Biochemical Pharmacology, Vol. 28, pp. 1163–1167.

0006-2952/79/0401-1163 $02.00/0

PHARMACOKINETICS AND HYPOLIPIDEMIC ACTIVITY OF CLOFIBRATE–NICOTINIC ACID COMBINATIONS IN RATS

MITCHELL N. CAYEN, WILLIAM T. ROBINSON, JEAN DUBUC and DUSHAN DVORNIK
Department of Biochemistry, Ayerst Research Laboratories, Montreal, Quebec, Canada H3C 3J1

(*Received* 15 *May* 1978; *accepted* 4 *October* 1978)

Abstract—Male albino rats were given various oral doses of clofibrate and nicotinic acid, both alone and in combination, daily for 1 week. In normal animals, the combination of drugs produced greater decreases in serum lipids, notably triglycerides, than equimolar doses of either drug alone. Synergistic decreases in serum triglycerides were observed in rats rendered hypertriglyceridemic with fructose and treated simultaneously with both drugs. The peak level of serum nicotinic acid was higher and the area under the serum nicotinic acid concentration–time curve (AUC) was 59 per cent greater when nicotinic acid was given with clofibric acid (CPIB) than when given alone. Pretreatment for 1 week with nicotinic acid slightly increased the apparent volume of distribution and the elimination half-life of intravenously injected CPIB. The presence of nicotinic acid in rat serum did not affect the protein binding of CPIB. The results show that the combined hypolipidemic effect of clofibrate and nicotinic acid in rats is accompanied by changes in the pharmacokinetics of the drugs, the most pronounced being a decrease in the rate of elimination of nicotinic acid.

The efficacy of the various antihyperlipidemic drugs usually depends upon the specific type of lipid disorder. Rational drug therapy of hyperlipoproteinemia should not only be assessed by understanding its effects on cholesterol and triglyceride metabolism, but also should be focused on the specific action of the given drug on lipoprotein metabolism. For example, nicotinic acid suppresses the release of very low density lipoproteins (VLDL) from the liver into the plasma, thereby reducing circulating levels of VLDL and its remnant, low density lipoproteins (LDL) [1]. The cholesterol lowering action of the anion exchange resins is associated with enhanced rate of LDL removal from the plasma [2]. The mode of action of clofibrate is as yet unclear; some reports suggest that it decreases the rate of VLDL production by the liver, while others show that it increases the catabolism of VLDL to LDL [3, 4].

The recent interest in the use of a combination of lipid-lowering drugs [5–7] derives from the concept that each drug would act at a different site of lipoprotein disposition. The advantage to the patient is that low doses of two drugs may elicit antihyperlipidemic activity similar to that from a higher dose of a single drug entity, resulting in reduced potential for side effects. In addition, combination drug therapy may produce a greater lowering of serum lipids than the usual dose of the single drug. Thus, it has been shown that combined therapy with clofibrate and nicotinic acid (or analogues) produced synergistic decreases in elevated plasma lipids [7, 8].

As far as we are aware, there have been no reports on the effect of combined treatment with clofibrate and nicotinic acid on the pharmacokinetics of the drugs. In this paper, we report on the blood levels and hypolipidemic activity of clofibrate and nicotinic acid in rats, showing that the combined effect on serum lipids was accompanied by changes in the rates of elimination of the drugs from the body. A portion of this work has appeared as an abstract [9].

MATERIALS AND METHODS

Animals and dose. Male albino Charles River CD rats, weighing 160–180 g, were fed Purina rat chow and were not fasted during any experiment. For oral administration, the compounds were suspended in 2% Tween-80 and administered by gastric intubation. For intravenous injection, nicotinic acid or the sodium salt of clofibric acid (*p*-chlorophenoxyisobutyric acid; CPIB) was dissolved in saline and injected into the caudal vein. Animals were killed by decapitation.

Analytical methods. Serum and liver cholesterol levels were determined by the method of Zlatkis *et al.* [10], as modified for the autoanalyzer. Phospholipid and triglyceride levels were measured by semiautomated techniques [11, 12]. Orally administered clofibrate is present in the blood as CPIB [13, 14]; the concentration of CPIB in serum was determined by a spectrophotometric method [15]. The serum levels of nicotinic acid were measured by an automated technique [16] based on the colorimetric method of Carlson [17]; data obtained by this method are not influenced by NAD compounds and only to a minor extent by free nicotinamide [17].

The two-tailed Student's *t*-test was used to determine the significance of difference between group means. For studies comprising more than two groups, one-way analysis of variance produced similar statistical differences as did Student's *t*-test.

Serum protein binding of CPIB or nicotinic acid was estimated by equilibrium dialysis [18]. Serum from treated rats was dialyzed against isotonic sodium phosphate buffer (pH 7.4) (containing [^{14}C]clofibric acid, sp. act. 91 μCi/mg or [^{14}C]nicotinic acid, sp. act. 136 μCi/mg) in a specially designed multi-cell stainless steel dialysis chamber. Each cell contained 1 ml serum and 1 ml buffer, and the duration of dialysis was 18 hr. Cells containing buffer on both sides of the dialysis membrane were used to demonstrate that equilibrium

had been attained with both radioactive compounds. The partition of radioactivity was used to calculate the binding of CPIB or nicotinic acid.

RESULTS

Effect of clofibrate and nicotinic acid on lipid levels in normal and hypertriglyceridemic rats. Rats were given clofibrate for 1 week at daily oral doses of 121, 73, 48 or 24 mg/kg; other groups of animals were given nicotinic acid at daily doses of 62, 37, 25 or 12 mg/kg for 1 week. These amounts corresponded to 0.5, 0.3, 0.2 or 0.1 m-mole/kg/day of both compounds for 1 week. Animals were killed 3 hr after the last dose, and serum and liver levels of cholesterol, phospholipids and triglycerides were measured. The same experimental protocol was also used in rats rendered hypertriglyceridemic by substituting the drinking water with a 10% fructose solution during the last 24 hr of treatment [19, 20].

None of the treatments altered food intake or body weight gain. The effects of clofibrate and nicotinic acid on serum lipid levels in normal rats are shown in Fig. 1. Clofibrate produced the expected [21] dose-related decreases in serum cholesterol, phospholipids and triglycerides; nicotinic acid decreased serum triglycerides but exhibited only weak cholesterol-lowering activity. Only at the highest dose (0.5 m-mole/kg/day) did nicotinic acid significantly reduce serum cholesterol ($P < 0.05$). The combination of clofibrate and nicotinic acid produced greater decreases in serum lipid levels than did either drug alone. The decreases in serum cholesterol produced by the combination were not significantly greater than that induced by clofibrate alone. However, serum phospholipids and triglycerides after the combination were significantly lower than after equimolar doses of either clofibrate or nicotinic acid. In certain instances, synergistic decreases were observed. For example, clofibrate at a daily dose of 0.1 m-mole/kg/day decreased serum triglycerides from 88.1 ± 6.53 mg/dl to 76.4 ± 8.40 mg/dl, a difference of 12 mg/dl, while the same dose of nicotinic acid lowered serum triglycerides to 83.2 ± 11.1 mg/dl, a difference of 5 mg/dl; neither of these decreases was statistically significant. If the combination of clofibrate and nicotinic acid were to elicit a change equal to the sum of the two components, the expected decrease would be 17 mg/dl. However, the observed level of serum triglycerides in rats given a combination of 0.1 m-mole/kg of clofibrate plus nicotinic acid was 50.0 ± 5.60 mg/dl, a statistically significant decrease ($P < 0.01$) and 38 mg/dl less than controls. Thus, the combination produced a synergistic decrease in serum triglycerides. Similar differences in serum phospholipids were also observed. As expected, clofibrate slightly elevated liver weight while nicotinic acid had no effect; none of the treatment regimens markedly altered liver lipid levels (Table 1).

The effects of the drugs on serum triglycerides in hypertriglyceridemic rats are shown in Fig. 2. No data were available for clofibrate at 0.1 m-mole/kg/day. As found in normal rats, clofibrate or nicotinic acid produced dose-dependent decreases in serum triglycerides, while equimolar doses of the combination produced substantially greater decreases than did either drug alone. At doses of 0.5 m-mole/kg/day, the decrease in serum triglycerides produced by the combination was significantly greater ($P < 0.05$) than that elicited by either drug.

Effect of nicotinic acid on the pharmacokinetics of CPIB. Rats were given daily oral doses of 0.5 m-mole/

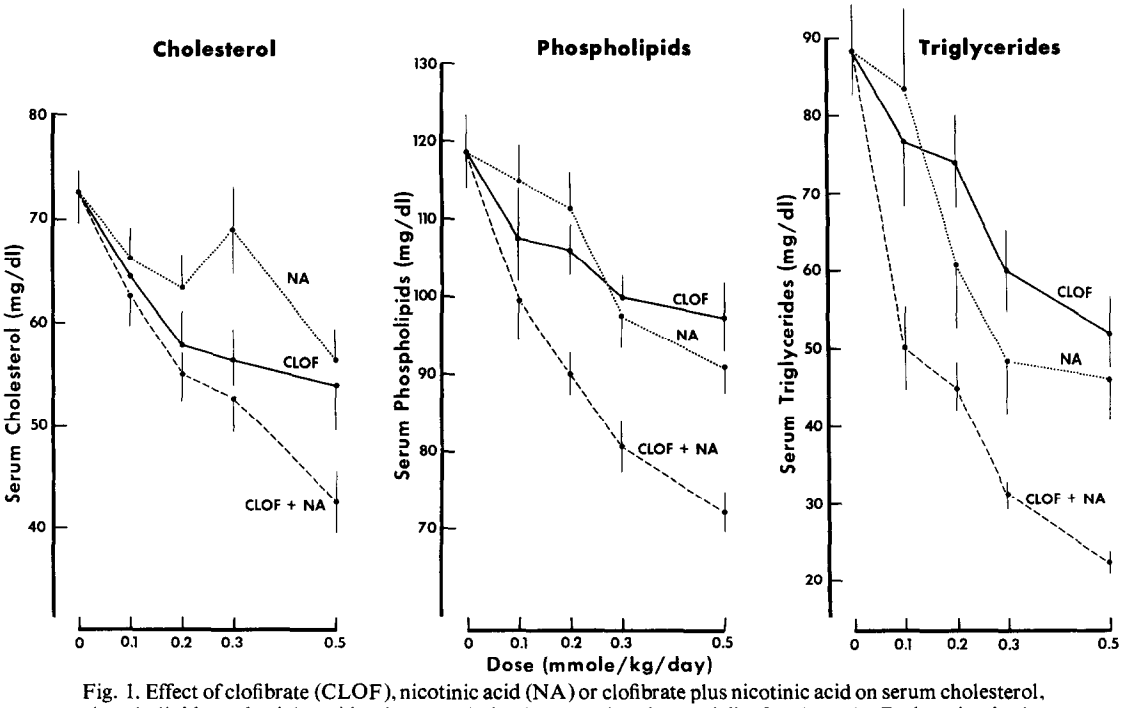

Fig. 1. Effect of clofibrate (CLOF), nicotinic acid (NA) or clofibrate plus nicotinic acid on serum cholesterol, phospholipids and triglycerides in rats. Animals were dosed p.o. daily for 1 week. Each point is the mean ± S.E. for eight rats/group.

Table 1. Effect of clofibrate and nicotinic acid on liver weight and liver lipids

Compounds	Dose* (m-mole/kg/day)	Liver wt (g)	Liver lipid levels (mg/100 g) Cholesterol	Phospholipids	Triglycerides
Control		9.99 ± 0.44	275 ± 7.6	3525 ± 58	560 ± 48.6
Clofibrate	0.5	11.62 ± 0.35†	246 ± 5.0†	3750 ± 65†	634 ± 47.6
	0.3	10.84 ± 0.26	254 ± 6.4	3775 ± 55‡	573 ± 41.1
Nicotinic acid	0.5	9.06 ± 0.30	299 ± 10.3	3750 ± 88	443 ± 36.4
	0.3	8.94 ± 0.48	301 ± 6.7†	3825 ± 63‡	458 ± 26.1
Clofibrate+ nicotinic acid	0.5	10.73 ± 0.19	237 ± 6.2†	3975 ± 53§	518 ± 35.5
	0.3	9.93 ± 0.25	255 ± 4.9	3675 ± 100	421 ± 21.5†

* Administered for 7 days. Data are presented as means ± S.E. for eight rats/group.
† $P < 0.05$.
‡ $P < 0.01$.
§ $P < 0.001$.

kg of nicotinic acid for 1 week; controls received Tween-80 daily. On the last day, all rats were injected intravenously with 0.5 m-mole/kg of the sodium salt of clofibric acid immediately after the oral dose of nicotinic acid or Tween-80. Groups of six rats were killed at 0.5, 1, 2, 4, 6, 8 and 12 hr after dosing, blood was collected, and serum levels of CPIB were measured.

The results in Fig. 3 show that chronic treatment with nicotinic acid slightly increased the serum half-life of CPIB. The serum half-life was calculated by least squares analysis and the area under the serum concentration-time curve (AUC) was calculated by the trapezoidal rule; computer programs were used in these calculations. In control rats, the half-life of approximately 5 hr was similar to that reported previously [14]. Pretreatment with nicotinic acid increased the serum half-life of CPIB to approximately 7 hr. The AUC of CPIB was 17 per cent greater in animals pretreated with nicotinic acid than in controls (given Tween-80). Nicotinic acid slightly decreased the total clearance of CPIB (Q = dose/AUC) from 43.8 to 37.5 $ml \cdot kg^{-1} \cdot hr^{-1}$. Since the relative increase in the half-life was greater than the increase in the AUC, nicotinic acid increased the apparent volume of distribution of CPIB ($V_{d\ area} = T\frac{1}{2} \cdot Q/0.693$) from 303 to 379 ml/kg.

In a second study, rats were given 0.5 m-mole/kg/day of clofibric acid, p.o. for 1 week, with and without daily oral doses of 0.5 m-mole/kg of nicotinic acid, and groups of five rats were killed at 1, 2, 3 and 6 hr after the final dose. The bioavailability of clofibric acid in rats is virtually identical to that of clofibrate [14]. Blood was collected and serum levels of CPIB were measured. The data in Fig. 4 show that the peak level and AUC (0–6 hr) of CPIB were somewhat higher in rats given both CPIB and nicotinic acid than in those given CPIB alone. However, none of the differences was statistically significant.

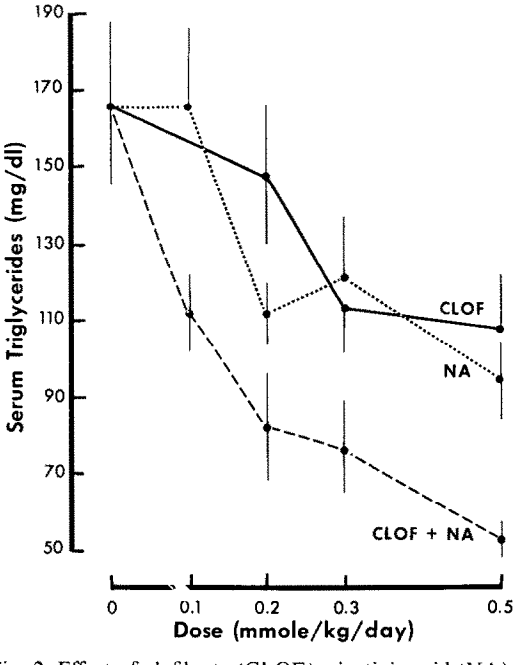

Fig. 2. Effect of clofibrate (CLOF), nicotinic acid (NA) or clofibrate plus nicotinic acid on serum triglycerides in rats rendered hypertriglyceridemic by substituting the drinking water with a 10% fructose solution during the last 24 hr of treatment. Compounds were administered p.o. daily for 1 week. Each point is the mean ± S.E. for eight rats/group. No data were available for clofibrate at 0.1 m-mole/kg/day.

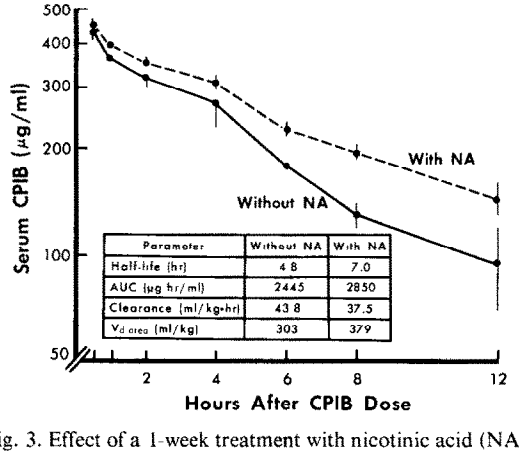

Fig. 3. Effect of a 1-week treatment with nicotinic acid (NA) (0.5 m-mole/kg/day, p.o.) on serum levels of clofibric acid (CPIB) after intravenous injection of a single dose of the sodium salt of CPIB (0.5 m-mole/kg). Controls received Tween-80 daily instead of nicotinic acid. Each point is the mean of six rats.

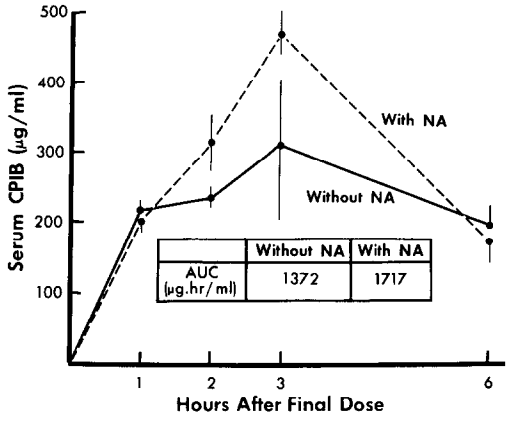

Fig. 4. Effect of a 1-week treatment with clofibric acid (CPIB) (0.5 m-mole/kg/day, p.o.) with and without nicotinic acid (NA) (0.5 m-mole/kg/day) on serum levels of CPIB. Each point is the mean of five rats.

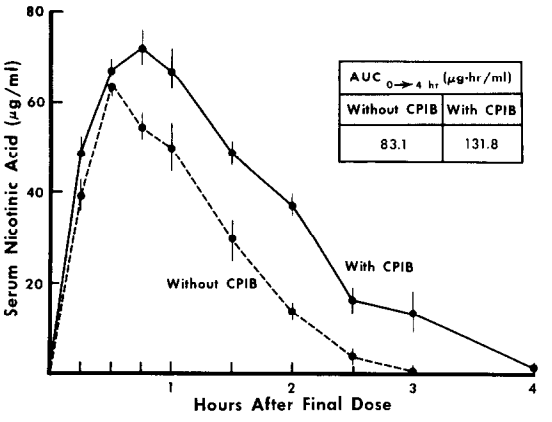

Fig. 6. Effect of a 1-week treatment with nicotinic acid (0.5 m-mole/kg/day, p.o.) with and without CPIB (0.5 m-mole/kg/day) on serum levels of nicotinic acid. Each point is the mean of five rats.

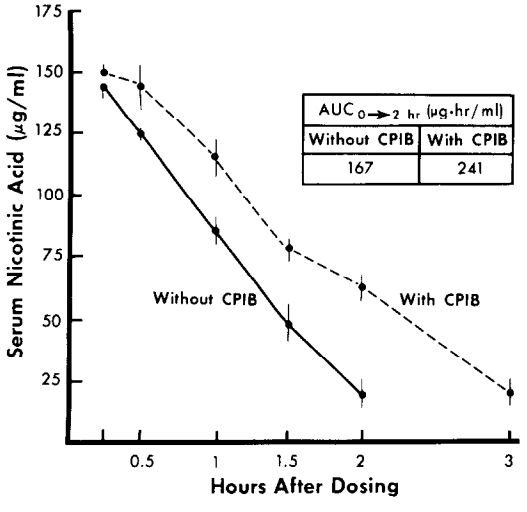

Fig. 5. Effect of a 1-week treatment with clofibric acid (CPIB) (0.5 m-mole/kg/day, p.o.) on serum levels of nicotinic acid after intravenous injection of a single dose of 0.5 m-mole/kg of nicotinic acid. Controls received Tween-80 daily instead of CPIB. Each point is the mean of five rats.

Effect of clofibric acid on the pharmacokinetics of nicotinic acid. Rats were given daily oral doses of 0.5 m-mole/kg of clofibric acid for 1 week; controls received Tween-80 daily. One hr after the last oral dose, all rats were injected intravenously with 0.5 m-mole/kg of nicotinic acid, and groups of five animals were killed at 0.25, 0.5, 1, 1.5, 2 and 3 hr thereafter. Serum levels of nicotinic acid are shown in Fig. 5. The differences at 1, 1.5 and 2 hr were statistically significant ($P < 0.05$). No serum nicotinic acid was detected at 3 hr in Tween-80-treated controls, while in CPIB-treated rats serum nicotinic acid at 3 hr after dosing averaged 20 μg/ml. The elimination of nicotinic acid appeared to follow zero-order kinetics. The AUC (0–2 hr) was 45 per cent greater in rats pretreated with CPIB than in controls.

Another study was conducted with rats treated orally for 1 week with 0.5 m-mole/kg/day of nicotinic acid with and without CPIB (0.5 m-mole/kg/day). Groups of five rats were killed at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3 and 4 hr after the final dose, and serum nicotinic acid was measured. Combined treatment with nicotinic acid and CPIB resulted in substantially higher serum levels of nicotinic acid than found in rats given nicotinic acid alone (Fig. 6). The AUC, calculated for the 0–4 hr

Table 2. Serum protein binding of clofibric acid and nicotinic acid after combined treatment*

	Clofibric acid (CPIB)				Nicotinic acid (NA)			
	Serum CPIB levels (μg/ml)		CPIB bound (%)		Serum NA levels (μg/ml)		NA bound (%)	
Hr after last dose	Without NA	With NA	Without NA	With NA	Without CPIB	With CPIB	Without CPIB	With CPIB
1	216	204	79.3	80.2	53	73	17.6	14.1
2	234	312	75.0	74.2	23	47	18.1	9.0
3	311	470	73.7	66.0	2	31	3.7	1.7
6	200	174	80.6	82.5	0	0		

* Rats were treated for 1 week with 0.5 m-mole/kg/day, p.o., with CPIB, nicotinic acid, or both drugs simultaneously. Animals were killed at various times after the final dose. A tracer amount of [^{14}C]CPIB (0.29 μg/ml) or [^{14}C]nicotinic acid (0.16 μg/ml) was added to the serum, and the protein binding was measured by equilibrium dialysis. Each value is the mean of five rats.

interval, was 59 per cent higher in rats given both drugs than in those given nicotinic acid alone.

Protein binding. In order to determine whether combined treatment affects the protein binding of CPIB or of weakly bound nicotinic acid, the extent of binding was determined in the serum of rats after 1 week of treatment with 0.5 m-mole/kg/day of clofibric acid and nicotinic acid, and the data were compared with those after treatment with either drug alone. The results in Table 2 show that simultaneous administration of the two drugs did not alter the extent of CPIB or nicotinic acid binding to serum protein.

DISCUSSION

The data show that a combination of clofibrate and nicotinic acid is more effective in reducing serum lipids, especially triglycerides and phospholipids, than either drug alone. Similar effects of clofibrate and nicotinyl alcohol on rat serum triglycerides have been reported by Simane and Nowak [22]. In the present study, a synergistic decrease in serum triglycerides was observed. The cholesterol-lowering activity of the combination was not as great as the hypotriglyceridemic activity. Nicotinic acid itself has weak cholesterol-lowering properties in rats [23], and the hypocholesterolemic activity of the combination was only slightly greater than that of clofibrate alone.

Combined treatment was accompanied by changes in the pharmacokinetics of the drugs. Regarding CPIB, the peak was somewhat higher and the elimination half-life was 2 hr longer when CPIB was given with nicotinic acid than when administered alone. However these differences were small when compared to the action of CPIB on the disposition of nicotinic acid. After the combination, the peak levels of nicotinic acid were higher and the AUC was substantially greater, resulting in greater bioavailability of the drug. Recent studies on the renal excretion of nicotinic acid in dogs have shown that the renal excretory mechanism of the drug is readily saturable and that net reabsorption can occur, depending upon the plasma concentration [24]. If such a mechanism were also operable in rats, and since CPIB is excreted mainly in the urine [14], CPIB may compete with nicotinic acid for the renal transport sites, thereby reducing the clearance of nicotinic acid. Such a competition may also partially explain the higher serum peak levels of nicotinic acid when given in combination with CPIB. Since elimination takes place as soon as absorption begins, decreased renal clearance could conceivably affect the shape of the absorption phase of the serum concentration–time curve, thereby resulting in higher peak levels of nicotinic acid.

The results of this study show that the combined effects of clofibrate and nicotinic acid on serum lipid levels in rats are accompanied by changes in the rate of elimination of the drugs, especially of nicotinic acid. This animal model provides data which can explain in pharmacokinetic terms the synergistic action of the drugs in hyperlipidemic subjects.

Acknowledgements—The authors wish to thank Dr. E. Greselin for his help with the animal experiments and Ms. Frances Boutin, Ms. Mae Wong and Ms. Jane Wylie for their technical assistance. The computer programs for the pharmacokinetic analyses were developed by Mr. Dale Jeffries.

REFERENCES

1. A. A. Magide, N. B. Myant and D. Reichl, *Atherosclerosis* **21**, 205 (1975).
2. R. I. Levy and T. Langer, in *Pharmacological Control of Lipid Metabolism* (Eds. W. L. Holmes, R. Paoletti and D. Kritchevsky), *Adv. Exp. Med. Biol.* Vol. 26, pp. 155–63. Plenum Press, New York (1972).
3. S. M. Grundy, in *Lipid Pharmacology* (Eds. R. Paoletti and C. J. Glueck), Vol. 2, pp. 127–59. Medicinal Chemistry Monographs. Academic Press, New York (1976).
4. T. A. Miettinen, in *Lipid Pharmacology* (Eds. R. Paoletti and C. J. Glueck), Vol. 2, pp. 83–125. Medicinal Chemistry Monographs. Academic Press, New York (1976).
5. P. F. Hansen, *Postgrad. med. J.* **51** (suppl. 8), 63 (1975).
6. R. I. Levy, *Postgrad. med. J.* **51** (suppl. 8), 60 (1975).
7. L. Oro, A. G. Olsson, S. Rossner and L. A. Carlson, *Postgrad. med J.* **51** (suppl. 8), 76 (1975).
8. B. Schaeffer and R. Kluthe, *Medsche Welt., Stuttg.* **26**, 655 (1975).
9. M. N. Cayen, W. T. Robinson and D. Dvornik, in *Drugs, Lipid Metabolism and Atherosclerosis* (Eds. D. Kritchevsky, A. Paoletti and W. L. Holmes) Adv. Exp. Med. Biol., Vol. 109, p. 371. Plenum Press, New York (1978).
10. A. Zlatkis, B. Zak and A. J. Boyle, *J. Lab. clin. Med.* **41**, 486 (1953).
11. M. Kraml, *Clinica chim. Acta* **13**, 442 (1966).
12. M. Kraml and L. Cosyns, *Clin. Biochem.* **2**, 373 (1968).
13. J. M. Thorp, *Lancet* **1**, 1323 (1962).
14. M. N. Cayen, E. S. Ferdinandi, E. Greselin, W. T. Robinson and D. Dvornik, *J. Pharmac. exp. Ther.* **200**, 33 (1977).
15. A. M. Barrett and J. M. Thorp, *Br. J. Pharmac. Chemother.* **32**, 381 (1968).
16. W. T. Robinson, L. Cosyns and M. Kraml, *Clin. Biochem.* **11**, 46 (1978).
17. L. A. Carlson, *Clinica chim. Acta* **13**, 349 (1966).
18. A. Goldstein, *Pharmac. Rev.* **1**, 102 (1949).
19. H. Bar-on and Y. Stein, *J. Nutr.* **94**, 95 (1968).
20. P. Hill, *Lipids* **5**, 621 (1970).
21. M. N. Cayen, J. Dubuc and D. Dvornik, *Proc. Soc. exp. Biol. Med.* **148**, 752 (1975).
22. Z. Simane and H. Nowak, *Atherosclerosis* **20**, 447 (1974).
23. D. Kritchevsky and S. A. Tepper, *J. Nutr.* **82**, 157 (1964).
24. P. B. Corr and D. G. May, *J. Pharmac. exp. Ther.* **192**, 195 (1975).