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ENHANCEMENT OF D-GLUCURONOLACTONE AND ACETALDEHYDE DEHYDROGENASE ACTIVITIES IN THE RAT LIVER BY INDUCERS OF DRUG METABOLISM

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Abstract—Three different fractions of D-glucuronolactone dehydrogenase were isolated from rat liver supernatant by means of CM and DEAE Sephadex ion exchange chromatography. These fractions also had acetaldehyde dehydrogenase activity. Intraperitoneal administration of phenobarbital, *p,p'*-DDT and 3-methylcholanthrene increased the enzyme activity in two of the fractions, while the third remained unaffected by these drugs. Enhanced activity was obtained with both substrates, but it varied in degree within the group of animals. The rats could be divided into poor, medium and high response groups. A change in the ratio of the D-glucuronolactone and acetaldehyde dehydrogenase activities was also found after the drug treatment. Spironolactone, which is also an inducer of drug metabolism, did not alter the activity of any of the enzyme fractions, at the dose used.

MARSH[1–3] reported in 1963 the conversion of D-glucuronolactone into D-glucaric acid by different tissue preparations, and he named the enzyme D-glucuronolactone dehydrogenase (EC 1.1.1.70). The initial product of this reaction, D-glucaro-(1, 4)-(6, 3)-dilactone, was spontaneously hydrolysed to both D-glucaro-(1,4)- and D-glucaro-(1,6)-monolactone, which were converted to D-glucaric acid. All these products, to a different extent, inhibit β-glucuronidase, and studies *in vivo* indicate that the D-glucaric acid pathway in fact controls β-glucuronidase activity and the hydrolysis of glucuronides.[4]

D-Glucaric acid excretion in urine is enhanced in animals and humans after the administration of various drugs.[5–8] Elevated activity of D-glucuronolactone dehydrogenase in the liver of mice has been reported during pregnancy[9] and after administration of norethisterone.[10] Hänninen and coworkers[11,12] demonstrated an increase of the enzyme activity in the rat liver after administration of phenobarbital and cinchophen, and the increase could be blocked by actinomycin D.

Studies concerning the induction of D-glucuronolactone dehydrogenase by different drugs have been sporadic and the results controversial.[6,13] In order to clarify the reason for the contradictory results in the literature, we have studied D-glucuronolactone dehydrogenase activity in rat liver after administration of the commonly used inducers of drug metabolism, i.e. 3-methylcholanthrene,[14] phenobarbital,[15] spironolactone,[16] and 1,1,1,-trichloro-2,2,bis(*p*-chlorophenyl)ethane (*p,p'*-DDT).[17] We have also measured aldehyde dehydrogenase (EC 1.2.1.3.) activity for comparison, since it has been suggested[18,19] that D-glucuronolactone is converted to D-glucaric acid by aldehyde dehydrogenase and not by a specific enzyme.

MATERIALS AND METHODS

All chemicals used were reagent grade, of the best commercially available quality. Solutions of acetaldehyde (Merck), D-glucuronolactone (Fluka) and NAD (Boehringer) were prepared before each measurement. Phenobarbital and pyrazole were obtained from Merck, 3-methylcholanthrene from Koch-Light Lab., *p,p'*-DDT from Fluka and olive oil from Fisher Co. Spironolactone was kindly donated by Orion Co. (Finland). Sephadex G-25, Carboxymethyl (CM,C50) and Diethylaminoethyl (DEAE,A50) Sephadex were prepared for use according to the directions of the manufacturer (Pharmacia, Sweden).

Treatment of the animals. Five male albino rats (*Rattus norvegicus*) weighing 100–200 g and fed *ad lib.* were used in each group. The rats were purchased as specific pathogen free Wistar/Af/Han/Mol/(Han 67) and represent the second generation, which has been outbred by the rotational mating system, in the Laboratory animal center of our University. Animals of approximately the same weight and when possible of the same litter were collected.

Drugs were administered intraperitoneally. The rats were sacrificed usually 24 hr after the last drug injection. Phenobarbital was dissolved (20 mg/ml) in 0·1 N NaOH, and the pH was adjusted to 8·6 by 1 N HCl. Phenobarbital (100 mg/kg) was given for 5 days. Control animals received the same volume of isotonic NaCl. Spironolactone (100 mg/kg) was suspended in olive oil (20 mg/ml) and administered, after thorough stirring before each injection, for 4 days. *p,p'*-DDT (100 mg/kg) was dissolved in olive oil (20 mg/ml) and administered for 4 days. The animals were sacrificed either 24 hr, or 2 weeks later. 3-Methylcholanthrene (20 mg/kg) was dissolved in olive oil (4 mg/ml) and administered for 2 days. Control animals for the spironolactone, *p,p'*-DDT and 3-methylcholanthrene groups were treated for the same time with the same volume of olive oil.

Preparation of the enzyme fractions. Livers were removed after stunning the rats with a blow on the head and bleeding them by cutting the renal vessels. All further procedures were carried out at 0–4°. The liver was blotted gently with paper and weighed. After mincing with scissors, it was homogenized in two volumes (w:v) of 0·25 M sucrose with a mechanically driven Potter–Elvehjem homogenizer with a Teflon pestle (400 rev/min, two strokes). The homogenate was centrifuged for 20 min at 10,000 *g* and the supernatant again at 125,000 *g* for 1 hr. The supernatant of the second centrifugation was designated the crude extract.

The two soluble fractions of aldehyde dehydrogenase reported by Shum and Blair,[20] were obtained by their method with slight modifications. Saturated ammonium sulphate solution (4°) was added slowly to the crude extract up to 40 per cent saturation, while the pH was kept constant at 7·4. The suspension was stirred gently for 15 min and then centrifuged at 10,000 *g* for 10 min. Ammonium sulphate was added slowly to the supernatant to give a final saturation of 60 per cent. After centrifugation the supernatant was discarded and 2 ml of 8 mM sodium phosphate buffer pH 6·8 were added to the sediment. Ammonium sulphate was removed by applying the redissolved protein to a 2 × 50 cm G-25 Sephadex column, equilibrated and eluted with 8 mM sodium phosphate buffer pH 6·8. The ammonium sulphate-free sample was first applied to a 2 × 30 cm CM Sephadex column, from which it was eluted by 8 mM phosphate buffer pH 6·8. The unadsorbed protein was then passed through a 2 × 40 cm DEAE Sephadex column. Some aldehyde dehydro-

genase activity was found in the void volume fractions. After washing the DEAE column with 8 mM buffer pH 6·8, the retained protein was subjected to a gradient elution by using 200 ml 8 mM and 200 ml 80 mM sodium phosphate buffer, pH 6·8. The second aldehyde dehydrogenase activity peak emerged during this procedure. After washing the CM column with 8 mM sodium phosphate buffer pH 6·8, it was eluted with a gradient (100 ml 8 mM and 100 ml 80 mM sodium phosphate buffer, pH 6·8). The elution revealed a third fraction of the enzyme, which came off with the first protein peak.

Experimental procedure for correlative studies. In routine studies on the isolated fractions of the soluble aldehyde dehydrogenase a different procedure was used than that described above. The samples were desalted by overnight dialysis in 6 l. of 8 mM sodium phosphate pH 6·8. The precipitated protein was removed by centrifugation at 10,000 *g* for 15 min, and the supernatant was then applied to a 1 × 30 cm CM Sephadex column. The effluent was passed through a 2 × 20 cm DEAE Sephadex column and the first 35 ml of the eluate were collected and designated as DEAE I enzyme fraction. After further thorough washing, 80 mM phosphate buffer pH 6·8 was applied and the first 30 ml were collected and designated as DEAE II enzyme fraction.

The same batch chromatography procedure was also used to remove the protein retained on the CM Sephadex by eluting the column with 80 mM phosphate buffer, pH 6·8. The first 10 ml were collected and designated as the CM enzyme fraction.

Measurement of the dehydrogenase activities. D-Glucuronolactone dehydrogenase activity was assayed by a modification of the method of Shum and Blair[20] for aldehyde dehydrogenase. The enzyme was measured at 38° and pH 7·8 in a final volume of 1 ml containing 80 mM sodium phosphate buffer, 0·4 mM NAD, 0·5 mM pyrazole and 28 mM D-glucuronolactone. The reaction was initiated by adding enzyme to the mixture, while a cuvette containing all the other reagents was used as a blank. The reduction of NAD was followed at 340 nm for at least 6 min, with a Perkin–Elmer 402 spectrophotometer connected to an external recorder Servogor 5 (Goerz Electro). The enzyme activity was calculated by using the molar extinction coefficient for reduced NAD, $6{\cdot}2 \times 10^3$.

Acetaldehyde dehydrogenase activity was measured by slightly modifying the method described by Shum and Blair[20] in a mixture containing 80 mM sodium phosphate buffer, 11 mM acetaldehyde, 0·4 mM NAD and 0·5 mM pyrazole at pH 7·8. The final volume was 1 ml and temperature 22°. The reaction was initiated by addition of NAD and a cuvette with all the other reagents was used as a blank.

Alcohol dehydrogenase activity was measured according to Racker,[21] by tracing the formation of NADH as above in a 1 ml cuvette containing 0·3 M ethanol, 0·15 mM NAD and 60 mM pyrophosphate buffer, pH 8·5 at 38°.

Pyrazole was included in acetaldehyde and D-glucuronolactone dehydrogenase measurements, in order to inhibit alcohol dehydrogenase,[22] although this enzyme was not always present in the fractions studied. It did not interfere with the D-glucuronolactone dehydrogenase assay.

Protein determination. Protein was determined by the method of Lowry *et al*,[23] using bovine albumin (Sigma) as reference. In studies of the elution pattern of the enzymes, we measured the absorption at 280 nm.

RESULTS

Elution profiles of the enzymes studied. Figure 1 shows the elution peaks of D-glucuronolactone and acetaldehyde dehydrogenase activities in the CM-Sephadex ion exchange chromatography of the liver cytosol fractions of an untreated (A) and a phenobarbital treated animal (B). Phenobarbital treatment considerably increased the activity. In control rats the enzyme activity with acetaldehyde as substrate was lower than with D-glucuronolactone. After phenobarbital treatment the ratio of acetaldehyde and D-glucuronolactone dehydrogenase activities was reversed. Phenobarbital treatment increased significantly the amount of total protein eluted together with the enzyme from the CM-column. The pattern of the DEAE I enzyme fraction was similarly changed after phenobarbital administration (Figs. 2 and 3). In the untreated animal the enzyme activity was low (Fig. 2). The two substrates gave similarly eluted peaks, but D-glucuronolactone dehydrogenase activity was higher. On the contrary, in the phenobarbital treated animal (Fig. 3) there was a high elevation of the enzyme activities for both substrates. Acetaldehyde dehydrogenase activity exceeded that of D-glucuronolactone dehydrogenase indicating again a reversal of the activity ratio with the two substrates. In this case there was only a minor change in the total protein eluting with enzyme.

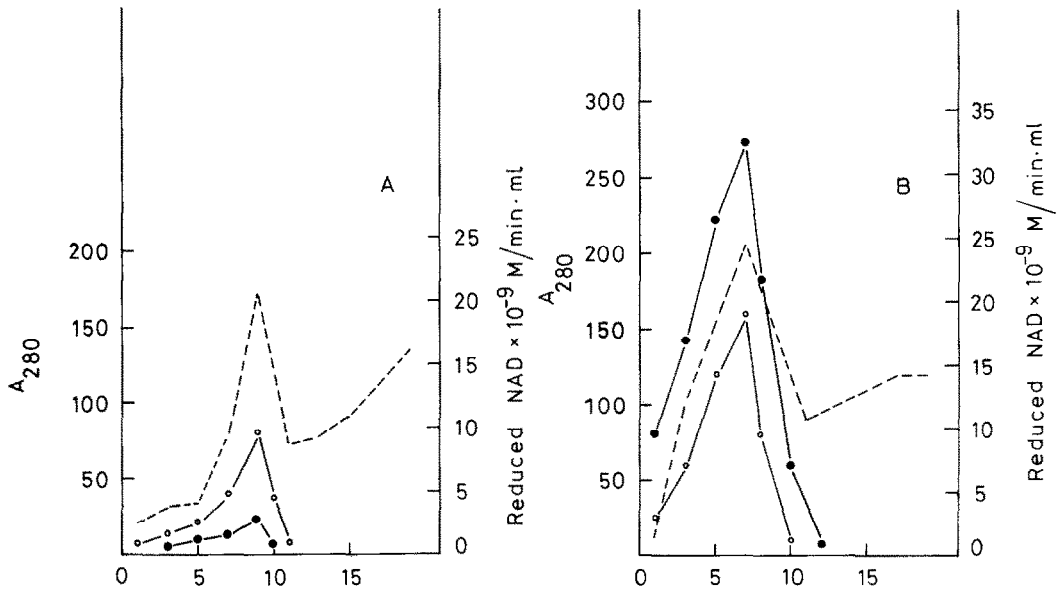

FIG. 1. CM Sephadex ion exchange chromatography of D-glucuronolactone and aldehyde dehydrogenase from the liver of an untreated (A) and a phenobarbital treated animal with a high response (B). The numbers on the abscissa are the fractions collected after initiation of the gradient elution. The volume of a fraction was 3 ml and the flow rate 24 ml/hr. Protein (——) was determined by measuring the absorption at 280 nm (× 10^{-3}). D-Glucuronolactone dehydrogenase (○) and aldehyde dehydrogenase (●) activities are expressed in nanomoles of reduced NAD/min and ml.

The elution profile of DEAE II enzyme fraction is presented in Figs. 2 and 3. Both untreated (Fig. 2) and treated (Fig. 3) animals possessed similar enzyme activity patterns and no changes were observed for the two substrates used after phenobarbital

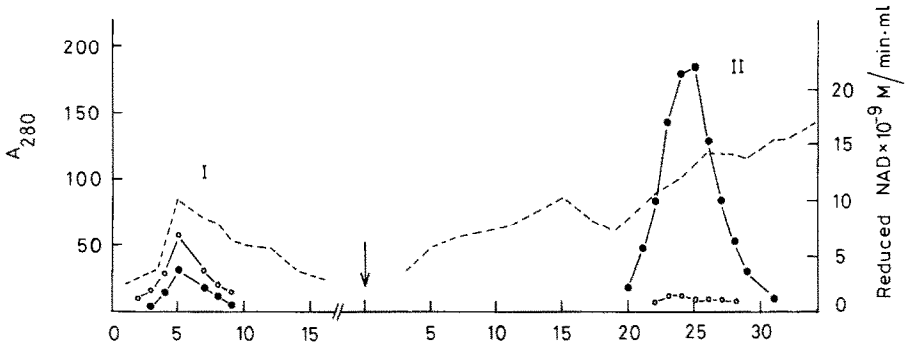

FIG. 2. Enzyme fractions I and II from a DEAE ion exchange chromatography of an untreated animal. Fractions of 6 ml were collected at a flow rate 24 ml/hr. The arrow shows the initiation of the gradient elution. Protein and enzyme activities are indicated as in Fig. 1.

administration. D-Glucuronolactone dehydrogenase activity was minimal in both cases.

Attempts to separate the D-glucuronolactone and acetaldehyde dehydrogenase activities by using gradients of different pH and ionic strength were without success. In a pH range from 6 to 7·5, the DEAE I enzyme fraction still remained unadsorbed and was eluted from the column containing D-glucuronolactone and acetaldehyde dehydrogenase activity with the same distribution pattern.

Induction of the dehydrogenases. Tables 1 and 2 show the activities of D-glucuronolactone and acetaldehyde dehydrogenase enzyme fractions after drug administration. Animals treated with phenobarbital and 3-methylcholanthrene exhibited a varying degree of increase in enzyme activity levels. The crude enzyme extract of 3-methylcholanthrene treated rats, when D-glucuronolactone was used as substrate however showed no variation in the degree of the response. Animals treated with *p,p'*-DDT

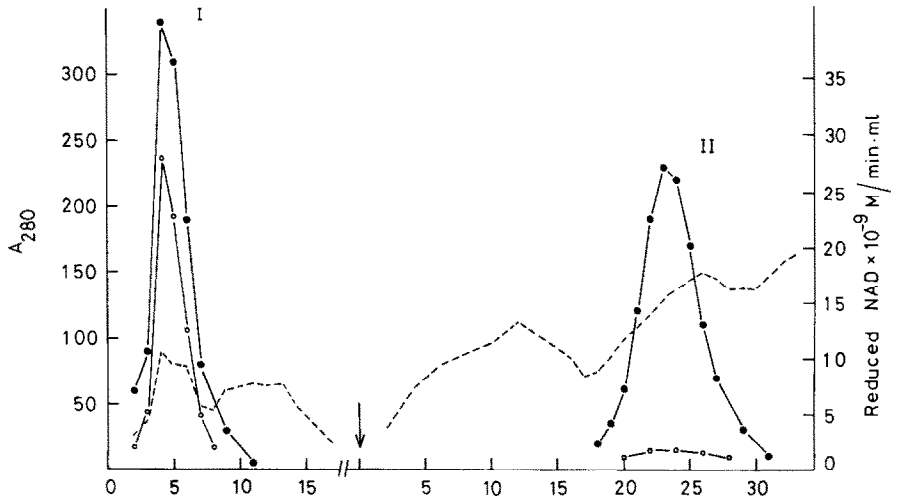

FIG. 3. Enzyme fractions I and II from a DEAE ion exchange chromatography of a phenobarbital treated animal with a high response. All parameters are expressed as in Fig. 2.

TABLE 1. THE EFFECT OF PHENOBARBITAL, 3-METHYLCHOLANTHRENE, *p,p'*-DDT, SPIRONOLACTONE AND OLIVE OIL ON THE ACTIVITY OF D-GLUCURONOLACTONE DEHYDROGENASE IN RAT LIVER*

Treatment	Activity of the enzyme fractions (nmoles of reduced NAD/min mg protein)			
	Crude extract	CM-enzyme	DEAE-enzyme I	DEAE-enzyme II
Phenobarbital	C 21·9 ± 7·5 (5) rr 29·2, 26·8 (2) Rr 57·8 (1) RR 80·0, 76·4 (2)	C 29·3 ± 2 (5) rr 48·4, 43·3 (2) Rr 57·7 (1) RR 69·7, 63·8 (2)	C 32·9 ± 3·4 (5) rr 56·5, 67·3 (2) Rr 116·5 (1) RR 135·1, 148·7 (2)	C 2·1 ± 0·6 (5) Treated 4·2 ± 2·4 (5)
3-Methylcholanthrene	C 17·9 ± 1·6 (5) Treated 42·9 ± 3 (5)	C30·1 ± 3·2 (5) rr 42·7 (1) Rr 57·5 ± 3·3 (3) RR 93·9 (1)	C 35·3 ± 3 (5) rr 41·4 (1) Rr 46·4 ± 0·3 (3) RR 78·1 (1)	C 4·0 ± 1·5 (5) Treated 5·8 ± 3 (5)
p,p'-DDT	C 18·1 ± 2·1 (5) rr 17·1 (1) Rr 76·3, 84·3 (2) RR 113·1, 135·4 (2)	C 28·8 ± 1·2 (5) rr 47·5 (1) Rr 61·7, 53·8 (2) RR 128·5, 124·7 (2)	C 44·5 ± 3·8 (5) rr 124·4 (1) Rr 195·1, 173·6 (2) RR 449·4, 405·7 (2)	C 2·4 ± 1 (5) Treated 3·0 ± 2 (5)
Spironolactone	C 50·3 ± 6·6 (5) Treated 49·9 ± 5·6 (5)	C 119·0 ± 7·6 (5) Treated 110·6 ± 3·9 (5)	C 124·8 ± 3·8 (5) Treated 133·0 ± 8·6 (5)	C 6·8 ± 2·8 (5) Treated 7·2 ± 2·7 (5)
Olive oil	C 13·0 ± 2·0 (5) Treated 50·3 ± 6·6 (5)	C 29·8 ± 2 (5) Treated 119·0 ± 7·6 (5)	C 32·9 ± 3·4 (5) Treated 124·8 ± 3·8 (5)	C 2·1 ± 0·6 (5) Treated 6·8 ± 2·8 (5)

* The magnitude of the response is indicated by the symbols rr, Rr and RR; control group (C). The *p,p'*-DDT control and experimental animals were sacrificed two weeks after the treatment. All other groups were studied 24 hr after the last injection. The results are expressed as the mean ± S.E.M. with the number of the animals in parentheses. All treatments, except spironolactone, were significantly different from the controls ($P < 0{\cdot}001$). The DEAE II enzyme fraction did not change significantly in any of the groups.

TABLE 2. THE EFFECT OF PHENOBARBITAL, 3-METHYLCHOLANTHRENE, *p,p'*-DDT, SPIRONOLACTONE AND OLIVE OIL ON THE ACTIVITY OF ACETALDEHYDE DEHYDROGENASE IN RAT LIVER*

Treatment	Activity of the enzyme fractions (nmoles of reduced NAD/min mg protein)			
	Crude extract	CM-enzyme	DEAE-enzyme I	DEAE-enzyme II
Phenobarbital	C 10·8 ± 0·9 (5) rr 23·8, 21·0 (2) Rr 103 (1) RR 203·2, 195·1 (2)	C 14·4 ± 0·4 (5) rr 12·1, 21·4 (2) Rr 113·4 (1) RR 231·1, 253·2 (2)	C 15·7 ± 1·2 (5) rr 144·3, 123·7 (2) Rr 809·7 (1) RR 1150·4, 1565·0 (2)	C 28·5 ± 2·7 (5) Treated 25·8 ± 3·6 (5)
3-Methylcholanthrene	C 8·3 ± 0·4 (5) rr 21·7 (1) Rr 97·8 ± 2·4 (3) RR 198·4 (1)	C 13·2 ± 4·2 (5) rr 38·8 (1) Rr 93·9 ± 2·4 (3) RR 208·2 (1)	C 19·3 ± 1 (5) rr 42·1 (1) Rr 76·6 ± 7·5 (3) RR 169·1 (1)	C 30·9 ± 2·2 (5) Treated 29·3 ± 3·8 (5)
p,p'-DDT	C 15·9 ± 1·8 (5) rr 13·0 (1) Rr 44·5, 44·9 (2) RR 166·2, 158·7 (2)	C 20·3 ± 1·8 (5) rr 15·9 (1) Rr 33·7, 30·7 (2) RR 72·8, 64·6 (2)	C 22·3 ± 0·8 (5) rr 109 (1) Rr 347·3, 339·8 (2) RR 1972·4, 1953·2 (2)	C 32 ± 2·6 (5) Treated 36·2 ± 7·5 (5)
Spironolactone	C 29·0 ± 1·5 (5) Treated 32·0 ± 1·5 (5)	C 38·3 ± 3·9 (5) Treated 36·9 ± 3·0 (5)	C 87·2 ± 6·6 (5) Treated 90·2 ± 8·1 (5)	C 31·8 ± 3·3 (5) Treated 33·3 ± 6·2 (5)
Olive oil	C 10·8 ± 0·9 (5) Treated 29·0 ± 1·5 (5)	C 14·4 ± 0·4 (5) Treated 38·3 ± 3·9 (5)	C 15·7 ± 1·2 (5) Treated 87·2 ± 6·6 (5)	C 29·3 ± 2·4 (5) Treated 31·8 ± 3·3 (5)

* For explanation see Table 1.

showed increased D-glucuronolactone and acetaldehyde dehydrogenase activities, two weeks after the administration of the drug (Tables 1 and 2). Rats which were studied 24 hr after the last injection failed to show any difference between control and experimental groups.

Spironolactone had no significant effect on the enzyme activities. The overall increase in the activity was similar to that obtained with olive oil treatment. There was no variation from animal to animal, or reversal for the two substrates used.

The activity of the DEAE II enzyme fraction, was not significantly altered by any of the drug treatments (Tables 1 and 2).

The variable response of the supernatant aldehyde dehydrogenase of rat liver to phenobarbital was first reported by Deitrich.[24] The symbols rr, Rr and RR introduced by this author to qualify the rats of poor, medium and high response respectively, are also used by us. Although the laborious separation of the different enzyme fractions did not permit an extensive study with more animals, consideration of all three groups with enhanced enzyme activity reveals a similar pattern of response to that described by Deitrich for phenobarbital. In an attempt to find out the frequency with which the various enzyme responses occur in our rat population we pooled the present results and we collected information from twenty more animals treated with phenobarbital. Acetaldehyde dehydrogenase was measured in the 125,000 *g* supernatant fraction of the liver. Approximately 40 per cent of the rats gave a poor response, 40 per cent gave a medium response and 20 per cent gave a high response.

Comparison of the values of the control groups with those of the experimental groups showed a reversal of the activity for the two substrates used. Although D-glucuronolactone dehydrogenase activity was higher in the control animals, the opposite was observed in the phenobarbital, 3-methylcholanthrene and *p,p'*-DDT treated animals,where acetaldehyde dehydrogenase activity predominated. The reversal was absent in all fractions of the olive oil treated rats (Tables 1 and 2).

The liver weights of control and treated rats are compared in Table 3. Statistically significant differences exist in the phenobarbital, spironolactone and *p,p'*-DDT treated groups, while 3-methylcholanthrene and olive oil treatment did not alter the liver weight.

TABLE 3. RELATIVE LIVER WEIGHTS AFTER DRUG TREATMENT

Treatment	Control	Experimental
Phenobarbital	3·96 ± 0·16 (5)	5·63 ± 0·47 (5)
Spironolactone	4·18 ± 0·20 (5)	4·86 ± 0·17 (5)
p,p' -DDT	4·01 ± 0·26 (5)	5·07 ± 0·13 (5)
3-Methylcholanthrene	4·20 ± 0·47 (5)	4·42 ± 0·35 (5)
Olive oil	3·96 ± 0·16 (5)	4·18 ± 0·20 (5)

The mean ± S.E.M., with the number of animals in parentheses, is given for the liver weight expressed as a percentage of the body weight. The phenobarbital, spironolactone and *p,p'*-DDT groups differ significantly from the controls ($P < 0·001$).

DISCUSSION

By using polarographic methods Sadahiro *et al.*[18] found that in aqueous solution D-glucuronolactone is partly in aldehyde form, and they suggested that D-glucuronolactone dehydrogenase might in fact be a non-specific aldehyde dehydrogenase.

Gupta and Chatterjee[25] found that incubation of D-glucuronolactone in the presence of aldehyde binding agents resulted in increased production of L-ascorbic acid. The aldehyde group in the D-glucuronolactone molecule is necessary therefore, if it is not to be involved in the metabolic pathway of L-ascorbic acid. The findings that both D-glucuronolactone and aldehyde dehydrogenase are inducible by phenobarbital[11,27] corroborate further the common enzyme hypothesis. Aarts and Hinnen-Bouwmans,[19] as well as Tonkes[26] have studied this hypothesis and found enough biochemical evidence to support the identity of these two enzymes. However, no effort has been made to study the different fractions of the soluble aldehyde dehydrogenase in connection with their activity towards both substrates. The existence of two fractions has been demonstrated in the liver of rabbit,[28] man,[29] mouse,[30] horse[31] and rat.[20] In our pilot experiments with cellulose acetate electrophoresis, using a modification of the disc-electrophoresis technique described by Robbins,[32] we found three bands with aldehyde dehydrogenase activity. At pH 8·5, one band moved to the cathode and two to the anode. We also separated three enzyme fractions by using CM and DEAE ion exchange chromatography. We took care to avoid contamination of our preparation with mitochondrial aldehyde dehydrogenase, by using isotonic sucrose solution and gentle homogenization techniques.

It is difficult to explain the reversal of the activity ratio towards the two substrates after inducer treatment. The possibility that the inducer is bound to the enzyme molecule, changing thus its affinity, seems improbable because of the dialysis and chromatographic procedures. *In vitro*, phenobarbital inhibits D-glucuronolactone dehydrogenase,[3] but not aldehyde dehydrogenase.[33] In our experiments, the crude enzyme extract of 3-methylcholanthrene treated animals did not exhibit any variation of activity pattern when D-glucuronolactone was the substrate, although such pattern was seen in the case of acetaldehyde and also in the rest of the fractions for both substrates. This favours the idea that traces of 3-methylcholanthrene in the crude extract may be inhibitory for D-glucuronolactone but not for acetaldehyde oxidation.

The view that this inversion of activity ratio denotes the existence of two separate enzymes is not supported by the rest of the results and especially by the observation that three different enzyme fractions exhibit non-separable activity for both substrates.

The increase of the D-glucuronolactone and acetaldehyde dehydrogenase activities after administration of olive oil alone can possibly be attributed to the existence of food additives in the oil. Phenolic antioxidants[34] are commonly added to olive oil and have been found to enhance drug metabolism. We could not obtain definite information on the existence of such an antioxidant in the oil used in our experiments. The response of the different animals to olive oil was similar, in contrast to the varied response to the other active compounds.

Induction by *p,p'*-DDT was detectable only two weeks after its administration. This is probably due to the slow rate of release of the drug from the adipose tissue, resulting in a long lag period until its metabolites reach stimulatory levels in the liver.

The findings that a number of common inducers of drug metabolism are also effective as inducers of D-glucuronolactone dehydrogenase activity in the rat liver is compatible with the existing evidence of increased D-glucaric acid in the urine after drug treatment. This increase has long been attributed to the enhanced activity of other

enzymes in the glucuronic acid pathway,[5,31,35] although Hänninen[11] could demonstrate an induction of D-glucuronolactone dehydrogenase itself, by using cinchophen or phenobarbital as model compounds. It appears that rats differ in their response to inducer administration. The frequency of the high responsiveness "gene" may vary in different rat colonies and this, together with differences in the enzyme assay, may explain the contradictory results. The use of urinary D-glucaric acid excretion as an index for the enhanced drug metabolism in the liver has been proposed.[9] Some inducers of drug metabolism like spironolactone appear, however, not to affect D-glucuronolactone dehydrogenase activity. There may be also other inducers lacking the effect on D-glucaric acid synthesis. Further studies on D-glucaric acid and on its use as an indicator of drug metabolic rate are needed.

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