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SHORT COMMUNICATIONS

Effect of protein depletion in guinea-pigs on glucuronate conjugation of chloramphenicol by liver microsomes

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UNDERSTANDING of human dietary protein deficiency has been sought by many investigators through the study of laboratory animal models. The liver is the organ that has been found to be most rapidly and profoundly affected by deprivation of dietary protein. Many reports of the effects of protein depletion on the livers of laboratory animals have been in general agreement as to the morphological and biochemical changes. These have been reviewed by Porta and Hartroft.[1] They include diminished size of the liver without diminution of number of cells, deposition of fat and glycogen, loss of total liver protein, loss of microsomal protein, loss of cytoplasmic RNA with reduction in the ribosome population, reduction in number and increase in size of mitochondria, and diminution and distortion of the endoplasmic reticulum.

Since the liver is the principal site of metabolism of most drugs, it might be expected that alteration of the capacity to metabolize drugs would be among the effects of protein depletion on hepatic functions. Several studies in mice and rats have shown that starvation or deprivation of protein diminishes the capacity of hepatic microsomes to metabolize a number of drugs that are oxidized by the NADPH-dependent mixed function oxidase system.[2–7] There has been little study of the effects of protein depletion on the conjugating activity of the liver. Feeding rats a protein-free diet for 4 days did not affect the capacity of liver slices to conjugate bilirubin with glucuronic acid.[8] Liver microsomes from rats deprived of protein for 7 days were reported to have greater than normal activity in conjugating *p*-nitrophenol and *o*-aminophenol with glucuronic acid.[9] In the same experiments, conjugation of sulfuric acid with *p*-nitrophenol was unaffected and with dehydroepiandrosterone lowered by protein depletion.

One of the drugs extensively used in areas of the world where protein–calorie malnutrition is a most serious problem is the antibiotic chloramphenicol. The principal pathway of metabolism of chloramphenicol in man is conjugation with glucuronic acid to yield the 3-D-glucosiduronic acid.[10] This conjugation entails reaction between the acceptor molecule and UDP-glucuronic acid (UDPGA) catalyzed by UDP-glucuronyltransferase. This enzyme is found in several tissues, but in mammals the greater part is in the liver, occurring in the microsomal fraction of homogenates.[11]

The present study concerns the effect of dietary protein depletion on the rate of conjugation of chloramphenicol by hepatic microsomes of the guinea-pig, an animal sensitive to protein deficiency and furnishing hepatic microsomes with a conjugation rate suitable for experimental measurements. Conjugation has been studied by observing the disappearance of unchanged chloramphenicol on incubation with microsomes and UDPGA.

Male guinea-pigs were placed on the diets at the age of about 3 weeks when they weighed 200–250 g. The high protein diet contained 22 % protein of mixed cereal, soyabean and milk origin and the low protein diet 8 % protein as casein. Both diets were adequate in other nutrients, minerals and vitamins. The animals were maintained on these diets for 4–6 weeks.

3-Methylcholanthrene in a daily dose of 20 mg/kg, i.p., in corn oil was given for 3 days and the liver experiment performed on the fourth day.

Liver microsomes were prepared by differential centrifugation of homogenates in 0·154 M KCl. The material not sedimented at 9000 *g* but sedimented at 100,000 *g* is termed the microsomal fraction. The microsomal pellet was resuspended in 0·1 M Tris buffer, pH 7·5, containing 0·01 M $MgCl_2$, 2 ml of buffer for each gram of liver.

An incubation contained 1·0 ml of the microsomal suspension, 50 μg of chloramphenicol in 0·2 ml of water, and 1 mg of the ammonium salt of UDPGA in 0·1 ml of water. An incubation identical except for replacement of the UDPGA by an equal volume of water served as a control for recovery of chloramphenicol when no conjugation was occurring. It was shown that no significant disappearance of chloramphenicol occurs in the absence of UDPGA. The tubes containing the incubation mixtures were shaken at 38° for 30 min.

After incubation, 1·0 ml of a 0·35 M solution of $ZnSO_4$ followed, after shaking, by 1·0 ml of a 0·16 M solution of $Ba(OH)_2$ was added to each tube, which was then centrifuged. A 2·0-ml portion

of the supernatant was extracted with 5 ml of ethyl ether. (In this partitioning, substantially all of the chloramphenicol is in the ether phase and all of the glucuronide in the aqueous phase.) Of the ether phase, 4·0 ml was evaporated with a stream of dry N_2.

Chloramphenicol was determined by gas chromatography with D(−)-*threo*-1-*p*-nitrophenyl-2-acetamido-1,3-propanediol serving as internal standard. Chloramphenicol in the residue of the ether extract together with the internal standard was converted to the ditrimethylsilyl ethers by treatment with hexamethyldisilazene and trimethylchlorosilane in pyridine. Samples of the silylated derivatives were introduced into the gas chromatographic column in CS_2 solution. The column, which was 1·2 m long and packed with OV-17 silicone on Gas-Chrom Q, was operated at 200° with a He flow of 50 ml/min. A hydrogen flame detector operated at 300° was used. Standard samples of chloramphenicol were included in each run. In the range covered, the ratio of chloramphenicol peak height to internal standard peak height was linearly related to the amount of chloramphenicol in the sample.

The results of the experiments are presented in Table 1. Protein depletion resulted in significantly lower body weight, liver weight, and liver weight as a proportion of body weight. The mean rate of conjugation per gram of liver was 25 per cent lower in the group on the low protein diet than in that on the high protein diet. However, owing to the large variation within each group, this difference is not significant at the 0·05 level of probability. Because of the lower liver weights in the protein-depleted group, the conjugation rates do differ significantly when expressed as rates per total liver.

TABLE 1. CONJUGATION OF CHLORAMPHENICOL BY MICROSOMES OF LIVERS FROM GUINEA-PIGS FED HIGH AND LOW PROTEIN DIETS WITH AND WITHOUT TREATMENT WITH 3-METHYLCHOLANTHRENE*

Diet and treatment	Body wt (g)	Liver wt (g)	Liver wt/ body wt	Rate of chloramphenicol conjugation	
				Per g liver ($\mu g\ hr^{-1}\ g^{-1}$)	Per total liver ($\mu g\ hr^{-1}$)
High protein	337 ± 21	15·0 ± 1·2	0·044 ± 0·002	106·0 ± 12·9	1561 ± 202
High protein + 3-MC	341 ± 12	14·5 ± 0·6	0·043 ± 0·001	157·0 ± 11·1	2235 ± 155
Low protein	239 ± 13	9·0 ± 0·4	0·038 ± 0·001	79·3 ± 12·0	707 ± 117
Low protein + 3-MC	224 ± 6	8·8 ± 0·4	0·039 ± 0·001	90·7 ± 12·0	796 ± 117

*There were twelve animals in each group. Values shown are means ± standard errors of the means.

3-Methylcholanthrene caused significant increase of conjugation rate in the guinea-pigs on the high protein diet. The mean rate per gram of liver was increased by 48 per cent. In the protein-depleted animals, on the other hand, the mean rate per gram of liver was only 14 per cent higher in the group treated with 3-methylcholanthrene, a difference that is not statistically significant.

These experiments indicate that the ability of the guinea-pig to detoxify chloramphenicol, as judged by the conjugating capacity of the whole liver, is impaired by protein depletion. Since the decreased size of the protein-depleted liver does not reflect a decrease in number of hepatocytes, it may be inferred that UDP-glucuronyltransferase activity per cell is reduced by protein depletion. If chloramphenicol were administered to guinea-pigs in proportion to body weight, a smaller percentage of the dose would be conjugated per hour in the depleted animals since depletion causes a greater proportional decrease in conjugation rate per liver than in body weight. Administration in a chronic schedule might be expected to result in accumulation of the unconjugated drug in higher concentrations in plasma and tissues.

Protein depletion not only reduces the UDP-glucuronyltransferase activity in the liver but also reduces the capacity of the liver to respond to induction of the enzyme by 3-methylcholanthrene.

There is much evidence of the multiplicity of UDP-glucuronyltransferase both between species of mammals and toward different substrates in the same species.[11] The multiplicity of the enzyme with regard to species is a reason for some reservations in applying the results of laboratory animal studies to clinical situations. Furthermore, human protein deficiency is accompanied by great variations in other components of the diet and is complicated by a multitude of physiological and environmental factors as well as by unrelated disease. While animal studies are valuable in furnishing warnings of potential dangers, there is a need for study of drug metabolism in patients with such conditions as kwashiorkor and marasmus. Nevertheless, our present experiments with guinea-pigs give an indication that impaired conjugation of chloramphenicol might be expected in clinical conditions of protein

depletion and that the usually prescribed doses might result in accumulation of toxic levels of the unconjugated drug.

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Transport and binding of decamethonium in mouse kidney slices

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MANY quaternary ammonium compounds are concentrated in renal slices by specialized transport processes, in many respects similar to those involved in the tubular secretion of these substances by the intact kidney.[1] The polymethylene-bisquaternary ammonium compounds, hexamethonium (C_6) and decamethonium (C_{10}) are concentrated in kidney cortex slices in various species: C_6 and C_{10} in chickens, C_6 in cats and C_{10} in rats and mice.[2–4] It was a characteristic result in these experiments, that slice-to-medium concentration ratio of C_6 or C_{10} continued to increase linearly over a period of time without reaching a steady state level even after an incubation of several hours.[2–4] The continued accumulation of C_6 and C_{10} with time indicates that efflux of these two methonium compounds from renal slices is very slow, as compared to influx. Recent results from our laboratory, showing that no C_{10} efflux occurs from mouse and rat kidney slices preloaded with C_{10}, confirm this suggestion.[5,6] McIsaac[3] has suggested that methonium compounds are bound to a specific carrier, which is capable of transporting quaternary ammonium compounds into renal tubule cells. The continued accumulation of methonium compounds, should, according to McIsaac,[3] be due to a strong binding of these substances to some intracellular structure. If the latter hypothesis is correct, a steady state ratio (influx equals efflux) between the concentration of methonium compound in renal slices and in external medium would not be obtained until equilibrium has been established between bound and unbound (freely diffusible) methonium compound in the tissue. The above-mentioned studies were only performed with relatively low (10^{-5} M or less) C_6 or C_{10} concentrations in external media.[2–6] In the present study C_{10} uptake by mouse kidney slices was investigated with a high C_{10} concentration in