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Effect of administration of clofibrate and clofenapate on kidney mitochondria of the rat

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THIS LABORATORY has previously reported[1] that the administration of the hypolipidaemic compound, clofenapate (methyl-2-[4-(*p*-chlorophenyl) phenoxy]-2-methylpropionate) to the rat increased the content of mitochondrial protein and the activities of catalase (hydrogen peroxide–hydrogen peroxide oxidoreductase, EC 1.11.1.6) and glycerolphosphate dehydrogenase (L-glycerolphosphate–acceptor oxidoreductase, EC 1.1.99.5) in the liver. In this respect it resembled the analogous compound clofibrate (ethyl-α-*p*-chlorophenoxyisobutyrate) which is widely used as an antihypercholesterolaemic drug. These compounds and their metabolites are excreted in the urine of man.[2] However, little information is available about the changes, if any, brought about by these drugs in the kidney. The results presented in this communication indicate a significant increase in kidney mitochondrial protein on administration of clofibrate or clofenapate to the rat. Kidney mitochondria from drug-fed animals showed better oxidation of NAD^+-linked substrates.

Male albino rats, weighing 140–160 g, drawn from the stock colony of this Institute were used. The composition of the normal diet and feeding schedule were the same as previously described.[3] After 7–10 days feeding on the normal diet, the experimental animals were given 0·5% (w/w) clofibrate or 0·005% (w/w) clofenapate mixed with the diet for the period indicated in the Tables. In the withdrawal experiments, the animals were maintained on a diet containing the drugs at the above concentrations for the time period indicated and were then replaced on the stock diet and kept on it for an equal period of time. The control animals were supplied with the normal diet. An equal number of control and experimental animals were killed and analysed at any one time with a view to keeping variations to a minimum.

The animals were stunned and killed by decapitation. The kidneys were removed and freed of capsule and medulla.[4] The cortex was homogenized in 0·25 M sucrose and the subcellular fractions sedimented as described earlier.[1,3] The nuclear and mitochondrial fractions were washed once with 0·25 M sucrose. The rate of oxygen uptake was measured polarographically with a Gilson KM oxygraph. The reaction medium contained 0·4 M mannitol, 50 mM Tris–HCl buffer, pH 7·4, 25 mM potassium phosphate buffer, pH 7·4, 25 mM KCl, 5 mM $MgCl_2$, 20 mM EDTA, 3·4 mg bovine serum albumin and 2–3 mg (protein) of freshly isolated mitochondria in a total reaction volume of 2 ml. Respiratory control and ADP/O[5] (ratio of ADP phosphoxylated to oxygen consumed) were determined as has already been described.[3] All other determinations and conditions of assay were the same as was earlier reported.[1,3]

Administration of clofibrate or clofenapate to the rat has been shown to increase the mitochondrial protein content of the liver 50–100 per cent, without appreciably affecting the protein content of the other subcellular components. The results presented in Table 1 show that the mitochondrial fraction of the kidney also responded in a similar manner to these drugs. Dietary administration of clofibrate increased the content of kidney mitochondrial protein by 38 per cent. Clofenapate also produced the same effect, but at one-hundredth the concentration of clofibrate. In control animals the mitochondrial protein accounted for 21 per cent of the total cellular protein, while in the drug-administered animals the mitochondrial fraction increased to 26–27 per cent of the total protein.

When the animals were returned to the normal diet, after the indicated period of drug administration, the protein in the kidney mitochondrial fraction returned to its normal value (Table 1). This indicates that the effect produced by these drugs on the kidney is reversible as observed previously[1] in the case of liver.

Administration of these drugs did not adversely affect the oxidative or phosphorylative activity of liver mitochondria.[1,3] The results obtained in Table 2 indicate that respiratory control and ADP/O of kidney mitochondria were not affected by the administration of these drugs. However, active

TABLE 1. EFFECT OF FEEDING RATS WITH CLOFIBRATE (0·5%) OR CLOFENAPATE (0·005%) ON THE SUBCELLULAR FRACTIONS OF KIDNEY

Subcellular fraction	Fed			Withdrawn		
	Normal	Clofibrate	Clofenapate	Normal	Clofibrate	Clofenapate
Nuclear	29 ± 4 (17)	33 ± 3 (18)	34 ± 4 (17)	26 ± 5 (17)	30 ± 5 (17)	29 ± 7 (18)
Mitochondrial	37 ± 4 (21)	51 ± 5* (27)	52 ± 5* (26)	32 ± 5 (20)	34 ± 7 (20)	32 ± 7 (20)
Supernatant	108 ± 13 (62)	103 ± 13 (55)	111 ± 12 (57)	98 ± 7 (63)	106 ± 10 (63)	101 ± 14 (62)

* Significant $P < 0·01$.

The rats were given clofibrate (0·5%) or clofenapate (0·005%) in the diet for 30 days. In the treatment marked "withdrawn" the animals were replaced on the drug-free diet and kept on it for 30 days. The values represent milligrams protein of the subcellular fraction per gram of fresh kidney. The values in parentheses are the percentage of protein, taking the total kidney protein to be 100. The results given are the mean ± S.D. of twelve independent determinations (animals). The "supernatant" refers to the post-mitochondrial fraction containing both microsomal and soluble proteins.

TABLE 2. EFFECT OF FEEDING THE RAT WITH CLOFIBRATE OR CLOFENAPATE ON OXIDATIVE PHOSPHORYLATION BY KIDNEY MITOCHONDRIA

Treatment	Drug	Glutamate + malate			Succinate		
		State 3 oxidation	RCI	ADP/O	State 3 oxidation	RCI	ADP/O
Fed	Control	45 ± 8	1·8	2·6	122 ± 17	1·7	1·9
	Clofibrate	83 ± 19	2·5	3·0	132 ± 20	2·4	2·0
	Clofenapate	64 ± 15	2·0	3·0	132 ± 31	2·0	2·0
Withdrawn	Control	45 ± 12	1·8	2·8	139 ± 19	2·4	1·8
	Clofibrate	39 ± 8	2·3	2·9	113 ± 17	2·6	1·6
	Clofenapate	36 ± 8	2·1	2·8	134 ± 29	2·7	1·5

The rats were given clofibrate (0·5%) or clofenapate (0·05%) in the diet for 20 days. In the treatment marked "withdrawn" the animals were replaced on the drug-free diet and kept on it for 20 days. "State 3" refers to active oxidation of substrate (nanogram-atoms O/minute/milligram mitochondrial protein) in the presence of phosphate acceptor.[5] The respiratory control index (RCI) is given as the ratio of the rate of oxidation in the presence of ADP (State 3) to that after the depletion of ADP (State 4).[5] The values are the mean of six independent determinations (animals). The S.D. is given for rate of oxygen uptake.

oxidation of a NAD^+-linked substrate (glutamate-malate) was 40–60 per cent higher in the mitochondria isolated from the kidneys of drug-fed animals than that from normal animals. On withdrawal of the drug from the diet the oxidative activity returned to its normal level (Table 2).

Administration of these drugs has been shown to increase the hepatic mitochondrial glycerophosphate dehydrogenase activity six- to ten-fold[1,6] and the hepatic catalase activity two-fold.[1,7] However, the activities of these enzymes in the kidney did not show a similar response (data not presented).

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Effect of *N*-hydroxy-2-acetylaminofluorene on ribonucleic acid and deoxyribonucleic acid synthesis

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ALTHOUGH the mechanism of liver carcinogenesis by 2-acetylaminofluorene (AAF) and its *N*-hydroxy metabolite (*N*-hydroxy-AAF) has not been fully elucidated, two general effects of the carcinogen have been recognized as being important in tumor induction. An early effect of the carcinogen is the acute hepatotoxicity (massive periportal necrosis) which is induced by *N*-hydroxy-AAF.[1] The hepatotoxicity, probably caused by the formation of AAF-*N*-sulfate in the liver, is followed by compensatory cell proliferation,[2] which is believed to be necessary for tumor formation.[3] Whether the role of hepatotoxicity is merely to induce cell proliferation, or is actually a part of the "initiation" step[3] is unclear. This problem has stimulated numerous investigators to study the initial effects of AAF and *N*-hydroxy-AAF on various biochemical processes. Because of their central role in the control of cell function, RNA and DNA synthesis have been given considerable attention. Presently there are conflicting reports in the literature on the effect of *N*-hydroxy-AAF on RNA synthesis in rat liver cells. Zieve and Gutmann[4] found that within 1 hr after a single injection of *N*-hydroxy-AAF to rats, RNA polymerase activity of isolated liver cell nuclei was inhibited as much as 80 per cent. Zieve[5] later reported approximately 70 per cent reduction in orotic acid incorporation into liver RNA *in vivo* 2 hr after an intraperitoneal injection of *N*-hydroxy-AAF. In contrast, Marsh and Drabkin[6] found no inhibition of liver RNA or DNA synthesis by normal rats after a single injection of *N*-hydroxy-AAF. They did find, however, that a single dose of *N*-hydroxy-AAF given 1 hr after partial hepatectomy inhibited RNA and DNA synthesis approximately 85 and 95 per cent, respectively, when measured 24 hr postoperatively. Marsh and Drabkin[6] suggested that the difference between their results and those of Zieve and Gutmann[4] was due to differences in the rate of absorption of the carcinogen in the two studies, since different solvents were used in administering the *N*-hydroxy-AAF. Levels of *N*-hydroxy-AAF in liver and plasma were found to be twice as high when the carcinogen was administered as a solution in propylene glycol, as used by Zieve and Gutmann,[4] when compared to the same dose given as a suspension in 1% gum acacia.[6]

In an attempt to resolve this discrepancy, we have examined the effects of *N*-hydroxy-AAF on RNA and DNA synthesis in liver slices from normal and partially hepatectomized male Holtzman rats. Metabolism of *N*-hydroxy-AAF by liver slices appears to be similar, if not identical, to that of liver cells *in vivo*.* Use of the slice obviates problems of rapid urinary and biliary excretion of the carcinogen as well as metabolism of the carcinogen by the flora of the digestive tract.[7]

* C. D. Jackson and C. C. Irving, unpublished work.