of the 7-HMBA sulphate treated S105 protein in the same manner as applied to the sulphotransferase-mediated binding of 7-HMBA. The synthetic sulphate reacted with L-cysteine, L-methionine and L-lysine at pH 7.4 to yield I, II and III, respectively. It also reacted with L-histidine to yield two ninhydrin-positive adducts. However, they were not found in the acid hydrolysate from S105 protein treated with 7-HMBA sulphate as well as with 7-HMBA in the presence of the PAPS-generating system.

The sulphuric acid ester group of 7-HMBA sulphate appears to behave as a leaving group, so that it may yield a stable 7-methylenecarbonium ion which reacts with the nucleophilic groups of proteins and amino acids. Very recently, both metabolically formed and synthetic sulphates of 7-HMBA and 12-hydroxymethyl-7-methyl-benz[*a*]anthracene (12-HMBA) have been demonstrated to react with DNA in a very similar manner [9]. In addition, sulphates of a series of arylmethanols such as benzyl alcohol, 1- and 2-hydroxymethylnaphthalenes, 1-hydroxy methylpyrene [4], 7-hydroxymethylbenz[*a*]anthracene [10] and 12-HMBA [9] have been shown to be reactive and mutagenic to *Salmonella* while sulphates of methanol, ethanol and *n*-propanol are much less reactive and non-mutagenic [4]. The present study may imply that the reason why DMBA as well as 7-HMBA is a potent adrenonecrogen [11] could be attributable to the facile protein binding of the reactive 7-HMBA sulphate formed in adrenals which are the well-known site of steroid sulphate biogenesis. A study on the metabolism and protein binding of the carcinogens in adrenals is in progress in our laboratory.

Laboratory of Drug Metabolism and Toxicology
Department of Hygienic Chemistry
Tokyo College of Pharmacy
Hachioji, Tokyo 192-03
Japan

TADASHI WATABE*
TSUNEO ISHIZUKA
YOSHIKI HAKAMATA
TOSHIKO AIZAWA
MASAKAZU ISOBE

REFERENCES

1. E. Boyland, P. Sims and C. Huggins, *Nature, Lond.* **207**, 816 (1965).
2. E. Boyland and P. Sims, *Biochem. J.* **95**, 780 (1965).
3. P. H. Jellinck and B. Goudy, *Science* **152**, 1375 (1966).
4. T. Watabe, T. Ishizuka, M. Isobe and N. Ozawa, *Science* **215**, 403 (1982).
5. P. G. Wislocki, K. M. Gadek, M. W. Chou, S. K. Yang and A. Y. H. Lu, *Cancer Res.* **40**, 3661 (1980).
6. C. Malaveille, H. Bartsch, B. Tierney, P. L. Grover and P. Sims, *Biochem. biophys. Res. Commun.* **83**, 1468 (1978).
7. J. A. Miller and E. C. Miller, in *Biochemical Reactive Intermediates* (Eds. D. J. Jollow, J. J. Kocsis, R. Snyder and H. Vainio), p. 6. Plenum Press, New York (1975).
8. T. Watabe, T. Ishizuka, N. Ozawa and M. Isobe, *Biochem. Pharmac.* **31**, 2542 (1982).
9. T. Watabe and T. Ishizuka, *J. pharm. Dyn.* **5**, S-57 (1982).
10. Y. Hakamata and T. Watabe, *Seikagaku, Tokyo* **54**, 889 (1982).
11. C. Huggins and S. Morii, *J. exp. Med.* **114**, 741 (1961).

* To whom correspondence should be addressed.

Biochemical Pharmacology, Vol. 32, No. 13, pp. 2122–2124, 1983.
Printed in Great Britain.

0006–2952/83 $3.00 + .00

Clofibrate-induced decrease in esterase 1 levels in the serum of the house mouse, *Mus musculus**

(*Received* 12 *October* 1982; *accepted* 13 *December* 1982)

Esterase 1, the so-called albumin esterase, is the principle carboxylesterase (EC 3.1.1.1) isozyme found in the plasma of the house mouse, *Mus musculus* [2]. Concomitant changes in esterase activity and in lipid absorption have been reported by several authors during the past 20 years [3–5]. It is possible that esterase 1 is the chylomicron-associated esterase which is seen in jejunal lymph, and which is transported with triglyceride into the plasma [6]. Clofibrate (chlorophenoxyisobutyrate) has been shown to have strong hypolipidemic effects in animals, including man [7–10]. Here we report the effects of clofibrate feeding upon plasma esterase 1 levels, which indicate another link between lipid levels and this esterase isozyme.

Male and female mice of our experimental line NMRI/Fre.mo-Es-1^f, carrying the allele for esterase 1F (ES-1F), were used in this study. Animals were subject to 12 hr : 12 hr light—dark cycles and had free access to Altromin® pellets and water; the pellets of the experimental group contained 0.5% (w/w) clofibrate. Mice were bled from the tail tips 14 days after the onset of clofibrate feeding. The blood was allowed to clot by standing at room temperature for 30 min, and after centrifugation the supernatant was assayed for total non-specific esterase activity using 4-nitrophenyl hexanoate as substrate. Activities were expressed as units (U)/ml (1 U is defined as 1 μmole of substrate hydrolysed/min at 25° at an initial substrate concentration of 0.13 mmole/l.). Samples of the supernatants were analysed by disc electrophoresis in 7.5% polyacrylamide gels. Levels of esterase 1 protein in the supernatant were assessed by rocket immunoelectrophoresis [11] using antiserum raised against esterase 1. Details of all the above procedures may be found in Otto *et al.* [2].

In female mice, the total serum esterase activity towards 4-nitrophenyl hexanoate fell to about 57% of the control activity following 14 days feeding with clofibrate (Table 1), a statistically significant difference ($P < 0.001$). In male mice, which have a lower control level of serum esterase [2], the results do not show a significant change.

The serum esterases were separated by disc electrophoresis (Fig. 1). The activity of esterase 1, indicated by the staining intensity, was reduced in both males and females following 14 days clofibrate feeding. Activities of other (largely unidentified) esterase bands did not appear to be affected by the treatment.

* This is communication 39 of a research program devoted to the cellular distribution, genetics and regulation of non-specific esterases. The previous paper in the series appeared elsewhere [1].

Table 1. Effect of clofibrate feeding on total serum esterase activity in male and female mice

	Male		Female	
	N*	Esterase activity† (U/ml)	N	Esterase activity (U/ml)
Control	4	16.24 ± 4.08	8	32.08 ± 5.01
Clofibrate	5	11.91 ± 4.05	9	18.28 ± 2.87

* N = number of animals.
† Mean values ± S.E.M.
Treatment period 14 days.

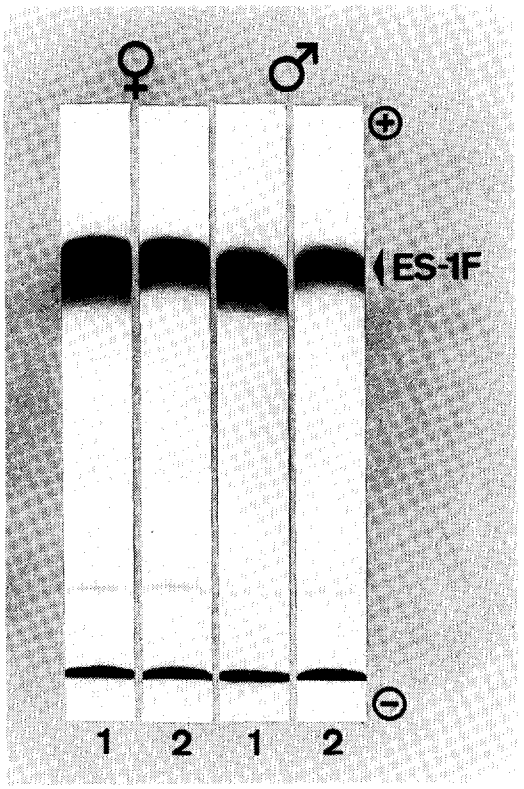

Fig. 1. Effect of clofibrate feeding on individual serum esterases: 1 = control; 2 = clofibrate-fed. Disc electrophoresis was carried out in 7.5% (w/v) polyacrylamide slab gels (76 mmole/l. Tris–HCl buffer, pH 8.9) after stacking in 3.1% (w/v) polyacrylamide (12 mmole/l. Tris–HCl buffer, pH 6.7). Electrode buffer contained 37 mmole/l. glycine in 5 mmole/l. Tris–HCl, pH 8.3. Each channel was loaded with 10 μl serum. Electrophoresis was carried out at 150 V for 30 min (stacking) followed by 300 V for approximately 2 hr, until tracking dye (bromophenol blue) had migrated to the bottom of the gel. The gels were stained for esterase activity with α-naphthyl acetate/fast red TR.

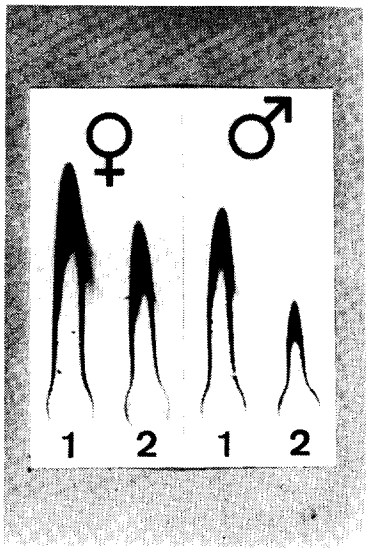

Fig. 2. Effect of clofibrate feeding on levels of esterase 1 protein: 1 = control; 2 = clofibrate-fed. Rocket immunoelectrophoresis was carried out in 1% (w/v) agarose gels (0.12 mole/l. Na–barbital buffer, pH 8.6), containing 10% (v/v) esterase 1 antiserum. The wells were loaded with 8 μl serum (diluted 1:9 with distilled water). After electrophoresis for 17 hr at 1 V/cm, the gels were stained for esterase activity with α-naphthyl acetate/fast red TR.

Clofibrate (20 μM) inhibited total serum esterase activity by 12% *in vitro*. This inhibition was clearly too low to account for the fall in serum esterase levels in the experimental animals. Rocket immunoelectrophoresis (Fig. 2) demonstrated that serum from both male and female experimental animals contained less esterase 1 protein than the corresponding controls. Thus the fall in serum esterase activity is at least partly due to a lowering of the amount of esterase 1 protein in the plasma.

Although certain experimental conditions have been shown to increase esterase activity in mice [12] and rats [13], this is the first report of an experimentally-induced reduction of plasma esterase levels. Raheja *et al.* [14] have shown that clofibrate reduces triglyceride and cholesterol levels in mice, and proposed that this effect was possibly due to an accelerated clearance of plasma lipids. The concomitant clofibrate-induced fall in esterase 1 levels strengthens our suggestion [6] that this esterase may participate in lipid transport in the plasma. It remains to be seen whether this fall in esterase levels is the result of a decreased rate of entry, or an increased clearance, of the enzyme from the blood stream.

The many components of the carboxylesterase isozyme system of the house mouse are coded for by a short segment on chromosome 8 [15]. The isozymes are expressed organ-specifically. Despite many years of intensive study, the biological function of these enzymes remains unknown. Our work suggests a possible new approach to the experimental study of this problem.

The results demonstrate that the decrease in serum esterase activity in clofibrate-treated mice is due to a reduction in the levels of esterase 1 protein circulating in the blood stream. Other esterases are not influenced by the treatment. The response of esterase 1 to clofibrate represents a further link between this enzyme and lipid levels.

Acknowledgements—We would like to thank Mrs M. Müller for performing the enzyme assays and Miss U. Wiehle for assistance with the photographs.

Abteilung für Chemische Pathologie
Universität Freiburg
Albertstraße 19
D-7800 Freiburg i.Br.F.R.G.

STEPHEN DE LOOZE
ADAM RONAI
OTTO VON DEIMLING*

REFERENCES

1. H.-C. Oehm, S. de Looze, A. Ronai and O. von Deimling, *Eur. J. Biochem.* **129**, 157 (1982).
2. J. Otto, A. Ronai and O. H. von Deimling, *Eur. J. Biochem.* **116**, 285 (1981).
3. A. M. M. Lewis and R. L. Hunter, *J. Histochem. Cytochem.* **14**, 33 (1966).
4. L. Vollrath, *Ergebn Anat. Entw Gesch.* **41**, 5 (1969).
5. I. Sauerbier, *Acta histochem.* **63**, 214 (1978).
6. A. Böcking and O. von Deimling, *Histochemistry* **75**, 377 (1982).
7. M. M. Best and C. H. Duncan, *Am. J. Cardiol.* **15**, 230 (1965).
8. S. O. Byers and M. Friedman, *Atherosclerosis* **11**, 373 (1970).
9. P. Segal, P. S. Roheim and H. A. Eden, *J. clin. Invest.* **51**, 1632 (1972).
10. M. N. Cayen, W. T. Robinson, J. Dubuc and D. Dvornik, *Biochem. Pharmac.* **28**, 1163 (1978).
11. C.-B. Laurell, *Analyt. Biochem.* **15**, 45 (1966).
12. C. L. Markert and R. L. Hunter, *J. Histochem. Cytochem.* **7**, 42 (1959).
13. K.-B. Augustinsson and B. Henricson, *Biochem. Pharmac.* **18**, 21 (1969).
14. K. L. Raheja, W. G. Linscheer and D. G. Patel, *Gen. Pharmac.* **13**, 49 (1982).
15. J. Peters, *Biochem. Genet.* **20**, 585 (1982).

* To whom correspondence should be addressed.

Biochemical Pharmacology, Vol. 32, No. 13, pp. 2124–2126, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + .00

Effect of mercuric chloride on cultured rat fibroblasts: survival, protein biosynthesis and binding of mercury to chromatin

(*Received* 8 *October* 1982; *accepted* 7 *February* 1983)

Exposure of animals to mercury leads to the accumulation of this metal in the cell nuclei [1]. The affinity of mercury for the nuclear material is high, its amount in the nuclei being considerable [2–4]. It has been established that mercury binds largely to the chromatin [3, 5–7], and the non-histone chromatin proteins are mainly responsible for this binding [4, 5]. Mercury studies on whole animals are difficult to interpret due to significantly different responses from tissue to tissue. Thus work on *in vitro* cell systems may reveal, more specifically, some of the cellular effects of this metal. However, little is known about the nature of mercury interaction with chromatin in cultured cells, except for a high incidence of chromosome aberrations induced by mercury in human lymphocytes [8], depression of DNA synthesis in mouse leukaemic [9] and HeLa cells [10], as well as reduction of rate of DNA replication in Chinese hamster ovary cells [11]. Experimental data on the binding of mercury by chromatin of cultured cells have been lacking until now.

This paper describes the effect of mercuric chloride on the survival of cultured fibroblasts and on the extent of mercury binding by the fibroblasts' chromatin.

Materials and methods

Experiments were performed on embryonic fibroblasts of Wistar rats. The fibroblasts were grown in a medium consisting of 75% MEM (Eagle's minimum essential medium) and 15% RTN (Hanks solution + 0.5% lactalbumin hydrolysate + 2% calf serum) supplemented with phenol red (for control of pH changes from the initial 7.2 to final 6.8), 10% fetal calf serum, 100 U penicillin/ml and 100 μg streptomycin/ml [12]. The fibroblasts were taken from skin tissue of 14–16-day old rat embryos subjected to digestion with 0.25% trypsin solution. Primary monolayer fibroblast culture was set up in rubber-stoppered 1-l. Legroux bottles at a concentration of 10^5 cells/ml and maintained for 6 days at 37°, changing the culture medium (100 ml) after every 3 days of incubation. The cultured cells were routinely passaged after 10–15 min trypsinization at 37°. Cell concentrations were determined using a haemocytometer, and cell viability was tested with 0.5% trypan blue. In the control cultures viability was found to be 92–96%. Cells after the third passage were used for experiments.

The effect of mercury on the survival of the cultured fibroblasts was studied by exposing them to a medium containing mercuric chloride in a concentration range from 5×10^{-7} to 1×10^{-4} M. Trypsinized cells passaged 2–3 times were used (amount: of 2×10^6 cells/bottle). Following 24-hr incubation, the culture medium was replaced by a fresh portion of medium containing mercuric chloride, and incubation was continued for another 24 hr. The cells were then suspended in Eagle's MEM medium and their concentration and viability were determined.

Incorporation of [^{14}C]leucine into proteins of the fibroblasts incubated in the presence of 10^{-6} and 10^{-5} M mercuric chloride was investigated in the monolayer cultures (2×10^6 cells). Following 23-hr incubation, [^{14}C]leucine (1.48 MBq) was added and incubation was continued for 1 hr (DL-[^{14}C]leucine; product of Institute of Nuclear Research, Warsaw, Poland; specific activity 389 MBq/mmole). After trypsinization and removal of non-incorporated labelled leucine by washing with culture medium, the cells were counted and monitored for viability, protein content and incorporation of radioactivity into acid-insoluble material after treatment of cell suspension with 10% trichloroacetic acid (1:1, v/v). The acid-insoluble precipitate was washed three times with trichloroacetic acid and dissolved in 1 ml NCS (Radiochemical Centre, Amersham, U.K.). The sample was neutralized with 0.1 ml of glacial