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CLOFIBRATE-INDUCED ALTERATIONS IN SERUM PROTEIN PATTERNS*†

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Abstract—Clofibrate when added to the diet at 0·05 to 1·25% (w/w) not only causes an apparently dose-dependent decrease in serum cholesterol of rats but also markedly affects the plasma protein pattern. An increase in albumin is detectable by cellulose acetate strip electrophoresis, acrylamide gel electrophoresis, and by extraction of albumin from plasma. Cellulose acetate strip electrophoresis reveals a decrease in the α_2-globulin fraction, while acrylamide gel electrophoresis indicates that there are manifold changes. Extractable seromucoid concentration declined from 440 mg/100 ml to 150 mg/ml as the dose of clofibrate increased. A concentration decrease in plasma glucose was also observed. Part of the decrement in seromucoid at low drug levels may be related to lessened haptoglobin concentration. The trypsin inhibitory capacity of the plasma was significantly decreased in what appeared to be a dose-dependent fashion. The decrease in seromucoid is consistent with the reduction in bound fraction of ribosomal RNA. Another explanation would appear to be required to explain the rise in albumin.

Clofibrate is an antihyperlipidemic agent used to reduce serum cholesterol and triglyceride levels [1]. In addition, clofibrate has been shown to decrease glycolytic enzyme activities in rat liver [2] and human jejunum [3], to increase the hepatic content of mitochondria in rats [4], to alter the activity of a number of oxidoreductase enzymes in rat liver [5] and to increase liver catalase synthesis [6]. Obviously, clofibrate has extensive effects on the synthesis of intrahepatic proteins. We, therefore, investigated the effects of clofibrate on serum proteins which are also synthesized by the liver [7].

MATERIALS AND METHODS

Clofibrate was obtained from Dr. George Brice (Ayerst Laboratories, New York). Male Fisher–Dunning rats weighing 150–200 g (Microbiological Associates, Walkersville, Md.), were housed in a room maintained at 22–24° and lighted from 0600 to 1800 hr. They were fed ground commercial rat food (Ralston Purina), to which varying amounts of clofibrate were added.

Plasma chlorophenoxyisobutyrate (CPIB) content was determined by the spectrophotometric method of Barrett and Thorp [8]. Clofibrate acid for standardization was obtained from Dr. Dvornik (Ayerst Laboratories, Montreal, Canada). Cellulose acetate strip electrophoresis was accomplished with a PhoroSlide (Millpore Corp., Bedford, Mass.) apparatus. The samples were run for 37 min to facilitate the separation of the α_1-fraction from albumin; the strips were stained with Ponceau-S and quantitated with a Phoro-Scope densitometer.

Acrylamide gel electrophoresis was carried out using commercially available gradient gels (3 20%) and electrophoretic equipment obtained from Ortec Inc. (Oak Ridge, Tenn.). Densitometric analysis was carried out using a model R Digiscreen (Gelman Instrument Co., Ann Arbor, Mich.).

Cholesterol and triglyceride was determined on an Auto Analyzer II system [9, 10] (Technicon Instrument Corp., Tarrytown, N.Y.).

Plasma glucose was determined using *ortho*-toluidine reagent [11].

Seromucoid and albumin were extracted by the procedure of Neuhaus *et al.* [12] and protein determined by an automated Lowry technique [13].

Serum haptoglobulin was determined by the procedure of Owen *et al.* [14].

Trypsin inhibitory capacity was assessed according to the method described by Eriksson [15]. No effect of CPIB on the assay itself was observed. Hepatic RNA was fractionated by the technique of Blöbel and Potter [16] and the RNA and DNA content was determined spectrophotometrically [17].

RESULTS

Plasma CPIB concentration increases as a function of the dietary content of the drug (Table 1). There is the anticipated increase in liver weight/100 g body weight and decrease in cholesterol concentration. In contrast, a biphasic relationship between plasma CPIB content and triglyceride concentration appears

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Table 1. Some metabolic effects of clofibrate in rats ($N = 10$)

% Clofibrate in diet	Plasma CPIB (μg/ml)	Liver wt (g/100 g body wt)	Cholesterol (mg/100 ml)	Triglycerides (mg/100 ml)	Glucose (mg/100 ml)
0		5·23 ± 0·23	56·7 ± 2·4	123·2 ± 15·2	155 ± 6
0·05	24 ± 4	5·35 ± 0·13	41·2 ± 2·1*	53·2 ± 3·0*	138 ± 4
0·25	129 ± 16	6·77 ± 0·42*	26·0 ± 0·7*	64·0 ± 6·9*	117 ± 3*
1·25	487 ± 57	7·17 ± 0·38*	17·8 ± 3·9*	88·8 ± 13·1	90 ± 5*

* Significantly different from controls, $P < 0{\cdot}001$.
† Significantly different from controls, $P < 0{\cdot}01$.

Table 2. Effect of clofibrate on serum protein pattern of rats ($N = 10$)

Clofibrate in diet (%)	Per cent				
	Albumin	α_1	α_2	β-γ	γ
0	49·4 ± 1·3	14·6 ± 1·2	13·9 ± 0·5	18·9 ± 1·4	3·2 ± 0·3
0·05	50·7 ± 0·5	10·9 ± 0·6*	12·7 ± 0·8	23·5 ± 1·7	2·2 ± 0·4
0·25	53·8 ± 1·3	13·9 ± 0·6	9·0 ± 0·3*	19·4 ± 1·3	4·1 ± 0·5
1·25	59·5 ± 1·6*	13·6 ± 0·7	5·4 ± 0·6*	18·3 ± 1·7	3·1 ± 0·6

* Significantly different from controls, $P < 0{\cdot}01$.
† Significantly different from controls, $P < 0{\cdot}001$.

to obtain. A 40 per cent decrease in plasma glucose was observed at the highest dose of the drug.

Table 2 summarizes the effect of clofibrate on serum protein pattern as revealed by cellulose acetate strip electrophoresis. There is a 20 per cent increase in albumin and a 60 per cent decrease in the α_2-fraction at 1·25% clofibrate. There is a significant decrease in the α_1-fraction and a seemingly complementary rise in the β-γ fraction at 0·05% clofibrate. Figure 1 shows sera run on acrylamide gels and stained for protein and glycoprotein and indicates that the effects of CPIB on plasma proteins may not be limited to albumin and a few proteins in the α_2-fraction. There appears to be a dose-dependent response with more extensive decrements in a number of protein bands at higher doses. Although not especially evident to the naked eye, densitometric analysis revealed a very significant increase in albumin from 1·9 to 2·7 g/100 ml in rats receiving 1·25% clofibrate as compared to controls. Less periodic acid-Schiff reactive material was observed just ahead of albumin where one would expect to find orosomucoid and α_1-antitrypsin. Despite the marked changes in serum protein pattern elicited by CPIB, there was no significant difference

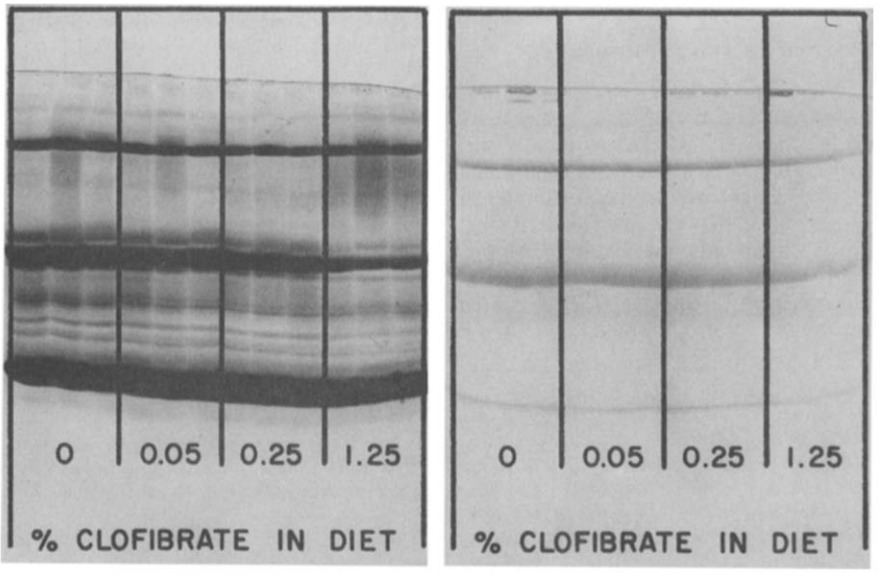

Fig. 1. Gradient acrylamide gel electrophoretic patterns. Three μl of serum was applied to the gel which was to be stained with amido schwarz for protein content and 20 μl to the gel which was stained with periodic acid-Schiff reagent to detect glycoproteins. The protein patterns are shown on the left, the glycoprotein on the right. The total serum protein concentration (g/100 ± 1 S.E.M.) was as follows: control, 6·28 ± 0·16; 0·05% clofibrate, 6·27 ± 0·07; 0·25% clofibrate, 6·48 ± 0·11; and 1·25% clofibrate, 6·26 ± 0·11.

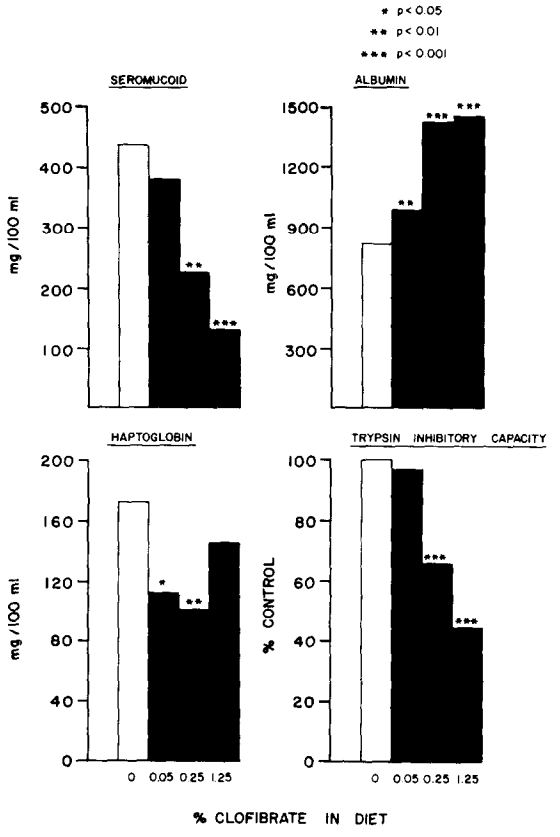

Fig. 2. Effect of clofibrate on selected protein components of serum. The means of six rats/group are depicted. The trypsin inhibitory capacity of control sera was 3·65 ± 0·19 (mean ± 1 S. D.) mg trypsin inhibited/ml of serum.

between the total serum protein concentration in treated vs control rats.

Since there were obvious decreases in glycoproteins, the sera were analyzed for seromucoid content. Even the lowest dose of clofibrate decreased seromucoid content somewhat, but significant dimunition was only observed at the higher doses (Fig. 2). Confirming the rise in albumin apparent by electrophoresis, there was almost a doubling of extractable albumin at the two highest doses of clofibrate, and even 0·05% clofibrate caused a significant elevation. The decrease in seromucoid at the lowest doses of clofibrate may be due to a fall in haptoglobin concentration, but at higher doses it would appear that changes in haptoglobin do not play a major role in this phenomenon. Trypsin inhibitory capacity decreased with increasing dose of clofibrate and might account in part for the decrement in seromucoid.

In many ways consistent with the changes in serum protein patterns just described were the alterations in hepatic RNA distribution, shown in Table 3. The concentration of total and soluble RNA/mg of DNA was not appreciably changed. However, free ribosomal RNA is significantly increased; this change was complemented by a reduction in the bound fraction, resulting in a significant change in the free-to-bound ribosome ratio.

DISCUSSION

Clofibrate induced the anticipated hepatic hypertrophy and decrease in cholesterol usually observed in animal studies, especially at higher doses of the drug [18]. The apparent biphasic relationship between clofibrate dose and triglyceride concentration is not totally unexpected in the light of the recent studies demonstrating that CPIB stimulates triglyceride synthesis in hepatocytes isolated from rats and squirrel monkeys [19]. The decrease in plasma glucose concentration is consonant with reports of clofibrate-induced decreases in hepatic glycogen content and certain glycolytic enzymes [2].

The effects of clofibrate on hepatic enzymes in general and catalase in particular led us to look at the effects of this drug on serum protein patterns. Since intrahepatic proteins are presumed to be synthesized on free ribosomes while proteins for export appear to be formed on the bound ribosomes [20], we hypothesized that significant changes in serum protein patterns might occur after clofibrate treatment. As expected such occurred, although not entirely as predicted. If one ascribes the decrease in seromucoids to a decrease in bound ribosomes, i.e. in the number of sites synthesizing serum proteins, then an alternative explanation is required to explain the rise in albumin, since albumin is also presumed to be formed on the rough endoplasmic reticulum [21]. It may be that CPIB affects transcription as well as translation giving rise to a different pattern of messenger RNA. Or, perhaps since albumin does not appear to contain any significant amounts of protein-bound carbohydrate, its synthesis may be affected differently than the bulk of the serum globulins, all of which have at least some bound carbohydrate [22]. If the decrease in plasma glucose we observed reflects the availability of sugars for attachment to nascent serum proteins and if sugar availability is rate limiting, one might expect depressed serum glycoprotein synthesis.

Table 3. Hepatic RNA distribution in rats ($N = 6$) fed 1·25% clofibrate in their diet

RNA fraction	mg RNA/DNA by days on diet		
	0	3	7
Total	2·64 ± 0·06	2·61 ± 0·03	2·53 ± 0·06
Soluble	0·65 ± 0·02	0·72 ± 0·01	0·71 ± 0·06
Ribomosal			
Free	0·36 ± 0·01	0·45 ± 0·03*	0·47 ± 0·03*
Bound	1·42 ± 0·10	1·27 ± 0·07	1·11 ± 0·10*
Bound/free ratio	(3·94 ± 0·33)	(2·82 ± 0·25)*	(2·36 ± 0·26)*

* Significantly different from control (day 0), $P < 0.01$.

Again, it may be that CPIB binding to albumin [23] lessens its degradation or affects its distribution.

Some if not all of the decrease in periodic acid-Schiff (PAS) reagent-positive material on acrylamide gels may be due to the decrease in seromucoids, a class of serum globulins containing large amounts of bound carbohydrate and comprised of proteins nominally referred to as acute phase globulins [22]. At 0·05% clofibrate the decrease in haptoglobin is almost sufficient to explain the drop in seromucoid content. However, at higher doses, changes in haptoglobin seem to play little part in the decrease in seromucoid. The dose-dependent decrements in trypsin inhibitory capacity may indeed augar a decrease in α_1-antitrypsin itself. Certainly there is less PAS-positive material at the site of the acrylamide gels where one might expect α_1-antitrypsin. However, α_2-macroglobulin also possesses antiprotease activity [24, 25] and a significant decrease in the α_2-fraction was observed in the cellulose acetate strip electrophoretic patterns. Orosomucoid or α_1-acid glycoprotein is another of the seromucoids and, although not assayed for specifically, again there was a decrease in PAS-positive material at its presumed location on acrylamide gels.

Although the decrease in the ratio of bound to free ribosomes is consistent with lessened seromucoid, it remains to be determined how CPIB affects RNA distribution and, subsequently, serum protein synthesis. In a whole animal, interpretation would be complicated by the fact that CPIB decreases corticosteroid production [26] and alters the balance of glucagon and insulin [27], hormones which are known to affect amino acid utilization and protein synthesis [28, 29]. To what extent CPIB acts through or in association with these or other hormones remains to be determined, but the fact remains that this drug can markedly alter serum protein synthesis.

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