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SHORT COMMUNICATION

Effects of dietary protein deficiency on the conjugation of foreign compounds in rat liver

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Although it is well known that the rate of metabolism of drugs may be altered by changes in various physiological and dietary conditions (see, for example, Kato, Takayanagi & Oshima, 1969), the specific effect of dietary protein level on drug metabolism has received relatively little attention.

It has recently been shown that the activities of microsomal enzyme systems responsible for some drug oxidations are markedly reduced in protein-deficient rats: these effects have been observed both *in vivo* (Kato, Oshima & Tomizawa, 1968) and with liver homogenate and microsomal preparations *in vitro* (McLean & McLean, 1966; Kato & others, 1968). Unpublished work by Dr. C. Furst, in this laboratory, is in agreement with a number of these findings.

The effect of protein deficiency on conjugation, another important pathway of drug metabolism, has not hitherto been investigated although a recent study of the effect of dietary protein on the glucuronidation of bilirubin in rat liver slices (Adlard, Lester & Lathe, 1969) suggests that this reaction is not affected by dietary protein level in the same way as is drug oxidation.

Preliminary results are now presented of the effects of protein-deficient diets on the rates of conjugation, as glucuronides or sulphates, of foreign compounds by liver transferases.

Method

Immature male Wistar rats (65–75 g), kept at 22–24°, were fed freely on a protein-free synthetic diet while control animals received an 18% casein synthetic diet (McLean & McLean, 1966). The control diet was restricted so that the calorie intake of both groups was approximately the same (experimental animals, 32 kcal (134 kJ)/100 g rat daily; control animals 36 kcal (150 kJ)/100 g daily). After seven days the rats were killed and their livers homogenized in 9 volumes of ice-cold 0·15M KCl. Each homogenate was then centrifuged (12 000 *g*, 10 min, 4°) and a microsomal fraction prepared from the resulting "low-speed" supernatant by centrifuging (105 000 *g*, 1 h, 4°). The "high-speed" supernatant was retained and the sedimented microsomes were resuspended in 0·15M KCl. They were recentrifuged (105 000 *g*, 1 h, 4°) and finally resuspended in sufficient 0·15M KCl so that 1 ml suspension was equivalent to 500 mg of liver.

Activities of conjugating enzymes were then assayed in the "low-speed" supernatant, "high-speed" supernatant and microsomal suspension. Glucuronyltransferase was assayed by measuring the rate of conjugation of *p*-nitrophenol (PNP), (Isselbacher, 1956) and *o*-aminophenol (OAP), (Dutton & Storey, 1962). Sulphotransferase was assayed by measuring the conjugation of PNP and dehydroepiandro-

sterone, DHEA, by a method based on that described for these substrates by Nose & Lipmann (1958) in which the sulphate conjugate was estimated as a complex with methylene blue.

Uridine diphosphoglucuronic acid (UDPGA) pyrophosphatase was assayed by the method of Ogawa, Sawada & Kawada (1966), the released glucuronic acid being determined according to Nir (1964). Protein was determined by the method of Miller (1959), using bovine serum albumin as a standard, liver fractions being diluted with 1% sodium deoxycholate (Juchau & Fouts, 1965).

Results

Animals fed the protein-free diet lost weight and showed other symptoms of protein deficiency including loss of hair and increased irritability when being handled. Their average calorie-intake per day was greater than maintenance requirements (Njaa, 1965); they are thus unlikely to have been calorie deficient. The control animals, on the other hand, increased steadily in weight and appeared to develop normally. The final body weights of the two groups of rats are given in Table 1. The liver weights of the two groups were the same but the protein contents (per g wet liver), of the whole liver homogenates and fractions from the protein-deficient rats, were lower than those of the control animals (Table 1).

Table 1. *Final body-weight, liver weight and protein content* (mean ± s.e.) *of liver fractions of rats fed either* 18% *protein diet or protein-free diet for* 7 *days*

	Final body weight (g)	Liver weight (g)	Protein content (mg/g wet liver)		
			Whole liver homogenate	Microsomal fraction	"High-speed" supernatant fraction
18% protein diet	90 ± 2 (10)	3·4 ± 0·3 (10)	204 ± 12 (5)	10·5 ± 1·0 (5)	73 ± 5·2 (5)
Protein-free diet	65 ± 2 (10)	3·0 ± 0·2 (10)	141 ± 7 (5)	8·0 ± 0·4 (3)	56 ± 4·5 (5)
% change with protein-free diet	−28*	−13	−31*	−26	−23*

* $P < 0{\cdot}05$ at least.

Figures in parentheses are numbers of observations; with protein determinations pairs of livers were pooled.

The activities of glucuronyltransferase in "low-speed" supernatant or microsomal fractions, were markedly elevated in the protein-deficient animals (Table 2). When the results are expressed as "n mol conjugated/g wet liver h^{-1}" the rate of conjugation of PNP in the "low-speed" supernatant was 59% higher. In the microsomal fraction, the conjugation of PNP was 64% higher, and of OAP, 71% higher than in the corresponding controls. The differences are even more marked when the results are expressed "per liver from 100 g body weight" (Fig. 1, Table 2) or "per mg microsomal protein" (Table 2).

Sulphotransferase activity in the "high-speed" supernatant fraction was not affected in the same manner (Table 2, Fig. 1). With DHEA as substrate the activity in protein-deficient rats was lower than in controls but with PNP as substrate it was not significantly affected.

Table 2. *Glucuronyltransferase and sulphotransferase activities in liver fractions of rats fed either 18% protein diet or protein-free diet for 7 days. Values in nmol compound conjugated per hour* (mean ± s.e. of 5 observations each on 2 pooled livers)

		Glucuronyltransferase			Sulphotransferase	
		"Low-speed" supernatant fraction	Microsomal fraction		"high-speed" supernatant fraction	
		PNP	PNP	OAP	PNP	DHEA
Activity per g wet liver	18% protein diet	3990 ± 330	894 ± 70	70 ± 10	2440 ± 170	750 ± 110
	Protein-free diet	6360 ± 380	1465 ± 72	120 ± 19	2000 ± 200	440 ± 60
	% change	+59†	+64†	+71*	−18	−42*
Activity per liver from 100 g body weight	18% protein diet	15 000 ± 1900	3380 ± 410	264 ± 33	9200 ± 1200	2810 ± 330
	Protein-free diet	29 400 ± 2800	6760 ± 590	554 ± 59	9200 ± 1200	2010 ± 270
	% change	+95†	+100†	+110†	0	−28
Activity per mg protein in fraction	18% protein diet	—	85 ± 10	6·7 ± 1·1	34 ± 3	10·2 ± 1·6
	Protein-free diet		183 ± 13	15·0 ± 2·5	36 ± 5	7·8 ± 1·2
	% change		+115†	+124†	+6	−31

PNP, *p*-nitrophenol; OAP, *o*-aminophenol; DHEA, dehydroepiandrosterone.
* $P < 0.05$. † $P < 0.01$, at least.

The most remarkable result of these experiments is the elevated glucuronyltransferase activity observed with the protein-deficient rats. This enzyme is microsomal, like the oxido-reductases involved in drug metabolism, yet the activity is affected by protein deficiency in a radically different way (Fig. 1).

This effect cannot be accounted for by differences in activity of microsomal UDPGA pyrophosphatase. Activities of this enzyme were not significantly different in the experimental and control animals, when assayed either at pH 8·9, the optimum pH for the pyrophosphatase, or at pH 7·4, the pH used for assay of glucuronyltransferase (at pH 8·9 controls showed 27·2 ± 4·9, experimental animals 24·8 ± 4·2 μ mol/g

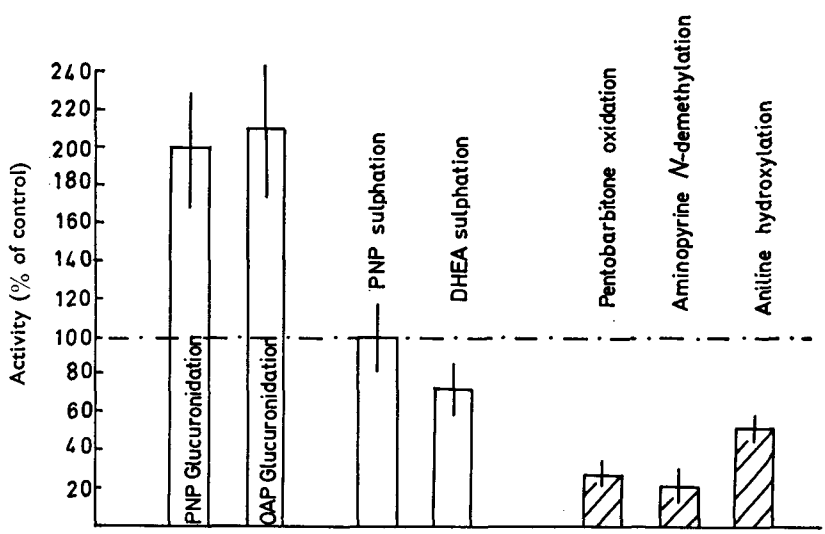

FIG. 1. Effects of protein-free diet on liver glucuronyltransferase and sulphotransferase activities (per liver from 100 g body weight, Table 2). Hatched columns are oxidoreductase activities of animals fed a protein-free diet (Kato, Oshima & Tomizawa, 1968). Control animals fed 18% protein diet. Vertical bars on top of columns indicate standard error.

wet liver h^{-1}). Furthermore, it is unlikely that the differences in glucuronyltransferase activity can be due to differences in activity of β-glucuronidase in the microsomal fractions from the two groups of animals. β-Glucuronidase is only weakly active at pH 7·4 and when a boiled solution of potassium hydrogen saccharate was added to assays of glucuronyltransferase in amounts sufficient to inhibit β-glucuronidase (Levvy, 1952), the differences between protein-deficient and control animals were unchanged.

Thus the results indicate that protein-deficiency induces a real increase in glucuronyltransferase activity. It is unlikely that this increase is due to synthesis of new enzyme protein, since the general level of protein synthesis is low in these animals. It is more likely to result from an increase in catalytic activity arising from alterations in the composition or structure of the endoplasmic reticulum, or both. Recent studies (Graham & Wood, 1969) have shown that the activity of guinea-pig liver microsomal UDP-glucuronyltransferase depends on the structural integrity of the microsomal membrane, particularly its phospholipid composition.

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