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EFFECT OF PROTEIN-FREE DIET ON UDP-GLUCURONYLTRANSFERASE AND SULPHOTRANSFERASE ACTIVITIES IN RAT LIVER

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Abstract—UDP-glucuronyltransferase and sulphotransferase activities in the livers of immature and adult male rats made protein-deficient by feeding protein-free diets for 7 days have been compared to corresponding activities in control rats.

Both immature and adult protein-deficient rats had higher microsomal UDP-glucuronyltransferase activities than control rats when the activities were measured with *p*-nitrophenol or with *o*-aminophenol as substrate. No significant difference in the activities of sulphotransferase, measured with *p*-nitrophenol or with dehydroepiandrosterone was observed.

These results indicate that in the liver of male rats UDP-glucuronyltransferase and sulphotransferase enzymes involved in the biotransformation of drugs, differ from the group of NADPH-dependent drug metabolising enzymes which are known to be markedly decreased in activity in protein-deficient rats.

IT IS well known that the rates of metabolism of drugs and foreign compounds by mammalian liver may be altered by changes in various physiological and dietary conditions,[1] but the specific effect of dietary protein level on drug metabolism has only recently received special attention.

Studies on the NADPH-dependent drug metabolism reactions of rat liver indicate that the activities of these enzyme systems are markedly reduced by protein deficiency.[2,4–6] Thus a protein-free diet fed to rats reduces the rate of aminopyrine *N*-demethylation to 20 per cent of normal[4] while *in vitro* and *in vivo* studies of Kato, Oshima and Tomizawa[6] have shown that the activities of NADPH-dependent enzymes are related to the protein content of the diet; very low drug metabolising activity was observed in rats fed a protein-free diet, while progressively higher activities were observed when the protein-content of the diet was raised to 50 per cent (i.e. well above the normal content of 18 per cent).

Investigations of a similar nature into the effect of protein level on enzyme systems catalysing conjugation reactions, e.g. with glucuronic acid and sulphate, have not yet been reported, although these reactions are equally important in the biotransformation of drugs and foreign compounds. In this paper we describe *in vitro* experiments on the effect of protein deficiency on conjugation with glucuronic acid (by UDP-glucuronyltransferase, a microsomal enzyme) and with sulphate (by sulphotransferase, a soluble enzyme) in rat liver. A preliminary account of part of this work has already been published.[7]

EXPERIMENTAL METHODS

Animals and diets

When rats are fed protein-deficient diets they reduce their food intake.[8,9] An attempt was therefore made to isolate the effects of protein-deficiency *per se* from the effects of concomitant sub-normal calorie intake levels. In the first part of this study, made on immature male Wistar rats, this was achieved by restricting the dietary intake of the control group, receiving a diet containing a normal percentage of protein, in order to make it isocaloric with that of the protein-deficient group. In the second part of the study, made on young adult male rats, the control group was allowed to feed *ad lib.* on a diet with a normal percentage of protein; but a group of rats on the protein-deficient diet was force-fed by stomach tube in an effort to raise their calorie intake to a level similar to that of the control group.

The experimental diets were based on the synthetic diet described by McLean and McLean[4] and contained either 18 per cent protein as casein or contained no protein, the casein being replaced by starch. The immature rats (65–75 g) were divided into two groups. Group ${}_0$I was fed *ad lib.* on the protein-free diet, whilst group ${}_{18}I_R$ received the 18 per cent protein-diet in restricted amounts. The average calorie intakes (per 100 g body weight) were 32 kcals/day and 36 kcals/day respectively. Young adult rats (125–150 g) were divided into three groups. Group ${}_0$A was fed the protein-free diet *ad lib.* Group ${}_{18}$A was fed the 18 per cent protein diet *ad lib.* and group ${}_0A_F$ was force-fed by stomach tube on a protein-free diet; this was given as a slurry (0·75 g/ml twice daily at 08.30 hr and 16.30 hr). The average calorie intake (per 100 g body weight) of these rats was: group ${}_0$A, 25 kcals/day; group ${}_{18}$A, 49 kcals/day; group ${}_0A_F$, 37 kcals/day. The diets were fed to all rats for 7 days. All animals had been fed prior to experimentation on commercial stock pellets, diet 41B.[10] The temperature of the animal house was 22–24°.

Preparation of tissues and liver factions

At 08.30 hr on the eighth day, animals were stunned by a sharp blow and livers rapidly removed. The livers were blotted, chilled, weighed and homogenized in ice-cold 0·15 M potassium chloride (MSE homogeniser) to give a final 10% (w/v) concentration. Livers were usually homogenized in pairs but those from the force-fed group, ${}_0A_F$, which contained fewer animals, were homogenized as single livers. The homogenates were centrifuged (12,000 *g*, 10 min, 4°), the pellet discarded and the supernatant centrifuged again (105,000 *g*, 1 hr, 4°). The resulting "high speed" supernatant was retained and stored at −18° (1–3 months) for sulphotransferase activity and protein content measurements, whilst the microsomal pellet was resuspended immediately with a glass-Teflon homogeniser (Potter–Elvehjem type) in a volume of ice-cold 0·15 M potassium chloride equal to the original volume of homogenate and recentrifuged at 105,000 *g* as before. The washed microsomal pellet was finally resuspended in sufficient ice-cold 0·15 M potassium chloride so that 1 ml of the suspension was equivalent to 500 mg wet liver.

Assays

UDP-glucuronyltransferase activity, with *p*-nitrophenol (PNP) as substrate, was assayed using a modification of the method of Isselbacher,[11] which measures PNP

disappearance. Incubations contained in a final volume of 0·6 ml: Tris buffer, pH 7·4, $4·2 \times 10^{-2}$ M; PNP, $2·2 \times 10^{-5}$ M; UDP-glucuronic acid (UDPGA), $4·9 \times 10^{-4}$ M; microsomal suspension 0·2 ml. In some experiments with high levels of UDPGA ($4·9 \times 10^{-3}$M) the concentration of PNP was $4·4 \times 10^{-5}$ M. In experiments with immature rats, tubes were incubated with shaking for 30 min at 37°. In subsequent experiments with adult rats, where the activities of UDP-glucuronyltransferase were higher, it was necessary to use an incubation time of only 20 min, in order to maintain time-linearity in the assay. Similarly, when the effect of high UDPGA levels on UDP-glucuronyltransferase were examined, an incubation time of 10 min was used. The reactions were stopped with 0·6 ml, 0·2 N trichloroacetic acid, the tubes centrifuged and 0·8 ml aliquots added to 3·2 ml, 0·5 N sodium hydroxide. The absorbance at 400 nm was then determined.

UDP-glucuronyltransferase activity, with *o*-aminophenol (OAP) as the substrate, was determined by the method of Dutton and Storey[12] in which the product of the reaction was measured spectrophotometrically. Incubations contained $4·9 \times 10^{-4}$ M UDPGA and 0·2 ml of microsomal suspension in a final volume of 0·6 ml and were incubated with shaking for 30 min at 37°. Control incubations contained no UDPGA.

Sulphate conjugation of dehydroepiandrosterone (DHEA) and of PNP was determined by a method based on that described for these substrates by Nose and Lipmann,[13] in which the sulphate conjugate is assayed as a complex with methylene blue. The incubation conditions were slightly modified so that the synthesis of the sulphate donor 3′-phospho-adenosine 5′-phosphosulphate (PAPS) by the yeast extract could be carried out in a separate, preliminary incubation. Aliquots of this reaction mixture containing standard quantities of PAPS were then used in the determination of sulphotransferase activity of rat liver. In the preliminary incubation, phosphate buffer, pH 7, 10^{-1} M, potassium sulphate, 10^{-2} M, magnesium sulphate, 10^{-2} M, ATP, 10^{-2} M and yeast sulphate-activating enzyme fraction, equivalent to 60 mg protein, were incubated in a volume of 10 ml for 1 hr. The reaction was stopped by the addition of 0·4 M neutralized EDTA to give a final EDTA concentration of 0·02 M.[14] The quantity of PAPS synthesized in such incubations was estimated by measuring the sulphate-dependent phosphate release[15] in parallel incubations carried out with Tris-maleate buffer, pH 7, instead of phosphate buffer. For the sulphotransferase activity measurements, incubations of 1 ml contained: phosphate buffer pH 7, 25 μmoles; cysteine hydrochloride, 5 μmoles; PNP, 0·4 μmole or DHEA 0·2 μmole added as solutions in propylene glycol; yeast incubation, 0·25 ml, equivalent to 125–150 nmoles PAPS; 0·2 ml rat liver "high-speed" supernatant fraction, previously dialysed overnight against phosphate buffer pH 7, 5×10^{-2} M, containing 2×10^{-3} M cysteine hydrochloride. The sulphate conjugate, after treatment with the methylene blue reagent, was estimated spectrophotometrically at 640 nm.

The degree of hydrolysis of UDPGA by UDPGA-pyrophosphatase present in microsomal suspensions was determined at pH 8·9, the optimum for this enzyme, by the method of Ogawa, Kawada and Sawada.[16] After termination of the reaction and treatment with charcoal to remove nucleotides, the glucuronic acid released was determined using the naphthoresorcinol method of Nir.[17] UDPGA-pyrophosphatase activity was also determined under conditions comparable to those used for the measurement of UDP-glucuronyltransferase. Incubations of 0·6 ml contained: $4·2 \times 10^{-2}$ M Tris buffer, pH 7·4; 0·2 ml microsomal suspension; $4·9 \times 10^{-4}$ M UDPGA,

and were incubated for 30 min at 37°. The method was otherwise the same as at pH 8·9.

The protein content of liver fractions (diluted 50–100-fold with1 % sodium deoxycholate[18]) was determined by the method of Miller,[19] using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

All animals fed the protein-free diet lost weight (Table 1) and showed other symptoms of protein-deficiency, e.g. increased irritability when being handled. The immature rats showed a pronounced loss of hair.

As expected[6,20] the protein-free diets produced a lower total protein, microsomal protein and soluble protein content in the liver than was observed in the control groups, fed 18 per cent protein diets. Adult rats fed the protein-free diet had lower liver weights than the control group but no similar effect was observed with the immature rats (Table 1).

Tables 2 and 3 show that the activities of UDP-glucuronyltransferase and sulphotransferase, unlike those of NADPH-dependent drug metabolising enzymes, are not markedly decreased by feeding protein-free diets to rats. On the contrary, a marked increase of UDP-glucuronyltransferase activity was observed in the protein deficient animals. These conclusions are valid when the data is expressed in terms of "activity per g liver", "activity per mg protein" in soluble or microsomal fraction or "activity per liver from 100 g body weight". On a "per g of wet liver" basis the conjugation of PNP and of OAP with glucuronic acid in immature rats was 64 and 71 per cent higher respectively than in the controls. In adult rats the corresponding figures for the group fed *ad lib.* on the protein-free diet, ${}_0A$, were PNP conjugation 24 per cent higher, OAP conjugation 56 per cent higher. For the force-fed group, ${}_0A_F$, PNP conjugation was 75 per cent higher and OAP conjugation 181 per cent higher than in the corresponding control group. These differences are even more marked when the results are expressed "per liver from 100 g body-weight" or "per mg microsomal protein", Table 2. The increases in UDP-glucuronyltransferase activity in immature and adult rats are quantitatively similar and thus this response to protein-free diets seems to be independent of age.

It has been reported that when rats are fed *ad lib.* on protein-free diets[8] or diets with amino-acid deficiencies[21] they reduce their calorie intake to sub-normal levels as an adaptive response. The livers from such animals appear histologically more normal than if the same diets had been force-fed in amounts equivalent to a normal calorie intake level. Force-feeding such diets thus induces a more severe dietary stress than when the same diets are given to rats *ad lib.* In the present study, if there is a relationship between the increase in activity of UDP-glucuronyltransferase and the degree of protein-deficiency, we might expect group ${}_0A_F$, the force-fed group, to have a higher UDP-glucuronyltransferase activity in the liver than group ${}_0A$, the group fed *ad lib.* since the calorie intake of group ${}_0A_F$, although 25 per cent lower than the control, group ${}_{18}A$, was 50 per cent greater than group ${}_0A$. As can be seen in Table 2, UDP-glucuronyltransferase activities of group ${}_0A_F$ are significantly higher than group ${}_0A$ for PNP conjugation. When expressed "per g liver" and "per liver from 100 g body-weight", $P<0{\cdot}01$. Expressed as "per mg microsomal protein", $P<0{\cdot}05$. (A similar effect may occur with OAP as substrate but the number of experiments made was

TABLE 1. INITIAL AND FINAL BODY-WEIGHT, LIVER-WEIGHT, LIVER-WEIGHT AS A PERCENTAGE OF BODY-WEIGHT AND PROTEIN CONTENT OF LIVER AND LIVER FRACTIONS OF RATS FED EITHER A PROTEIN-FREE DIET OR AN 18 PER CENT PROTEIN DIET

Group*	Body-weight (g)		Liver-weight (g)	Liver-weight as % final body-weight	Liver protein (mg/g wet liver)		
	Initial	Final			Homogenate	Microsomal fraction	Soluble fraction
$_0$I	71 ± 2 (10)	65 ± 2 (10)	3·0 ± 0·2 (10)	4·61 ± 0·19 (10)	141 ± 7 (5)	8·0 ± 0·4 (3)	56 ± 5 (5)
$_{18}I_R$	70 ± 2 (10)	90 ± 2 (10)	3·4 ± 0·3 (10)	3·78 ± 0·29 (10)	204 ± 12 (5)	10·5 ± 1·0 (5)	73 ± 5 (5)
$_0$A	127 ± 3 (12)	104 ± 3 (12)	4·6 ± 0·2 (12)	4·4 ± 0·1 (12)	153 ± 8 (6)	12·2 ± 0·7 (6)	86 ± 5 (6)
$_{18}$A	129 ± 3 (12)	164 ± 14 (12)	8·7 ± 0·2 (12)	5·3 ± 0·1 (12)	181 ± 11 (6)	17·2 ± 1·5 (6)	99 ± 7 (3)
$_0A_F$	132 ± 3 (8)	116 ± 4 (8)	5·1 ± 0·2 (8)	4·4 ± 0·1 (8)	144 ± 8 (8)	12·6 ± 0·4 (8)	77 ± 3 (8)

Mean values ± S.E. Figures in parentheses are numbers of observations.

* Code for groups: A, adult rats; I, immature rats; O, protein-free diet; 18, 18 per cent protein diet; R, restricted-feeding; F, force-fed. Groups not marked R or F are fed *ad lib.*

TABLE 2. UDP-GLUCURONYLTRANSFERASE ACTIVITIES IN LIVER MICROSOMES FROM RATS FED EITHER AN 18 PER CENT PROTEIN DIET OR A PROTEIN-FREE DIET

		Immature rats					
		p-nitrophenol conjugation			*o*-aminophenol conjugation		
	Group*		% difference from group $_{18}I_R$	P value		% difference from group $_{18}I_R$	P value
Activity per g wet liver	$_{18}I_R$	894 ± 70 (5)			70 ± 10 (5)		
	$_{0}I$	1465 ± 72 (5)	+ 64	<0·01	120 ± 19 (5)	+ 71	<0·05
Activity per mg protein in fraction	$_{18}I_R$	85 ± 10			6·7 ± 1·1 (5)		
	$_{0}I$	183 ± 13	+ 115	<0·01	15·0 ± 2·5 (5)	+ 124	<0·01
Activity per liver from 100 g body weight	$_{18}I_R$	3380 ± 410			264 ± 33 (5)		
	$_{0}I$	6760 ± 590	+ 100	<0·01	554 ± 59 (5)	+ 110	<0·01

		Adult rats					
		p-nitrophenol conjugation			*o*-aminophenol conjugation		
	Group*		% difference from group $_{18}A$	P value		% difference from group $_{18}A$	P value
Activity per g wet liver	$_{18}A$	1780 ± 55 (6)			107 ± 18 (4)		
	$_{0}A$	2210 ± 80 (6)	+ 24	<0·01	167 ± 36 (4)	+ 56	<0·1
	$_{0}A_F$	2800 ± 130 (8)	+ 57	<0·001	301 ± 95 (4)	+ 181	<0·05
Activity per mg protein in fraction	$_{18}A$	103 ± 9			6·2 ± 1·2		
	$_{0}A$	181 ± 12	+ 75	<0·001	13·7 ± 3·1	+ 121	<0·05
	$_{0}A_F$	222 ± 13	+ 115	<0·001	23·9 ± 7·3	+ 286	<0·05
Activity per liver from 100 g body weight	$_{18}A$	9490 ± 350			568 ± 98		
	$_{0}A$	9725 ± 440	+ 3	N.S.	736 ± 162	+ 30	<0·25
	$_{0}A_F$	12,405 ± 700	+ 31	<0·01	1331 ± 410	+ 134	<0·1

Activities are mean values nmoles substrate conjugated per hour ± S.E. Figures in parentheses are numbers of observations. P values obtained using Student *t*-test.

* Code for groups: A, adult rats; I, immature rats; O, protein-free diet; 18, 18 per cent protein-diet; R, restricted feeding; F, force-fed. Groups not marked R or F are fed *ad lib.* N.S.—difference not significant (P > 0·05).

TABLE 3. SULPHOTRANSFERASE ACTIVITIES IN SOLUBLE FRACTION OF LIVER FROM RATS FED EITHER AN 18 PER CENT PROTEIN DIET OR A PROTEIN-FREE DIET

	Group*	*p*-nitrophenol conjugation	% difference from group $_{18}I_R$	P value	Dehydroepiandrosterone conjugation	% difference from group $_{18}I_R$	P value
Immature rats							
Activity per g wet liver	$_{18}I_R$	2240 ± 170 (5)			750 ± 110 (5)		
	$_0I$	2000 ± 200 (5)	−18	N.S.	440 ± 60 (5)	−42	<0·05
Activity per mg protein in fraction	$_{18}I_R$	34 ± 3			10·2 ± 1·6		
	$_0I$	36 ± 5	+ 6	N.S.	7·8 ± 1·2	−31	N.S.
Activity per liver from 100 g body weight	$_{18}I_R$	9200 ± 1200			2810 ± 330		
	$_0I$	9200 ± 1200	0		2010 ± 270	−28	N.S.
Adult rats							
Activity per g wet liver	$_{18}A$	2700 ± 246 (4)			350 ± 54 (4)		
	$_0A$	2300 ± 300 (4)	−15	N.S.	300 ± 77 (4)	−14	N.S.
	$_0A_F$	2100 ± 210 (4)	−22	N.S.	380 ± 59 (4)	+ 9	N.S.
Activity per mg protein in fraction	$_{18}A$	27·3 ± 3·0			3·54 ± 0·60		
	$_0A$	26·7 ± 3·9	− 2	N.S.	3·49 ± 0·92	− 2	N.S.
	$_0A_F$	27·3 ± 2·9	0	N.S.	4·94 ± 0·79	+40	N.S.
Activity per liver from 100 g body weight	$_{18}A$	14,340 ± 1370			1860 ± 295		
	$_0A$	10,130 ± 1415	−29	<0·05	1320 ± 345	−29	N.S.
	$_0A_F$	9290 ± 1070	−35	<0·01	1680 ± 277	−10	N.S.

Activities are mean values nmoles substrate conjugated per hour ± S.E. Figures in parentheses are numbers of observations. P values obtained using Student *t*-test.

* Code for groups: A, adult rats; I, immature rats; O, protein-free diet; 18, 18 per cent protein-diet; R, restricted feeding; F, force-fed. Groups not marked R or F fed *ad lib*. N.S.—difference not significant (P > 0·05).

insufficient to establish this conclusively.) Thus, protein-deficiency alone increases the level of UDP-glucuronyltransferase activity, but when protein-deficiency is accompanied by a subnormal calorie intake a much smaller increase of enzyme activity is observed.

Sulphotransferase activity in the "high speed" supernatant fraction of liver, Table 3, was relatively unaffected by protein-deficiency. In the immature rat experiment DHEA conjugation was significantly lower in protein-deficient rats when expressed "per g liver" but PNP conjugation was the same in the two groups of immature rats. In the experiments with adult rats sulphotransferase activities in the protein-deficient groups, ${}_0A$ and ${}_0A_F$, measured with both PNP and DHEA as substrates were not significantly different from the control group ${}_{18}A$ when expressed "per g liver" or "per mg soluble protein". When these results are expressed "per liver from 100 g body-weight", the sulphotransferase levels in the protein-deficient groups are significantly lower than in the control group, with PNP as substrate, and this is mainly due to the lower ratio of liver-weight to body-weight in groups ${}_0A$ and ${}_0A_F$.

In order to examine the possibility that the apparent diet-dependent changes in the activity of UDP-glucuronyltransferase are wholly or partly due to change of microsomal UDPGA-pyrophosphatase levels, this enzyme was assayed in both immature and adult rats. There was no significant difference in microsomal UDPGA-pyrophosphatase activity between the two groups of immature rats when the enzyme activity was measured at its pH optimum, 8·9 (Group ${}_{18}I_R$, 27·2 $\pm$4·9 and group ${}_0I$, 24·8 $\pm$ 4·2 μmoles UDPGA hydrolysed per g wet liver per hour $\pm$S.E.). With adult rats, UDPGA pyrophosphatase activity was measured in microsomes at pH 7·4 under conditions comparable to those employed for the assay of UDP-glucuronyltransferase. No significant differences were observed between the three groups of rats (Group ${}_{18}A$, 3·08 $\pm$ 0·38; group ${}_0A$, 2·46 $\pm$ 0·27 and group ${}_0A_F$, 2·40 $\pm$ 0·23 μmoles UDPGA hydrolysed per g wet liver per hour $\pm$ S.E.). These results indicate that it is unlikely that differences in UDPGA-pyrophosphatase activity are the cause of the observed differences in UDP-glucuronyltransferase activity between control and protein-deficient rats. Further support for this conclusion was obtained in experiments in which UDP-glucuronyltransferase was assayed in microsomes from livers of adult rats using a concentration of UDPGA ten times higher than that used in the routine assays (where large concentrations of UDPGA were not economically feasible). With the higher UDPGA concentration any effect of UDPGA-pyrophosphatase is expected to be lower because of the closer approach to saturation of UDP-glucuronyltransferase with respect to this substrate. In spite of this the activities of UDP-glucuronyltransferase in protein-deficient rats were still markedly higher than in control rats. For example in one experiment with PNP as substrate, the relative UDP glucuronyltransferase activities (per g wet liver) at the lower UDPGA concentration ($4·9 \times 10^{-4}$ M) were ${}_{18}A$, 1·0; ${}_0A$, 1·43; ${}_0A_F$, 1·64 whilst at the high UDPGA concentration ($4·9 \times 10^{-3}$ M) the corresponding relative activities were ${}_{18}A$, 1·0; ${}_0A$, 1·68; ${}_0A_F$, 1·79. Similar results were obtained with *o*-aminophenol as substrate.

Another possible source of apparent difference of UDP-glucuronyltransferase activity between the various groups of rats is differences in the level of β-glucuronidase activity which although optimally active at pH 5·5, might hydrolyse some glucuronide conjugate at pH 7·4. However, boiled solutions of potassium hydrogen saccharate, known to contain a highly specific inhibitor of β-glucuronidase,[22] had no significant

effect on activity of UDP-glucuronyltransferase in microsomes from control and protein-deficient rats and it was concluded that β-glucuronidase is not sufficiently active in these preparations to interfere with UDP-glucuronyltransferase activity measurements.

Thus it is apparent that protein deficiency in rats causes a real increase in UDP-glucuronyltransferase activity. There is some evidence that conjugation of PNP and OAP with glucuronic acid, the substrates used in this investigation, is catalysed by separate enzymes.[23] If this is so, then the results suggest that acute protein deficiency produces a general increase in UDP-glucuronyltransferase activities. Adlard, Lester and Lathe[24] found no alteration in the rate of conjugation of bilirubin with glucuronic acid in an *in vitro* liver slice system when rats had been fed a protein-free diet for 4 days. However, factors other than the activity of the UDP-glucuronyltransferase are important in determining the rate of glucuronide conjugation in liver slice systems, e.g. the rate of synthesis of UDPGA.

The biochemical changes underlying the higher than normal UDP-glucuronyltransferase activities in protein deficient rats are not clear. Such activities would result if these rats were able to maintain the normal amount of enzyme protein in the liver (or even increase it) because the body-weight, liver weight and liver protein contents of these animals is below normal. However, this seems unlikely in view of the general decrease in protein content of the liver and liver fractions brought about by the protein-free diet.

It seems more likely that the increased levels in microsomal fractions from protein deficient rats is due either to increased catalytic activity of the enzyme or perhaps to increased stability *in vitro* or indeed to both these factors. Recent studies[25] have shown that the activity of guinea-pig liver microsomal UDP-glucuronyltransferase depends on the structural integrity of the microsomal membrane, particularly its phospholipid components. One may speculate that increased catalytic activity in protein deficient rats might result indirectly from an effect of the diet on the structure of the endoplasmic reticulum. Such changes of membrane structure might also affect enzyme stability; however, we found that the ratio of UDP-glucuronyltransferase activity in the microsomal fraction, where some activity is lost during preparation, to that in the 12,000 *g* supernatant fraction of the liver homogenate was the same in protein-deficient and normal rats, suggesting that difference of stability is not a major factor.

The foregoing observations bring out an important difference between liver conjugation reactions with glucuronic acid and sulphate and the NADPH-dependent drug metabolising system. The latter group of enzymes in both male and female rats is considerably decreased in activity by protein deficient diets. Kato, Oshima and Tomizawa[6] observed that after 4 days of feeding a protein-free diet to male rats the rate of metabolism of several drugs by NADPH-dependent enzymes was decreased both *in vivo* and in microsomal preparations *in vitro*. With pentobarbital oxidation and aminopyrine *N*-demethylation the rate of metabolism *in vitro* was only 20–25 per cent of that observed with control rats fed on 18 per cent protein diet. McLean and McLean[4] in similar feeding studies found that the rate of aminopyrine *N*-demethylation and benzopyrine hydroxylation in rat liver was below 20 per cent of that observed with control animals fed a 30 per cent protein diet. Unpublished observations from this laboratory* have shown that feeding a 3 per cent protein diet to male rats for 4

* Dr. C. Furst (1967).

days decreased the *N*-demethylation of aminopyrine and the hydroxylation of aniline to 30 per cent and reduction of *p*-nitrobenzoic acid to 50 per cent of that observed with control rats fed a diet containing 30 per cent protein. These observations have indicated that *increasing* the protein content of the diet given to rats produces effects similar to those seen after the administration of phenobarbital. Liver weight and liver microsomal protein are increased together with the NADPH-dependent drug metabolising enzyme activities. They have led to the view that the level of protein in the diet influences the rate of NADPH-dependent drug metabolism by regulating the biosynthesis of microsomal protein.[6] Kato and Takanaka[3] have suggested that such a control is mediated through the anabolic action of circulating androgenic hormones. Under normal conditions it is assumed that androgenic hormones maintain the activities of NADPH-dependent drug metabolising enzymes of liver microsomes and that these enzymes are easily decreased in activity by abnormal physiological states such as protein-deficiency, starvation[5] and alloxan diabetes.[26] The present investigations and others indicate that the activity of UDP-glucuronyltransferase is not under this type of control. The activity of UDP-glucuronyltransferase in male rats is not decreased by reducing protein-intake; neither is it decreased in activity by starvation[27] or by alloxan diabetes.[28]

The rate of conjugation of drugs and foreign compounds with glucuronic acid and with sulphate in the intact liver depends on the activity of the respective transferases and also on the availability of the respective substrates UDPGA and PAPS. These factors together with others such as the rate of hydrolysis of conjugated compounds by β-glucuronidase and sulphatase in the liver, will be important in determining the rate and extent of conjugation of a drug or foreign compound prior to its elimination from the body. It seems probable that the rate of sulphate conjugation *in vivo* is lower than normal in protein deficient rats. Although we have shown that sulphotransferase activity is normal in such animals, the availability of sulphate ions (and therefore of PAPS), usually derived from dietary protein, has been reported to be a factor limiting the rate of drug sulphation in rats fed protein-deficient diets.[29] A similar dependence on dietary protein does not apply with respect to glucuronide conjugation. UDPGA is synthesised from glucose-l-phosphate which is present, for example as a constituent of liver glycogen. In protein-deficient rats the content of glycogen in the liver is higher than normal.[4] In immature and adult male rats the higher than normal activity of UDP-glucuronyltransferase suggests that conjugation with glucuronic acid may proceed at an elevated rate *in vivo*. In fact this possibility seems highly likely since the glucuronide conjugating system in rats has a high capacity for handling drugs and foreign compounds[30] and the rate of glucuronide formation seems to depend primarily on the concentration of drug or metabolite requiring conjugation and on the catalytic activity of UDP-glucuronyltransferase.[30,31]

It can be expected that the fate of many drugs and foreign compounds will differ from normal in protein-deficient rats and as a consequence the pharmacological activity and toxicity of such substances will alter. Experiments are in progress on the effect of protein-free diets on drug metabolism in rats with a view to obtaining information on the efficiency of glucuronic acid and sulphate conjugation *in vivo*.

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