Differential Effect of Clofibrate on Inflammation-Induced Alterations in Plasma Proteins in the Rat*

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Daily intramuscular injections of clofibrate begun 6 h before the initiation of inflammation induced by the subcutaneous injection of turpentine exerted a differential, dose-dependent inhibition of the anticipated acute-phase globulin response. Specifically, clofibrate at 140 mg/kg muted the increase in α_2 -macrofoetoprotein, but did not affect that of seromucoid or haptoglobin and only transiently inhibited the rise in copper and the rebound in transferrin. A higher dose, 280 mg/kg, markedly suppressed α_2 -macrofoetoprotein appearance and the rebound in transferrin, somewhat inhibited the increase in seromucoid and haptoglobin and only transiently affected the rise in plasma copper; 420 mg of clofibrate/kg very nearly abolished the appearance of α_2 -macrofoetoprotein, markedly suppressed the transferrin rebound and the increases in seromucoid and haptoglobin and again only transiently affected the increase in copper. Clofibrate did not diminish the localized inflammatory response, did not cause microscopically detectable liver damage and did not prevent the hypozincaemia, hypoalbuminaemia and enhanced amino acid uptake by liver usually associated with inflammation. Thus it is unlikely that clofibrate exerted its dose-dependent selective inhibition by muting the initial stimulus or by impairing hepatic metabolism. This seemingly selective action of clofibrate on plasma-protein alterations during inflammation may provide a means of elucidating the function of individual acute-phase globulin during disease. Clofibrate of itself, apart from inflammation, produced decreases in plasma zinc, copper, transferrin and seromucoid and an increase in hepatic amino acid uptake that were to some extent dependent on the dose of the drug.

Antihyperlipidaemic drugs appear to affect various aspects of metabolism in a manner antithetical to inflammation, injury or infection (Canonico *et al.*, 1977). For example, whereas inflammatory diseases usually result in increased plasma acute-phase protein concentration and synthesis (Neuhaus *et al.*, 1966; Sarcione, 1970; Powanda *et al.*, 1972, 1975), clofibrate [2-(4-chlorophenoxy)-2-methylpropanoic acid ethyl ester], which is used clinically to treat hyperlipidaemia (Berkowitz, 1971), markedly decreases the plasma concentration of seromucoid, caeruloplasmin and trypsin-inhibitory capacity in healthy rats (Powanda *et al.*, 1976, 1978). It has been proposed

that the alterations that occur in selected plasma proteins during inflammation producing injuries or diseases are of benefit to the host and are part of an integrated system of host defence and/or repair that is regulated by factors derived from phagocytic cells (Powanda, 1977). Clofibrate, with its seemingly opposite action on plasma acute-phase globulins, might offer a way of testing the hypothesis that alterations in these proteins are of value to the host during disease, especially if the drug were selective in its effect.

In order to assess the effect of clofibrate on selected plasma-protein alterations during inflammation, a sterile abscess was generated by the subcutaneous injection of turpentine. Turpentine was used rather than an infection since clofibrate had been demonstrated to be protective against *Streptococcus pneumoniae* in rats (Powanda & Canonico, 1976) and thus one might not be able to distinguish clofibrate's effect on the spread and severity of infection from its effects on host metabolism. The intramuscular route of drug administration was chosen rather than

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intubation or via the diet to obviate differences in intake or gastrointestinal absorption due to inflammation.

Plasma zinc concentration and amino acid uptake by liver were also measured in addition to light-microscopic analysis of the site of injection and light- and electron-microscopic analyses of the liver to ensure that clofibrate did not act by suppressing the localized inflammatory response or by damaging the liver, the major site of acute-phase globulin synthesis.

Materials and Methods

Clofibrate was obtained from Dr. Georg Brice (Ayerst Laboratories, New York, NY, U.S.A.). Male Fisher-Dunning rats weighing 175–224 g (Microbiological Associates, Walkersville, MD, U.S.A.) were housed in a room maintained at 22–24°C and lit from 06:00 h to 18:00 h. The rats were fed on commercial chow (Ralston Purina Co., St. Louis, MO, U.S.A.) *ad libitum* until the start of each experiment, at which time food was removed to obviate differences in nutrient intake due to inflammation. The turpentine used was commercial grade (Warner-Graham Co., Baltimore, MD, U.S.A.).

Plasma zinc and copper were determined by atomic-absorption spectrophotometry (Pekarek *et al.*, 1970). Rat plasma albumin and transferrin were quantified by a semi-automated nephelometric specific-antibody technique (Bostian *et al.*, 1976). The antisera were purchased from Cappel Laboratories, Cochranville, PA, U.S.A. Seromuroid was measured as an indicator of overall acute-phase-globulin response (Neuhaus *et al.*, 1966) in addition to the specific acute-phase proteins, haptoglobin (Owen *et al.*, 1960) and α_2 -macrofetoprotein (Weimer & Benjamin, 1965). Amino acid flux into liver was determined by measur-

ing the uptake of amino[^{14}C]isobutyric acid uptake (Wannemacher *et al.*, 1971). The plasma clofibric acid concentration was measured spectrophotometrically (Bruderlein *et al.*, 1975).

Tissue samples from the site of turpentine injection and of the liver were formalin-fixed, paraffin-embedded, stained with Haematoxylin and Eosin and examined to assess whether clofibrate affected lesion development and whether clofibrate and/or turpentine caused liver damage. Sections of liver were also prepared for electron-microscopic evaluation.

The protocol used for each study is shown in Table 1. Statistical significance of data was assessed by one-way analysis of variance, except for the data in Table 6, for which a *t* test was used.

Results

Turpentine-induced inflammation resulted in a significant decrease in plasma zinc concentration that persisted throughout the study. Clofibrate neither prevented nor exacerbated the inflammation-induced decrease. Clofibrate itself, however, also caused a significant decrease in plasma zinc, which appeared to be dose-dependent (Table 2).

Inflammation resulted in a 3–4-fold enhancement of amino acid uptake by liver within 18 h, which fell to about a 2-fold increase in hepatic amino acid uptake by 66 h. Clofibrate neither abated nor exacerbated the inflammation-induced hepatic uptake of amino acid, but did of itself produce an augmented uptake (Table 3).

The sterile abscess generated by turpentine resulted in a marked decrease in the plasma concentration of albumin. Clofibrate did not have a marked effect on albumin concentrations, either alone or in the presence of a turpentine abscess (Table 4).

Table 1. *Protocol*

The number of animals in each group is given in parentheses. The same protocol was used for each study; only the amount of clofibrate injected was changed. 0.9% NaCl was injected subcutaneously (s.c.) into group I, and, since the dilutions of clofibrate were made in mineral oil, groups I and III received mineral oil intramuscularly (i.m.).

	Group ...	I	II	III	IV
Treatment		Control	Clofibrate	Turpentine	Clofibrate + turpentine
Day 1					
09:00h, no. of animals given amino[^{14}C]isobutyrate (s.c.) ...		(24)	(24)	(24)	(24)
Day 2					
09:00h, clofibrate (i.m.);		—	+	—	+
15:00h, turpentine (s.c.)		—	—	+	+
(all animals deprived of food)					
Days 3–5					
09:00h, no. of animals killed ...		(8)	(8)	(8)	(8)
Inject remaining animals with clofibrate		—	+	—	+

Table 2. *Effect of clofibrate on plasma zinc concentration*

Animals were treated and killed according to the protocol given in Table 1. Plasma zinc concentrations were determined by atomic-absorption spectrophotometry. The mean values $\pm$ S.E.M. for the plasma zinc concentrations for eight animals/group are shown. * $P < 0.01$, ** $P < 0.001$ (versus control).

Time (h) after turpentine injection	Dose of clofibrate (mg/kg) Group	[Plasma zinc] ($\mu\text{g/dl}$)		
		140	280	420
18	Control	137 $\pm$ 4	121 $\pm$ 5	144 $\pm$ 3
	Clofibrate	143 $\pm$ 5	129 $\pm$ 4	110 $\pm$ 6**
	Turpentine	89 $\pm$ 12**	55 $\pm$ 5**	61 $\pm$ 3**
	Clofibrate + turpentine	90 $\pm$ 4**	62 $\pm$ 3**	54 $\pm$ 3**
42	Control	147 $\pm$ 4	123 $\pm$ 5	124 $\pm$ 6
	Clofibrate	129 $\pm$ 2**	96 $\pm$ 3**	78 $\pm$ 7**
	Turpentine	64 $\pm$ 2**	69 $\pm$ 4**	50 $\pm$ 2**
	Clofibrate + turpentine	57 $\pm$ 2**	74 $\pm$ 5**	53 $\pm$ 3**
66	Control	152 $\pm$ 4	112 $\pm$ 3	105 $\pm$ 3
	Clofibrate	130 $\pm$ 3**	88 $\pm$ 6*	62 $\pm$ 5**
	Turpentine	85 $\pm$ 4**	68 $\pm$ 5**	58 $\pm$ 3**
	Clofibrate + turpentine	71 $\pm$ 3**	72 $\pm$ 5**	44 $\pm$ 5**

Table 3. *Effect of clofibrate on hepatic amino acid uptake*

Animals were treated and killed according to the protocol (Table 1). 24 h before the first injection of clofibrate, rats were injected subcutaneously with 1 μCi of amino[^{14}C]isobutyrate/100 g body weight. After killing and exsanguination, livers were removed, perfused with chilled 0.9% NaCl and 1 g samples taken. The liver sample was homogenized in 2 vol. of 0.9% NaCl containing 150 mg of sulphosalicylic acid/ml and then centrifuged. Duplicate 0.1 ml portions were counted for radioactivity. The mean values $\pm$ S.E.M. for eight animals/group are shown, except for the 42 h samples given 280 mg of drug/kg. * $P < 0.01$, ** $P < 0.001$ (versus control).

Time (h) after turpentine injection	Dose of clofibrate (mg/kg) Group	^{14}C radioactivity (d.p.m./mg of liver)		
		140	280	420
18	Control	21.0 $\pm$ 3.0	24.5 $\pm$ 3.3	36.4 $\pm$ 5.6
	Clofibrate	34.4 $\pm$ 3.8*	51.7 $\pm$ 4.9**	60.6 $\pm$ 6.8*
	Turpentine	72.6 $\pm$ 10.6**	95.7 $\pm$ 10.6**	106.0 $\pm$ 21.4**
	Clofibrate + turpentine	96.0 $\pm$ 12.2**	105.5 $\pm$ 11.8**	157.2 $\pm$ 21.2**
42	Control	13.5 $\pm$ 1.0	—	44.1 $\pm$ 5.3
	Clofibrate	27.0 $\pm$ 2.9	—	83.3 $\pm$ 12.3*
	Turpentine	52.8 $\pm$ 2.9**	—	99.5 $\pm$ 21.0*
	Clofibrate + turpentine	74.9 $\pm$ 9.9*	—	97.1 $\pm$ 17.1*
66	Control	14.3 $\pm$ 1.5	30.8 $\pm$ 8.9	36.3 $\pm$ 4.7
	Clofibrate	30.5 $\pm$ 3.2**	92.6 $\pm$ 9.3**	78.2 $\pm$ 11.2*
	Turpentine	32.3 $\pm$ 3.7**	72.7 $\pm$ 12.0**	77.8 $\pm$ 12.8*
	Clofibrate + turpentine	47.1 $\pm$ 7.6**	79.4 $\pm$ 33.9**	106.8 $\pm$ 2.9*

Inflammation caused a biphasic response in plasma transferrin concentration. There was an initial decrease at 18h, followed by a return to control values at 42h and an increase over control values at 66h. The highest dose of clofibrate totally prevented the return to, and overshoot of, control values in turpentine-injected animals, whereas the lower doses

of the drug suppressed, but did not entirely prevent, the biphasic response of transferrin. All concentrations of clofibrate by themselves caused a decrease in transferrin (Table 5).

Figs. 1, 2 and 3 summarize the data regarding the effect of clofibrate on inflammation-induced increases in plasma copper, seromuroid, haptoglobin

Table 4. *Effect of clofibrate on plasma albumin concentration*

Animals were treated and killed according to the protocol (Table 1). Plasma albumin concentration was quantified by nephelometric determination of extent of reaction with antisera to rat albumin. The mean values $\pm$ S.E.M. for eight animals/group are shown. * $P < 0.001$ versus control; ** $P < 0.001$ versus turpentine-injected.

Time (h) after turpentine injection	Dose of clofibrate (mg/kg) ... Group	[Albumin] (g/dl)		
		140	280	420
18	Control	2.78 $\pm$ 0.05	2.50 $\pm$ 0.12	2.50 $\pm$ 0.7
	Clofibrate	2.89 $\pm$ 0.08	2.60 $\pm$ 0.04	2.54 $\pm$ 0.14
	Turpentine	1.83 $\pm$ 0.08*	1.93 $\pm$ 0.05*	1.73 $\pm$ 0.06*
	Clofibrate + turpentine	1.96 $\pm$ 0.12*	1.85 $\pm$ 0.05*	1.60 $\pm$ 0.06*
42	Control	2.61 $\pm$ 0.08	2.83 $\pm$ 0.13	2.40 $\pm$ 0.08
	Clofibrate	2.78 $\pm$ 0.05	2.15 $\pm$ 0.07*	2.34 $\pm$ 0.03
	Turpentine	1.20 $\pm$ 0.05*	1.53 $\pm$ 0.07*	1.21 $\pm$ 0.06*
	Clofibrate + turpentine	1.42 $\pm$ 0.09*	2.01 $\pm$ 0.04*,**	1.40 $\pm$ 0.05*
66	Control	2.86 $\pm$ 0.06	2.80 $\pm$ 0.11	2.16 $\pm$ 0.07
	Clofibrate	3.02 $\pm$ 0.09	2.66 $\pm$ 0.10	1.90 $\pm$ 0.11
	Turpentine	1.14 $\pm$ 0.04*	1.62 $\pm$ 0.05*	1.27 $\pm$ 0.03*
	Clofibrate + turpentine	1.53 $\pm$ 0.09*	1.60 $\pm$ 0.03*	1.54 $\pm$ 0.04*,**

Table 5. *Effect of clofibrate on plasma transferrin concentration*

Animals were treated and killed according to the protocol (Table 1). Plasma transferrin concentration was quantified by nephelometric measurement of extent of reaction with antisera to rat transferrin. The mean values $\pm$ S.E.M. for eight animals/group are shown. * $P < 0.01$, ** $P < 0.001$ versus control; † $P < 0.01$, ‡ $P < 0.001$ versus turpentine-injected.

Time (h) after turpentine injection	Dose of clofibrate (mg/kg) ... Group	[Transferrin] (mg/dl)		
		140	280	420
18	Control	513 $\pm$ 18	475 $\pm$ 22	550 $\pm$ 11
	Clofibrate	555 $\pm$ 22	481 $\pm$ 22	475 $\pm$ 27
	Turpentine	392 $\pm$ 13**	357 $\pm$ 11**	326 $\pm$ 9**
	Clofibrate + turpentine	370 $\pm$ 36**	319 $\pm$ 10**	288 $\pm$ 15**
42	Control	492 $\pm$ 18	444 $\pm$ 11	520 $\pm$ 6
	Clofibrate	410 $\pm$ 9**	299 $\pm$ 9**	388 $\pm$ 9**
	Turpentine	500 $\pm$ 22	442 $\pm$ 9	473 $\pm$ 14
	Clofibrate + turpentine	317 $\pm$ 11**,†	311 $\pm$ 5**,‡	334 $\pm$ 18**,‡
66	Control	465 $\pm$ 20	356 $\pm$ 9	445 $\pm$ 10
	Clofibrate	282 $\pm$ 9**	214 $\pm$ 10**	283 $\pm$ 24**
	Turpentine	468 $\pm$ 44	464 $\pm$ 8**	520 $\pm$ 10**
	Clofibrate + turpentine	247 $\pm$ 15*	266 $\pm$ 16**,‡	298 $\pm$ 13**,‡

and α_2 -macrofoetoprotein. In general, low concentrations of clofibrate (140mg/kg) had little or no effect on seromucoid and haptoglobin concentrations and had only a transient effect on copper in inflamed rats. However, a marked inhibition in the appearance of α_2 -macrofoetoprotein occurred at this dose. α_2 -Macrofoetoprotein concentrations were also suppressed at higher doses of clofibrate (280 and 420mg/kg). In addition, these concentrations of clofibrate also caused a dose-dependent inhibition of

inflammation-induced changes in seromucoid and haptoglobin. Clofibrate of itself induced decreases in plasma copper and seromucoid concentration.

Although both groups receiving clofibrate received the same dose on a weight basis, those animals that were injected with turpentine generally had significantly lower plasma concentrations of clofibrate at time of death (Table 6).

Necropsy of a separate group of animals that had been given turpentine, clofibrate or clofibrate and

Fig. 1. Effects of clofibrate (140 mg/kg) on (a) plasma copper, (b) α_2 -macrofoetoprotein, (c) seromuroid and (d) haptoglobin. Group I (□), untreated (0.9% NaCl) control; group II (▨), clofibrate; group III (■), turpentine control; group IV (■), clofibrate + turpentine. Significance is expressed in reference to appropriate control, i.e. group I versus group II; group III versus group IV. The turpentine-injected rats (group III) are significantly different ($P < 0.01$) from untreated controls (group I) at 18, 42 and 66 h with regard to α_2 -macrofoetoprotein, seromuroid and haptoglobin, and at 42 and 66 h with respect to copper. * $P < 0.01$, ** $P < 0.001$ versus controls.

turpentine indicated that all rats receiving turpentine subcutaneously had comparable acute diffuse necrotizing dermatitis whether or not the animals had also been injected with clofibrate intramuscularly. Neither clofibrate nor turpentine could be seen to cause liver lesions of any note on light- or electron-microscopic analysis.

Discussion

Clofibrate appears to selectively, in a dose-dependent manner, attenuate the acute-phase globulin responses to inflammation in rats. Light-microscopic assessment of the site of turpentine injection and of the liver gave no evidence that clofibrate acted either by diminishing the localized inflammatory response or by damaging the liver, the most prominent site of acute-phase globulin synthesis. A decrease in plasma zinc, with an increase in hepatic zinc content, and enhanced amino acid flux into liver, are symptomatic of inflammation (Powanda *et al.*, 1973). This zinc and amino acid redistribution appears to vary as

a function of the severity of the inflammation (Powanda *et al.*, 1975; Berendt *et al.*, 1977). Hypoalbuminaemia is also a concomitant of inflammation (Neuhaus *et al.*, 1966). The decreases in plasma zinc and albumin concentration and the increase in hepatic amino acid uptake were comparable, in fact nearly identical, in the turpentine-injected and clofibrate-treated turpentine-injected animals. These data are consistent with the conclusion from light-microscopic analysis that the severity of the localized inflammation was not muted by clofibrate.

The lower plasma concentrations of clofibric acid found in the inflamed rats compared with those given clofibrate alone may be related to the hypoalbuminaemia that occurs during inflammation. Decreased plasma albumin concentration may lead to a more rapid metabolism/elimination of this drug (Koch-Weser & Sellers, 1976), since over 90% of the plasma content of clofibric acid is bound to albumin (Tillement *et al.*, 1974).

Zinc and amino acid redistribution as well as altered plasma protein concentration/synthesis during

Fig. 2. Effects of clofibrate (280mg/kg) on (a) plasma copper, (b) α_2 -macrofoetoprotein, (c) seromucoid and (d) haptoglobin. Group I (□), untreated (0.9% NaCl) control; group II (▨), clofibrate; group III (■), turpentine control; group IV (■), clofibrate+turpentine. Significance is expressed in reference to appropriate control, i.e. group I versus group II; group III versus group IV. Group III is significantly different ($P < 0.01$) from group I at 18, 42 and 66h with regard to α_2 -macrofoetoprotein, seromucoid and haptoglobin, and at 42 and 66h with respect to copper. * $P < 0.01$, ** $P < 0.001$ versus controls.

inflammation, infection and trauma appear to be regulated by a substance(s) released from stimulated phagocytes (Powanda, 1977; Kampschmidt, 1978). It has been proposed that there is but a single mediator of all of the above metabolic sequelae of inflammation (Merriman *et al.*, 1977; Kampschmidt, 1978). If there is but a single mediator, it is unlikely that clofibrate inhibited mediator release. For, if mediator release were inhibited, it would be difficult to see how essentially comparable effects could be produced in turpentine-injected and clofibrate-treated turpentine-injected animals with regard to decreases in plasma zinc and albumin, and hepatic amino acid uptake, yet differential effects with regard to acute-phase globulins.

Increased synthesis and plasma concentration of acute-phase proteins occur during various forms of inflammation (Weimer & Benjamin, 1965; Neuhaus *et al.*, 1966; Sarcione, 1970; Powanda, 1977). The increase in plasma acute-phase globulin concentration is for the most part the result of synthesis *de novo* (Chandler & Neuhaus, 1964; Maung *et al.*, 1964; Neuhaus *et al.*, 1966; Zeineh *et al.*, 1972) occurring primarily in the liver (Neuhaus *et al.*, 1962; Neuhaus

& Liu, 1964; John & Miller, 1969; Sarcione, 1970). In the absence of microscopically detectable liver damage, one might presume that overall liver function is not impaired.

The selective dose-dependent inhibition of increases in various plasma acute-phase globulins during inflammation by clofibrate contrasts with the general undifferentiated inhibition produced by colchicine, which appears to act by restricting egress of already formed proteins from the liver through colchicine's effect on microtubules (Redman *et al.*, 1975; Banerjee *et al.*, 1976; Pipeleers *et al.*, 1977). Regulation of protein synthesis may be accomplished in various ways: (a) through preferential replication of a gene; (b) at the point of transcription of a gene into RNA; (c) during processing of the RNA; (d) in the course of transport of RNA from nucleus to cytoplasm; (e) by attachment of mRNA to ribosomes and initiation of protein synthesis; (f) as a function of stability of a particular mRNA; (g) during processing of protein into final form, e.g. through proteolytic cleavage or the addition of sugars to a glycoprotein.

Clofibrate may act at one or more of these putative

Fig. 3. Effects of clofibrate (420mg/kg) on (a) plasma copper, (b) α_2 -macrofoetoprotein, (c) seromuroid and (d) haptoglobin. Group I (□), untreated (0.9% NaCl) control; group II (▨), clofibrate; group III (■), turpentine control; group IV (■) = clofibrate+turpentine. Significance is expressed in reference to appropriate control, i.e. group I versus group II; group III versus group IV. Group III is significantly different ($P<0.01$) from group I at 18, 42 and 66h with regard to α_2 -macrofoetoprotein, seromuroid and haptoglobin, and at 42h with respect to copper. * $P<0.01$, ** $P<0.001$ versus controls.

Table 6. Plasma clofibrate content at time of necropsy

Animals were treated and killed according to the protocol (Table 1). Clofibrate was determined spectrophotometrically. The mean values $\pm$ S.E.M. for clofibrate for six to eight animals/group are shown. * $P<0.01$, ** $P<0.001$ versus clofibrate-treated alone.

Time (h) after turpentine injection	Dose of clofibrate (mg/kg) Group	[Plasma clofibrate] (g/dl)		
		140	280	420
18	Control	272 $\pm$ 36	354 $\pm$ 76	380 $\pm$ 40
	Clofibrate+turpentine	120 $\pm$ 18*	234 $\pm$ 36	259 $\pm$ 63
42	Control	212 $\pm$ 32	735 $\pm$ 105	1049 $\pm$ 87
	Clofibrate+turpentine	76 $\pm$ 5**	241 $\pm$ 56**	423 $\pm$ 83**
66	Control	312 $\pm$ 51	993 $\pm$ 106	1183 $\pm$ 122
	Clofibrate+turpentine	141 $\pm$ 76*	205 $\pm$ 48**	241 $\pm$ 83**

control points. Since α_2 -macrofoetoprotein appearance in plasma during inflammation appears to require the expression of a foetal gene (Sarcione, 1970), its diminished response may involve suppression or repression of that gene or the signal which

regulates that gene. Clofibrate is capable of increasing the amount of free ribosomal RNA and decreasing the amount of bound ribosomal RNA in liver (Powanda *et al.*, 1976). If free ribosomes are primarily responsible for the synthesis of intrahepatic proteins,

and bound ribosomes for the synthesis of proteins to export (Redman, 1969; Hicks *et al.*, 1969), then such a change in the ratio of bound to free ribosomes may diminish the synthesis of secreted proteins. The fact that clofibrate does not appear to affect albumin concentration/synthesis may be related to the apparent difference in intracellular distribution between newly synthesized albumin and the glycoproteins (Redman & Cherian, 1972). The fact that clofibrate itself does not diminish the plasma-albumin concentration suggests that the release of newly formed proteins is not blocked.

Clofibrate can selectively decrease certain hepatic glycolytic enzymes (Zakim *et al.*, 1970), and, either as a result, or at least concomitantly, decreases the plasma glucose concentration (Powanda *et al.*, 1976). This alone might affect the synthesis of many acute-phase globulins, particularly those of the seromucoid fraction, since these contain large amounts of various sugars. Conceivably clofibrate could also inhibit one or more of the glycosyltransferases essential to glycoprotein synthesis (Wagner & Cynkin, 1971; Redman & Cherian, 1972).

Alternatively clofibrate might affect hepatic protein synthesis indirectly by its effect on amino acid availability, hormones or adenylate cyclase (Ruegamer *et al.*, 1969; Greene *et al.*, 1970; Fulton & Hsia, 1972; Fedele *et al.*, 1976). If amino acid availability does exert some regulatory effect on hepatic synthesis of plasma proteins (Rothschild *et al.*, 1969; Morgan & Peters, 1971), the fact that clofibrate enhances amino acid flux into liver should stimulate rather than depress plasma protein production. Clofibrate exerts some effect on the metabolism of steroid-hormone precursors (Fulton & Hsia, 1972) and in certain instances insulin (Fedele *et al.*, 1976). Both cortisol and insulin are thought to influence acute-phase-plasma-protein synthesis (John & Miller, 1969). Were clofibrate to significantly decrease cortisol production, then the pronounced attenuation of the α_2 -macrofoetoprotein response relative to that of the other acute-phase proteins may be explicable in terms of a differential requirement for corticosteroids in the synthesis of these proteins. It is known that adrenalectomy abolishes the α_2 -macrofoetoprotein response to leucocyte endogenous mediator without blocking the increase in haptoglobin (Thompson *et al.*, 1976).

Whatever mechanism of action, the fact that clofibrate appears to attenuate selectively, in a dose-dependent fashion, plasma acute-phase globulin concentration/production during inflammation may allow elucidation of the role of certain acute-phase globulins during inflammation/infection/trauma. For example, selective suppression of α_2 -macrofoetoprotein by low doses of clofibrate should make it possible to test whether this protein affects the

development of immunity during infection or on vaccination or regulates immunity so that antibodies against self are not formed in response to damaged tissue.

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References

- Banerjee, D., Manning, C. P. & Redman, C. M. (1976) *J. Biol. Chem.* **251**, 3887-3892
- Berendt, R. F., Long, G. G., Abeles, F. B., Canonico, P. G., Elwell, M. R. & Powanda, M. C. (1977) *Infect. Immun.* **15**, 586-593
- Berkowitz, Z. D. (1971) *J. Am. Med. Assoc.* **218**, 1002-1005
- Bostian, K. A., Blackburn, B. S., Wannemacher, R. W., Jr., McGann, V. G., Beisel, W. R. & Dupont, H. L. (1976) *J. Lab. Clin. Med.* **87**, 577-585
- Bruderlein, H., Robinson, W. T., Kraml, L. M. & Dvornik, D. (1975) *Clin. Biochem.* **8**, 261-272
- Canonico, P. G., Rill, W. & Ayala, E. (1977) *Lab. Invest.* **37**, 479-486
- Chandler, A. M. & Neuhaus, O. W. (1964) *Am. J. Physiol.* **206**, 169-173
- Fedele, D., Tiengo, A., Muggeo, M., Nosadini, R. & Crepaldi, G. (1976) *Atherosclerosis* **25**, 191-198
- Fulton, J. E., Jr. & Hsia, S. L. (1972) *J. Lipid Res.* **13**, 78-85
- Greene, H. L., Herman, R. H. & Zakim, D. (1970) *Proc. Soc. Exp. Biol. Med.* **134**, 1035-1038
- Hicks, S. J., Drysdale, J. W. & Munro, H. N. (1969) *Science* **164**, 584-585
- John, D. W. & Miller, L. L. (1969) *J. Biol. Chem.* **244**, 6134-6142
- Kampschmidt, R. F. (1978) *J. Reticuloendothel. Soc.* **23**, 287-297
- Koch-Weser, J. & Sellers, E. M. (1976) *N. Engl. J. Med.* **294**, 311-316
- Maung, M., Baker, D. G. & Murray, R. K. (1964) *Life Sci.* **3**, 1349-1354
- Merriman, C. R., Pulliam, L. A. & Kampschmidt, R. F. (1977) *Proc. Soc. Exp. Biol. Med.* **154**, 224-227
- Morgan, E. H. & Peters, T., Jr. (1971) *J. Biol. Chem.* **246**, 3500-3507
- Neuhaus, O. W. & Liu, A. (1964) *Proc. Soc. Exp. Biol. Med.* **117**, 244-248
- Neuhaus, O. W., Balegno, H. F. & Milauskas, A. T. (1962) *Am. J. Physiol.* **202**, 257-260

- Neuhaus, O. W., Balegno, H. F. & Chandler, A. M. (1966) *Am. J. Physiol.* **211**, 151–156
- Owen, J. A., Better, F. C. & Hoban, J. (1960) *J. Clin. Pathol.* **13**, 163–164
- Pekarek, R. S., Burghen, G. A., Bartelloni, P. J., Calia, F. M., Bostian, K. A. & Beisel, W. R. (1970) *J. Lab. Clin. Med.* **76**, 293–303
- Pipeleers, D. G., Pipeleers-Marichal, M. A., Sherline, P. & Kipnis, D. M. (1977) *J. Cell Biol.* **74**, 341–350
- Powanda, M. C. (1977) *Am. J. Clin. Nutr.* **30**, 1254–1268
- Powanda, M. C. & Canonico, P. G. (1976) *Proc. Soc. Exp. Biol. Med.* **152**, 437–440
- Powanda, M. C., Wannemacher, R. W., Jr. & Cockerell, G. L. (1972) *Infect. Immun.* **6**, 266–271
- Powanda, M. C., Cockerell, G. L. & Pekarek, R. S. (1973) *Am. J. Physiol.* **225**, 399–401
- Powanda, M. C., Cockerell, G. L., Moe, J. B., Abeles, F. B., Pekarek, R. S. & Canonico, P. G. (1975) *Am. J. Physiol.* **229**, 479–483
- Powanda, M. C., Henriksen, E. L., Ayala, E. & Canonico, P. G. (1976) *Biochem. Pharmacol.* **25**, 785–788
- Powanda, M. C., Blackburn, B. S., Bostian, K. A., Fowler, J. P., Hauer, E. C. & Pekarek, R. S. (1978) *Biochem. Pharmacol.* **27**, 125–127
- Redman, C. M. (1969) *J. Biol. Chem.* **244**, 4308–4315
- Redman, C. M. & Cherian, M. G. (1972) *J. Cell Biol.* **52**, 231–245
- Redman, C. M., Banerjee, D., Howell, K. & Palade, G. E. (1975) *J. Cell Biol.* **66**, 42–59
- Rothschild, M. A., Oratz, M., Mongelli, J. & Schreiber, S. S. (1969) *Am. J. Physiol.* **216**, 1127–1130
- Ruegamer, W. R., Ryan, N. T., Richert, D. A. & Westergeld, W. W. (1969) *Biochem. Pharmacol.* **18**, 613–624
- Sarcione, E. J. (1970) *Biochemistry* **9**, 3059–3062
- Thompson, W. L., Abeles, F. B., Beall, F. A., Dinterman, R. E. & Wannemacher, R. W., Jr. (1976) *Biochem. J.* **156**, 25–32
- Tillement, J.-P., Zini, R., d'Athis, P. & Vassent, G. (1974) *Eur. J. Clin. Pharmacol.* **7**, 307–313
- Wagner, R. R. & Cynkin, M. A. (1971) *J. Biol. Chem.* **246**, 143–151
- Wannemacher, R. W., Jr., Powanda, M. C., Pekarek, R. S. & Beisel, W. R. (1971) *Infect. Immun.* **4**, 556–562
- Weimer, H. E. & Benjamin, D. C. (1965) *Am. J. Physiol.* **209**, 736–744
- Zakim, D., Paradini, R. S. & Herman, R. H. (1970) *Biochem. Pharmacol.* **19**, 305–310
- Zeineh, R. A., Barrett, B., Niemirowski, L. & Fiorella, B. J. (1972) *Am. J. Physiol.* **222**, 1326–1332