DBCO p.o. at doses ranging from 0.3 to 30 mg/kg/day, and cholesterogenic activity was measured in liver homogenates incubated with [^{14}C]acetate or with [^{3}H]mevalonate. Cholesterol biosynthesis was suppressed in a dose-dependent manner in livers of rats treated with DBCO for 1 week (Table 3). The primary site of suppression was prior to the formation of mevalonate; a secondary site was observed after mevalonate. However, upon extending the treatment with DBCO to 3 weeks, acetate incorporation into cholesterol returned to that found in untreated rats used as control (Table 3).

In view of the suppressed rate of cholesterol biosynthesis in rats treated with DBCO for 1 week, we have also determined the effect on cholesterol biosynthesis of DBCO added directly to normal rat liver homogenates. As shown in Table 4, at a final concentration of 1×10^{-4} M, DBCO had no effect on cholesterol formation from acetate or mevalonate.

A comparison of the effects of different doses of DBCO on serum and liver cholesterol levels and on hepatic cholesterol synthesis (from [^{14}C]acetate) in rats treated with DBCO for 1 week is presented in Fig. 3. While 1 mg/kg/day significantly reduced the cholesterol levels in the serum, a daily dose of 10 mg/kg was required to elevate cholesterol in the liver. DBCO suppressed hepatic cholesterol synthesis at doses ranging from 3 to 30 mg/kg/day.

Effect of prolonged treatment. Serum lipid and liver cholesterol levels were also measured in male rats treated with 10 mg/kg/day of DBCO for periods ranging from 4 days to 4 weeks. As presented in Table 5, liver cholesterol was elevated in animals treated with DBCO for 4–7 days, but returned to normal in rats treated for 2–4 weeks. At the same time, serum cholesterol was lower after 1 week than after 2, 3 and 4 weeks of treatment. However, in all treated

Table 1. Food intake and liver lipid levels in male rats treated for 1 week with DBCO*

Dose (mg/kg/day)	Weight gain (g/7 days)	Average food intake (g/rat/day)	Liver weight (g)	Liver lipids (mg/100 g)		
				Cholesterol	Phospholipids	Triglycerides
Control	51 ± 1.9	18.9	9.3 ± 0.24	229 ± 5	3275 ± 75	485 ± 37
0.3	47 ± 2.2	18.0	9.4 ± 0.20	231 ± 6		
1	37 ± 3.6†	16.3	8.8 ± 0.21	219 ± 8		
3	26 ± 3.7‡	14.6	9.3 ± 0.30	250 ± 18	3200 ± 75	457 ± 40
10	2 ± 2.4‡	11.6	7.6 ± 0.23‡	427 ± 43‡		
30	−1 ± 2.5‡	9.6	8.1 ± 0.20‡	455 ± 29‡	3250 ± 75	326 ± 19†

* Results are expressed as mean ± S.E.M. for ten rats/group.
† $P < 0.05$.
‡ $P < 0.001$.

Table 2. Serum lipid levels in male rats treated for 1 week with DBCO*

Dose (mg/kg/day)	Serum lipids (mg/dl)						
	Cholesterol			Phospholipids			
	LDL	HDL	Total	LDL	HDL	Total	Triglycerides
Control	23 ± 0.8	35 ± 0.6	58 ± 0.9	37 ± 0.1	85 ± 0.7	122 ± 3.5	88 ± 4.7
0.3	20 ± 1.4	35 ± 2.1	54 ± 2.1	42 ± 2.5	92 ± 3.5†	133 ± 4.5	83 ± 4.9
1	16 ± 0.9‡	28 ± 1.3‡	43 ± 2.4‡	45 ± 0.5	77 ± 2.78	122 ± 4.2	80 ± 5.0
3	15 ± 1.2‡	19 ± 2.0‡	34 ± 3.3‡	37 ± 2.2	73 ± 9.2	110 ± 8.5	84 ± 4.8
10	15 ± 0.8‡	5 ± 0.6‡	20 ± 1.7‡	30 ± 0.2‡	42 ± 1.5‡	72 ± 4.5‡	56 ± 8.48
30	11 ± 0.8‡	4 ± 0.5‡	15 ± 2.2‡	25 ± 2.5‡	35 ± 1.5‡	59 ± 3.0‡	47 ± 8.1§

* Results are expressed as mean ± S.E.M. for ten rats/group.
† $P < 0.05$.
‡ $P < 0.001$.
§ $P < 0.01$.

Table 3. Hepatic cholesterol synthesis in male rats treated with DBCO*

Duration of treatment	Dose (mg/kg/day)	No. of rats	[^{14}C]acetate		[^{3}H]mevalonate	
			Cholesterol (dis./min/ mg nitrogen)	Change from controls (%)	Cholesterol (dis./min. mg nitrogen)	Change from controls (%)
1 week	Control	10	337 ± 71		10,200 ± 700	
	30	10	6 ± 5†	−98	960 ± 130†	−91
1 week	Control	7	680 ± 142		22,400 ± 1,600	
	3	7	59 ± 11†	−91	11,300 ± 1,100†	−50
	1	7	773 ± 54	+14	26,200 ± 1,700	+17
	0.3	7	610 ± 76	−10	22,800 ± 1,770	+2
3 weeks	Control	9	833 ± 158			
	10	9	526 ± 119	−37		
	3	9	626 ± 126	−25		
	1	9	794 ± 245	−5		

* Liver homogenates were prepared and incubated with 5 μCi sodium [2-^{14}C]acetate with or without 0.67 μCi [^{3}H]mevalonate (and appropriate cofactors). Cholesterol was isolated, purified and counted as its 5,6-dibromo derivative [3, 9].

† $P < 0.001$.

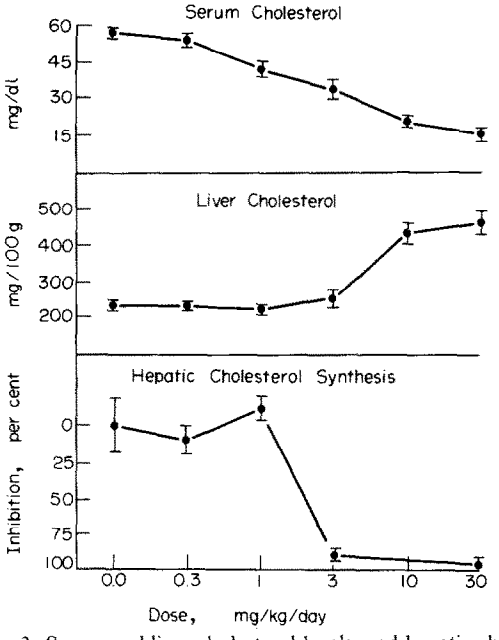

Fig. 3. Serum and liver cholesterol levels, and hepatic cholesterol synthesis (from [^{14}C]acetate *in vitro*) in rats treated for 1 week with different doses of DBCO.

rats, serum cholesterol remained significantly lower than in controls. This difference in serum cholesterol levels was due in part to an increase of LDL cholesterol to normal levels in rats treated for 2 to 4 weeks: HDL cholesterol remained low in all rats. The magnitude of phospholipid lowering was inversely proportional to the duration of treatment. This was due to a shift of phospholipids from HDL to LDL; while phospholipid levels in the HDL fraction remained significantly lower in all treated rats, prolonged treatment increased the phospholipids in the LDL fraction.

Effect of DBCO on lipid metabolism in female rats. Female rats (weighing 170–180 g) were given p.o. a 2% Tween 80 suspension containing 3, 10 or 30 mg/kg/day of DBCO for 1 week. Like in male rats, treatment of female rats with DBCO produced dose-dependent decreases in food intake, body weight gain, serum cholesterol and phospholipids, liver weight, liver triglycerides and hepatic cholesterol synthesis, as well as an elevation in liver cholesterol (data not shown). However, in contrast to the hypotriglyceridemic effect in males, DBCO either increased or produced no effect on serum triglycerides in female rats. This apparent sex dependence of the effects of DBCO was then reinvestigated and rats of both sexes (ten rats/group weighing 70–80 g) were given p.o. 10 or 30 mg/kg/day of DBCO for periods ranging from 4 to 28 days. The effects of DBCO on serum and liver cholesterol and phospholipids were similar in all animals. The most pronounced hypolipidemic response was observed in rats treated with DBCO for 4 days. When the treatment was extended, LDL cholesterol (Fig. 4) and phospholipid levels were increased, thus partly reversing the lipid-lowering effect of DBCO. However, there were marked sex-dependent differences in the effect on serum triglyceride levels. In male rats, while short-term treatment with DBCO decreased the triglycerides, marked increases

Table 4. Effect of DBCO on hepatic cholesterol synthesis *in vitro**

Group	Radioactivity (dis./min)/incubation			
	Nonsaponifiable lipids		Cholesterol	
	[^{14}C]acetate	[^{3}H]mevalonate	[^{14}C]acetate	[^{3}H]mevalonate
Control	90,000	213,000	46,000	164,000
DBCO (1×10^{-4} M)	103,000	198,000	51,000	160,000

* A pooled liver homogenate was prepared from normal male rats and incubated simultaneously with 4.8 μCi [2-^{14}C]acetate and 0.25 μCi [^{3}H]mevalonate, appropriate cofactors, and DBCO at a final concentration of 1×10^{-4} M, and the cholesterogenic activity was measured [9]. Cholesterol was isolated, purified and counted as its 5,6-dibromo derivative [3]. Results presented are averages of duplicate incubations.

Table 5. Serum and liver cholesterol, and serum phospholipids in male rats treated chronically with 10 mg/kg/day of DBCO*

Days of treatment	Serum lipids (mg/dl) Cholesterol LDL	Cholesterol HDL	Cholesterol Total	Phospholipids LDL	Phospholipids HDL	Phospholipids Total	Liver cholesterol (mg/100 g)	Liver cholesterol (mg/liver)
0 (Control)	20.6 ± 0.76	37.5 ± 1.61	58.1 ± 2.00	32.7 ± 2.00	88.3 ± 2.25	121.0 ± 3.00	239 ± 3.9	28.1 ± 0.72
4	14.1 ± 1.31†	6.4 ± 1.52†	20.7 ± 2.55†	20.5 ± 2.00†	38.5 ± 2.50†	58.8 ± 4.25†	327 ± 25.7§	39.3 ± 3.67‡
7	18.6 ± 2.04	3.3 ± 0.87†	22.0 ± 2.64†	42.0 ± 5.50	35.2 ± 2.50†	77.2 ± 7.29†	339 ± 14.1§	35.1 ± 1.94‡
14	23.0 ± 2.21	3.2 ± 0.22†	25.9 ± 2.19†	54.0 ± 5.50‡	36.0 ± 1.50†	90.0 ± 7.29†	301 ± 12.7§	29.3 ± 1.77
21	18.8 ± 1.14	10.1 ± 1.96†	29.0 ± 1.25†	49.0 ± 5.00‡	56.0 ± 5.25†	105.0 ± 2.50†	279 ± 4.4§	24.6 ± 1.23
28	23.3 ± 2.15	7.6 ± 0.65†	30.9 ± 2.65†	53.5 ± 4.25†	47.0 ± 2.25†	100.5 ± 4.75†	292 ± 10.2§	29.0 ± 1.77

* Rats (initial body weight, 70–80 g) treated for 4 days received the vehicle (2% Tween-80) daily for 24 days and then DBCO for 4 days; the timetable for the other groups was arranged in a similar manner, so that all animals were killed at the same time. Results are expressed as mean ± S.E.M. for ten rats/group.

† $P < 0.001$.

‡ $P < 0.01$.

§ $P < 0.05$.

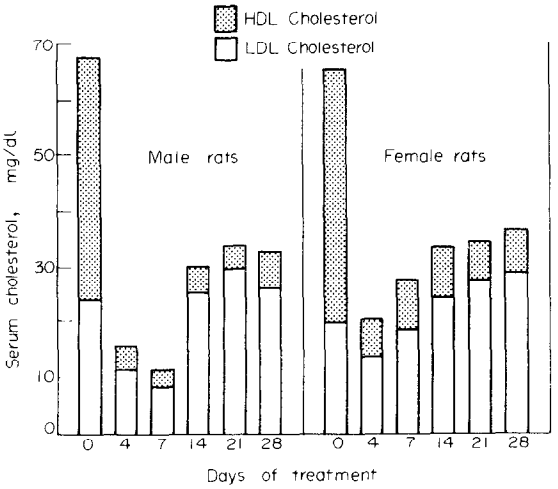

Fig. 4. Effect of duration of DBCO treatment (30 mg/kg/day) on LDL and HDL cholesterol in sera of male and female rats.

occurred when treatment was prolonged (Fig. 5); the initial decrease in serum triglycerides was dose dependent. In contrast, DBCO treatment increased serum triglycerides at all time intervals in female rats and, the longer the treatment, the greater the increase (Fig. 5). DBCO lowered liver triglycerides in both male and female rats.

Estrogenicity. Male rats were treated for 3 weeks with 1, 3 and 10 mg/kg/day of DBCO and the weights of the adrenals, seminal vesicles, dorsolateral prostate and levator ani were measured. Treatment with DBCO resulted in dose-dependent increases in adrenal weight and decreases in weights of the accessory sex organs (Table 6). These changes, coupled with lower food intake and body weight gain, are suggestive of estrogenic activity. As described in an accompanying report [11], the estrogenicity of DBCO was corroborated in female rats.

DISCUSSION

The hypocholesterolemic effect which we observed in normal rats treated with DBCO p.o. for 1 week was similar to that reported earlier by Piantadosi *et al.* [1] in rats treated for 5 days: DBCO lowered serum cholesterol at doses as low as 1 mg/kg/day. We have also found that, in rats receiving DBCO for 1 week, the fall in serum cholesterol was accompanied by an increase in liver cholesterol and a concomitant suppression of hepatic cholesterol synthesis.

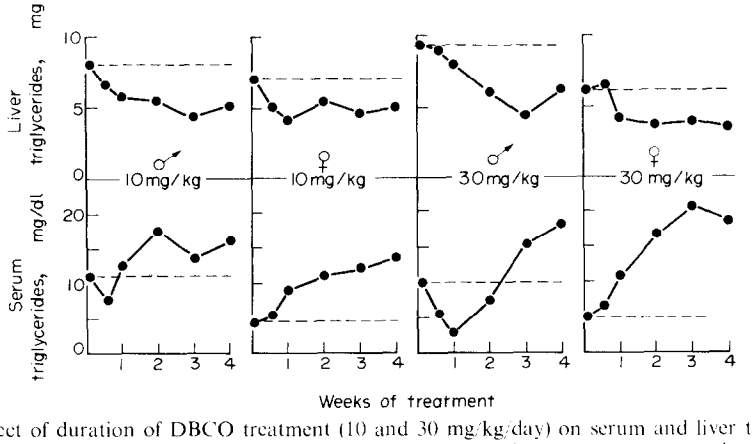

Fig. 5. Effect of duration of DBCO treatment (10 and 30 mg/kg/day) on serum and liver triglycerides of male and female rats. Dashed line represents triglyceride levels in control rats. Each point is the mean of ten rats/group.

Table 6. Weights of adrenals and accessory sex organs of male rats treated for 3 weeks with DBCO

Compound	Dose (mg/kg/day)	Tissue wt (mg/100 g body wt)			
		Adrenals	Seminal vesicles	Dorsolateral prostate	Levator ani*
Control		14 ± 0.4	94 ± 7.4	33 ± 2.0	180 ± 9
DBCO	1	17 ± 0.6†	65 ± 3.4‡	24 ± 2.1‡	127 ± 9†
	3	23 ± 0.8†	35 ± 5.2†	11 ± 1.5†	76 ± 10†
	10	27 ± 1.1†	18 ± 1.5†	7 ± 0.9†	45 ± 3†
17β-Estradiol§	0.15	29 ± 0.6†	41 ± 2.9†	10 ± 1.2†	55 ± 2†

* Includes bulbocavernosus muscle.
† $P < 0.001$.
‡ $P < 0.01$.
§ 17β-Estradiol was given subcutaneously.

However, when treatment with DBCO was prolonged, the hypocholesterolemic effect was diminished, and liver cholesterol levels and hepatic cholesterol synthesis returned to normal. Since DBCO had no effect on cholesterol biosynthesis when added directly to normal rat liver homogenate, it is likely that the suppression of hepatic cholesterol synthesis found after 1 week of treatment with DBCO was due to the increased cholesterol content in the liver.

As in male rats, oral administration of DBCO to female rats produced similar changes in food intake, weight gain, serum cholesterol and phospholipids, liver lipid levels and hepatic cholesterol synthesis. However, the effect of DBCO on serum triglycerides was sex dependent. Thus, in male rats, serum triglycerides were lowered on short-term treatment with DBCO and elevated when treatment was prolonged. In contrast, DBCO increased serum triglycerides in female rats, independently of duration of treatment. If the effect of DBCO on serum triglycerides is associated with its estrogenicity, then the observed sex dependence is in accordance with our findings that short-term administration of estrogens causes an increase in serum triglycerides in female rats [12], but not in male rats.* This phenomenon may be related to the sex dependence of the triglyceride secretion rate by rat liver [10].

Treatment of male rats with DBCO increased the weight of the adrenals and decreased the weights of the accessory sex organs; food intake body weight gain were also suppressed.

*M. N. C., unpublished observations

It was concluded that DBCO is a potent hypocholesterolemic agent in rats, its effectiveness tending to decrease on prolonged treatment. The cholesterol-lowering activity of DBCO was accompanied by changes in tissue weights which were suggestive of estrogenicity.

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REFERENCES

1. C. Piantadosi, I. H. Hall, J. L. Irvine and G. L. Carlson, *J. med. Chem.* **16,** 770 (1973).
2. G. L. Carlson, I. H. Hall, G. S. Abernethy and C. Piantadosi, *J. med. Chem.* **17,** 154 (1974).
3. E. Schwenk and N. T. Werthessen, *Archs Biochem. Biophys.* **40,** 334 (1952).
4. A. Zlatkis, B. Zak and A. J. Boyle, *J. Lab. clin. Med.* **41,** 486 (1953).
5. M. Kraml, *Clinica chim. Acta* **13,** 442 (1966).
6. M. Kraml and L. Cosyns, *Clin. Biochem.* **2,** 373 (1969).
7. A. Ferrari, *Ann. N.Y. Acad. Sci.* **87,** 792, (1960).
8. K. W. Walton and P. J. Scott, *J. clin. Path.* **17,** 627 (1964).
9. M. N. Cayen and D. Dvornik, *Can. J. Biochem.* **46,** 179 (1968).
10. M. L. Watkins, N. Fizette and M. Heimberg, *Biochim. biophys. Acta* **280,** 82 (1972).
11. M. N. Cayen, J. Dubuc, M. L. Givner, E. Greselin and C. Revesz, *Biochem. Pharmac.* **25,** 1543 (1976).
12. P. Hill and D. Dvornik, *Circulation* **40,** III–106 (1969).