May & Baker Ltd., Canada. This work was supported by a grant from the Federal Health and Welfare Department.

Division of Neurological Sciences, A. JAKUBOVIC
Department of Psychiatry, E. G. MCGEER
University of British Columbia, P. L. MCGEER
Vancouver, B.C. V6T 1W5,
Canada

REFERENCES

1. C. C. Scott, E. G. Robbins and K. K. Chen, *J. Pharmac. exp. Ther.* **93**, 282 (1948).
2. S. E. Smits and M. B. Myers, *Res. Commun. Chem. Path. Pharmac.* **7**, 561 (1974).
3. H. Isbell and A. J. Eisenman, *Fedn Proc.* **7**, 162 (1948).
4. H. R. Sullivan, S. E. Smits, S. L. Due, R. E. Booher and R. E. McMahon, *Life Sci.* **11**, 1093 (1972).
5. H. R. Sullivan, S. L. Due and R. E. McMahon, *J. Pharm. Pharmac.* **27**, 728 (1975).
6. S. Irwin, P. H. Blachly, J. Marks, E. Carlson, J. Loewen and N. Reade, Problems on Drug Dependence, Committee on Problems on Drug Dependence (Natn. Acad. Sci., Washington, 1973), pp. 579–94.
7. W. Ling, V. C. Charuvastra, S. C. Kaim and C. J. Klett, *Archs gen. Psychiat.* **33**, 709 (1976).
8. P. Cushman Jr., *N.Y. St. J. Med.* **72**, 1261 (1972).
9. T. J. Cicero, R. D. Bell, W. G. Wiest, J. H. Allison, K. Polakoski and E. Robins, *N. Engl. J. Med.* **292**, 882 (1975).
10. F. Azazi, A. G. Vagenakis, C. Longcope, S. H. Ingbar and L. E. Braverman, *Steroids* **22**, 467 (1973).
11. J. J. Mendelson and N. K. Mello, *Clin. Pharmac. Ther.* **17**, 529 (1975).
12. J. H. Mendelson, C. E. Inturrisi, P. Renault and E. C. Senay, *Clin. Pharmac. Ther.* **19**, 371 (1976).
13. J. H. Mendelson, R. E. Myer, J. Ellingboe, S. M. Mirin and M. McDougle, *J. Pharmac. exp. Ther.* **195**, 296 (1976).
14. T. J. Cicero, C. E. Wilcox, R. D. Bell and E. R. Meyer, *J. Pharmac. exp. Ther.* **198**, 340 (1976).
15. J. A. Thomas and J. T. Dombrosky, *Archs int. Pharmacodyn. Thér.* **215**, 215 (1975).
16. D. J. Smith and J. M. Joffe, *Nature, Lond.* **253**, 202 (1975).
17. J. M. Joffe, J. M. Peterson, D. J. Smith and L. F. Soyka, *Res. Commun. Chem. Path. Pharmac.* **13**, 611 (1976).
18. R. N. Morris and J. R. Davis, *Archs int. Pharmacodyn. Thér.* **162**, 432 (1966).
19. A. R. Means and P. F. Hall, *Endocrinology* **82**, 587 (1968).
20. P. J. Kirby, G. A. Langford and J. R. Davis, *Fedn Proc.* **28**, 774 (1969).
21. J. L. Dufau, J. K. Catt and T. Tsuruhara, *Endocrinology* **90**, 1032 (1972).
22. J. L. Dufau, K. Watanabe and J. K. Catt, *Endocrinology* **92**, 6 (1973).
23. M. A. Hollinger, *Biochem. Pharmac.* **19**, 2701 (1970).
24. M. J. Free, in *The Testis* (Eds. A. D. Johson, W. R. Gomes and N. L. Vandemark), pp. 125–92. Academic Press, New York (1970).
25. J. R. Davis and G. A. Langford, in *The Testis* (Eds. A. D. Johson, W. R. Gomes and N. L. Vandemark), pp. 259–306. Academic Press, New York (1970).
26. I. P. Lee and R. L. Dixon, *Toxic. appl. Pharmac.* **23**, 20 (1972).
27. A. Jakubovic and P. L. McGeer, in *Marihuana: Chemistry, Biochemistry, and Cellular Effects* (Ed. G. G. Nahas), pp. 223–41. Springer-Verlag, New York (1976).
28. V. Monesi, *Expl. Cell Res.* **39**, 197 (1965).
29. C. Mendelson, M. Dufau and K. Catt, *Biochim. biophys. Acta* **411**, 222 (1975).
30. A. E. Robinson and F. M. Williams, *J. Pharm. Pharmac.* **23**, 353 (1971).
31. A. Jakubovic and P. L. McGeer, Abstracts 7th Annual Meeting American Society for Neurochemistry, Vancouver B.C. (1976), p. 149.
32. B. A. Judson, W. H. Horns and A. Goldstein, *Clin. Pharmac. Ther.* **20**, 445 (1976).
33. D. Buchenauer, M. Turnbow and M. A. Peters, *J. Pharmac. exp. Ther.* **189**, 66 (1974).
34. N. R. Scott and J. J. Ryan, 5th Natn. Conf. on Methadone Treatment, Washington, D.C. (1973), pp. 17–19.
35. M. M. Davis, B. S. Brown and J. T. Glendinning, 5th Natn. Conf. on Methadone Treatment, Washington, D.C. (1973), pp. 1153–64.
36. C. Zelson, *New Engl. J. Med.* **288**, 1393 (1973).
37. C. Zelson, S. J. Lee and M. Casalino, *New Engl. J. Med.* **289**, 1261 (1973).

Biochemical Pharmacology, Vol. 27, pp. 125–127. Pergamon Press, 1978. Printed in Great Britain.

Clofibrate-induced alterations in zinc, iron and copper metabolism*

(*Received* 4 *February* 1977; *accepted* 19 *April* 1977)

Clofibrate (*p*-chlorophenoxyisobutyrate) is generally considered an antihyperlipidemic agent; however, it has also been demonstrated recently to alter serum protein patterns [1]. Since a number of serum proteins bind or transport trace metals, we studied the effects of various levels of dietary clofibrate on plasma zinc, iron and copper and some of the proteins associated with these elements. We also measured the liver and muscle content of zinc, iron and copper to assess if clofibrate altered their concentration.

METHODS

Clofibrate was obtained from Dr. George Brice (Ayerst Laboratories, New York, U.S.A.). Male, Fisher–Dunning rats weighing 175–255 g (Microbiological Associates, Walkersville, MD, U.S.A.) were housed in a room maintained at 22–24° and lighted from 6:00 a.m. to 6:00 p.m. The rats

* In conducting the research described in this report, the investigators adhered to the *Guide for the Care and Use of Laboratory Animals*, as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

were fed for 1 week ground commercial rat food (Ralston Purina) to which varying amounts of clofibrate (0.05, 0.25 and 1.25%) dissolved in 95% ethanol were added. Control rats received ground chow to which ethanol was also added. The food was air-dried before being fed to the rats.

Plasma and tissue trace metal concentrations were determined by atomic absorption spectrophotometry [2]. Serum iron and total iron-binding capacity were also measured by a semiautomated colorimetric procedure [3]. Tissue samples were prepared for trace metal determinations by adding 1 ml of 25% aqueous tetramethylammonium hydroxide [4], then heating at 65° for 30 min with frequent mixing. One ml of double distilled water was then added. Further dilutions were made if necessary. Fresh plasma was assayed for ceruloplasmin by measuring serum oxidase activity [5]. Plasma albumin, α_2-macroglobulin and transferrin were quantitated by a semiautomated nephelometric specific antibody technique [6].

RESULTS

A significant decrease in plasma zinc was detectable after the rats were fed the 1.25% clofibrate diet for 1 week. Significant decrements in plasma copper were observed at the two highest dietary levels of clofibrate. In contrast to plasma zinc and copper, no alteration in plasma iron concentration was found at any level of the drug used. Clofibrate (1.25%) also produced a significant decrease in hepatic zinc and a marked increase in hepatic iron. Otherwise there were no changes of any importance in the liver or muscle concentration of zinc, iron or copper (Table 1).

Plasma oxidase activity, indicative of ceruloplasmin concentration, was significantly decreased at the two highest levels of the drug to about the same extent that plasma copper was decreased. Plasma transferrin was decreased at all concentrations of clofibrate and markedly so at the highest level of the drug, while albumin was slightly increased at 1.25% clofibrate. α_2-Macroglobulin content, which had to be expressed as per cent of control in the absence of a standard, showed a slight reduction at 0.25% clofibrate but none at 1.25% (Table 2).

Serum iron concentration, measured colorimetrically, was lower than that detected in plasma by atomic absorption but neither method revealed a significant alteration at any level of the drug used (Table 3). In contrast, total iron binding capacity decreased at 0.25 and 1.25%, while the per cent saturation, as expected, increased. Sera were also assayed for transferrin and again the concentration of this protein was significantly decreased at the two highest levels of the drug.

DISCUSSION

The present study demonstrates that clofibrate can selectively decrease the plasma concentration of two trace metal carrier proteins, transferrin and ceruloplasmin, while not seeming to affect the concentration of a third, α_2-macroglobulin. Reflecting the decrease in ceruloplasmin, the plasma copper concentration decreases, as might be expected, since 90–95 per cent of the plasma copper has been found to exist tightly bound to ceruloplasmin [7, 8]. In contrast, despite a 65 per cent decrease in the iron-binding protein, transferrin [9], there is no significant decrease in the plasma concentration of iron. Instead the degree of saturation of the transferrin with iron increases and the hepatic concentration of iron also increases. The latter finding may reflect the decrease in iron transport capacity, but it is also possible that the reduction in ceruloplasmin may contribute to this accumulation of iron within the liver, since ceruloplasmin appears to act as a ferro-oxidase facilitating the transfer of stored iron to transferrin [10].

Table 1. The effect of clofibrate on tissue trace metal concentrations*

Dietary concn of clofibrate (%)	Trace metal concn (μg/100 ml or μg/g)								
	Plasma			Liver			Muscle		
	Zinc	Iron	Copper	Zinc	Iron	Copper	Zinc	Iron	Copper
0 (Control)	165 ±3	237 ±10	121 ±3	14.5 ±0.4	39.7 ±1.8	1.59 ±0.05	7.26 ±0.31	7.22 ±0.39	0.55 ±0.02
0.05	160 ±3	206 ±7	117 ±2	16.3 ±0.8	42.8 ±2.2	1.76 ±0.08	7.43 ±0.14	6.82 ±0.20	0.53 ±0.01
0.25	166 ±3	202 ±6	104 ±3†	16.4 ±0.4	45.7 ±1.7	1.93 ±0.09	7.87 ±0.50	7.07 ±0.29	0.45 ±0.02
1.25	127 ±4†	216 ±12	83 ±2†	12.7 ±0.4†	61.1 ±3.8†	1.79 ±0.06	8.31 ±0.92	7.98 ±0.52	0.51 ±0.03

* Concentration is expressed as mean ± S. E. (N = 8).
† $P < 0.01$ vs control.

Table 2. Effect of clofibrate on plasma protein concentrations*

Dietary concn of clofibrate (%)	Ceruloplasmin† (mg/100 ml)	Albumin‡ (g/100 ml)	Transferrin‡ (mg/100 ml)	α_2-Macroglobulin‡ (% of control)
0 (Control)	51.3 ± 1.4	2.62 ± 0.03	600 ± 10	100
0.05	47.6 ± 0.8	2.46 ± 0.07	518 ± 19§	97
0.25	42.7 ± 0.3§	2.80 ± 0.06	336 ± 13§	86
1.25	35.3 ± 1.1§	3.07 ± 0.09§	195 ± 4§	98

* Concentration is expressed as mean ± S. E.
† N = 8.
‡ N = 10.
§ $P < 0.01$ vs control.

Table 3. Effect of clofibrate on serum iron concentration and iron-binding capacity*

Dietary concn of clofibrate (%)	Iron (μg/100 ml)	Total iron binding capacity (μg/100 ml)	Saturation (%)	Transferrin (mg/100 ml)
0 (Control)	152 ± 9	615 ± 19	24.7 ± 1.1	595 ± 13
0.05	174 ± 7	680 ± 24	25.6 ± 1.4	568 ± 13
0.25	150 ± 9	425 ± 23†	35.7 ± 2.4†	373 ± 12†
1.25	149 ± 10	156 ± 7†	90.2 ± 4.4†	204 ± 6†

* Values are expressed as mean ± S. E. (N = 10).
† $P < 0.01$ vs control.

The decrease in plasma zinc remains to be explained, since α_2-macroglubulin, a zinc metalloprotein which firmly binds about 40 per cent of the plasma zinc [11], is not decreased and albumin, to which a large proportion of plasma zinc is loosely bound [12, 13], is somewhat increased. Clofibrate also causes a reduction in the liver concentration of zinc, perhaps by affecting zinc metallothionein synthesis, and thus the decline in plasma concentration may merely be the result of a redistribution to achieve a new systemic equilibrium. One might also speculate that the depression in plasma zinc reflects the marked decrease in transferrin, which is known to bind zinc [13], or is caused by the displacement of zinc from albumin by clofibrate [14].

The mechanism of action by which clofibrate appears to selectively decrease the plasma concentration of serum proteins is not known. Clofibrate decreases the ratio of bound to free RNA [1] consistent with a decrease in serum protein production in general, but this would not explain the selective aspect of clofibrate inhibition. It may be that clofibrate affects transcription as well as translation, giving rise to a different pattern of messenger RNA. An effect on transcription is suggested by the fact that clofibrate inhibits the *de novo* synthesis of α_2-macrofetoprotein in the rat during turpentine-induced inflammation [15]. Alternatively, clofibrate may also selectively alter the secretion of plasma proteins from the liver.

In any event, clofibrate may prove useful in further elucidating the mechanisms [5] which regulate the plasma concentration of ceruloplasmin and transferrin as well as of a number of non-metallo acute-phase globulins in both healthy and infected animals.

Acknowledgements—The authors wish to acknowledge the editorial assistance of Mrs. Phebe W. Summers and the secretarial aid of Mrs. Diane Finneyfrock and Ms. Linda Stup.

* Current address: Human Nutrition Laboratory, United States Department of Agriculture, Grand Forks, ND 58201.

United States Army Medical Research Institute of Infectious Diseases, Frederick, MD 21701, U.S.A.

MICHAEL C. POWANDA
BILLY S. BLACKBURN
KAREN A. BOSTIAN
JOHN P. FOWLER
EDWARD C. HAUER
ROBERT S. PEKAREK*

REFERENCES

1. M. C. Powanda, E. L. Henriksen, E. Ayala and P. G. Canonico, *Biochem. Pharmac.* **25**, 785 (1976).
2. R. S. Pekarek, W. R. Beisel, P. J. Bartelloni and K. A. Bostian, *Am. J. clin. Path.* **57**, 506 (1972).
3. T. J. Giovanniello, G. DiBenedetto, D. W. Palmer and T. Peters, Jr., *J. Lab. clin. Med.* **71**, 874 (1968).
4. L. Murthy, E. E. Mender, P. M. Eller and H. G. Petering, *Analyt. Biochem.* **53**, 365 (1973).
5. A. A. Ravin, *J. Lab. clin. Med.* **58**, 161 (1961).
6. K. A. Bostian, B. S. Blackburn, R. W. Wannemacher, V. G. McGann, W. R. Beisel and H. L. DuPont, *J. Lab. clin. Med.* **87**, 577 (1976).
7. G. S. Shields, H. Markowitz, G. E. Cartwright and M. M. Wintrobe, in *Metal Binding in Medicine* (Eds. M. J. Seven and L. A. Johnson), pp. 259–64. J. Lippincott, Philadelphia (1960).
8. M. D. Poulik and M. L. Weiss, in *The Plasma Proteins* (Ed. F. W. Putnam), 2nd Edn Vol. II, pp. 51–108. Academic Press, New York (1975).
9. G. Sandor, *Serum Protein in Health and Disease*, p. 640. Williams & Wilkins, Baltimore (1966).
10. H. P. Roeser, G. R. Lee, S. Nacht and G. E. Cartwright, *J. clin. Invest.* **49**, 2408 (1970).
11. A. F. Parisi and B. L. Vallee, *Biochemistry* **9**, 2421 (1970).
12. E. L. Giroux and R. I. Henkin, *Biochim. biophys. Acta* **273**, 64 (1972).
13. G. W. Evans and T. W. Winter, *Biochem. biophys. Res. Commun.* **66**, 1218 (1975).
14. J.-P. Tillement, R. Zini, P. d'Athis and G. Vassent, *Eur. J. clin. Pharmac.* **7**, 307 (1974).
15. M. C. Powanda, B. S. Blackburn, J. P. Fowler and F. B. Abeles, *J. Cell Biol.* **70**, 138a (1976).