STIMULATION OF PROTEIN AND PLASMA ALBUMIN SYNTHESIS IN A CELL-FREE SYSTEM FROM LIVERS OF NEPHROTIC RATS*

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An accelerated hepatic synthesis of plasma proteins in rats with nephrosis, produced by means of anti-kidney sera, has been demonstrated in the intact animal (1), the isolated, perfused liver (2), and in liver slices (3). We have now investigated the incorporation of labeled amino acids into microsomal protein by an anaerobic microsome-supernatant fluid system similar to that employed by Campbell et al (4). The microsome-supernatant fluid system from nephrotic liver has been found to be about twice as active as that from the control. The RNA content of microsomes from nephrotic liver was elevated; the ratio of RNA to protein was increased from 0.12 in the control to 0.20 in the nephrotic microsomes. However, the increase in activity of the nephrotic system appeared to be largely due to the 100,000 g supernatant fraction.

RESULTS AND DISCUSSION

The specific activity of the protein from both the microsome and supernatant fractions from the nephrotic rat livers was greatly increased (Table 1). This increase is not due to a change in protein content of either fraction as may be calculated from data on specific activity and total incorporation of isotope in Table 1. Neither can it be accounted for by a decrease

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in the pool size of free amino acids, since this is not significantly altered in nephrotic liver (6). The experiments reported were highly reproducible quantitatively, regardless of the amino acids used (L-leucine-U-C^{14}, isoleucine-U-C^{14} or a mixture of L-amino acids-C^{14} from a chlorella protein hydrolysate), and the nephrotic system was found to be from 1.5 to 2.5 times more active than the control system.

TABLE 1

In Vitro Incorporation of C^{14}-Amino Acids into Protein of the Soluble and Microsomal Fractions from Livers of Control and Nephrotic Rats*

	Soluble Fraction		Microsomes	
	Specific Activity, cpm/mg	Total Incorporation, cpm	Specific Activity, cpm/mg	Total Incorporation, cpm
Control	54	44	226	275
Nephrotic	94	71	765	771

*The incubation mixture consisted of 0.4 ml. of microsomal suspension (corresponding to microsomes from 1 gm. of liver), 0.3 ml. of supernatant fraction (105,000 g), 0.3 ml. of medium containing phosphoenolpyruvate (PEP), ATP (cf. 4) and 0.3 microcurie of C^{14}-chlorella protein hydrolysate. Incubation was carried out for 40 minutes under nitrogen at 37° C. Casein hydrolysate was added at the end of the incubation, the system cooled to 0° and centrifuged for 45 minutes at 105,000 g to isolate microsomes and supernatant fluid. In all cases duplicate samples were incubated at 0° C for 40 minutes and the amount of radioactivity found in the purified protein, which was generally less than 10 per cent of that in the 37° sample, was subtracted from the latter. Protein was isolated by dissolving the microsomes in 0.4 per cent sodium deoxycholate and precipitating with cold 5 per cent trichloroacetic acid. Nucleic acid was removed with hot trichloroacetic acid and lipid removed with ethanol and ether. The protein was dissolved in 0.1 N NaOH, re-precipitated with trichloroacetic acid, redissolved in NaOH and assayed for radio-activity as previously described (1, 3). Protein was measured by the Folin procedure as modified by Lowry et al (5).

The relative effectiveness of nephrotic microsomes and supernatant fluid in stimulating protein labeling was assessed in the experiments reported in Table 2.

TABLE 2

In Vitro Incorporation of C^{14}-Amino Acids and Isoleucine-U-C^{14} into Microsomal Protein from Livers of Control and Nephrotic Rats.

Time of incubation at 37°C was 20 minutes. With the exception of isotopes employed, the medium was as in Table 1. (Control microsomes, C_m, control supernatant fraction, C_S, nephrotic microsomes, N_m, and nephrotic supernatant, N_S).

Experiment	Specific Activity, cpm/mg
1. $C_m + C_S$	443*
2. $N_m + N_S$	915*
3. $C_m + N_S$	780*
4. $N_m + C_S$	540*
5. $C_m + C_S$	142†
6. $C_m + C_S + N_S$	208†

* Labeled with C^{14}-chlorella protein hydrolysate

† Labeled with Isoleucine-U-C^{14}. In experiment No. 6, 0.15 ml. of nephrotic supernatant fraction was added to 0.15 ml. of control supernatant fraction, whereas in experiment No. 5, 0.3 ml. of control supernatant fraction was used.

The complete nephrotic system, $N_m + N_S$, was more than twice as active as the control (experiments No. 1 and 2, Table 2). Substitution of the nephrotic for the control supernatant fraction increased the level of incorporation by 77 per cent, (experiments No. 1 and 3), whereas substitution of the nephrotic supernatant by control supernatant decreased the activity in comparison with the complete nephrotic system (experiments No. 2 and 4). Furthermore, when the nephrotic supernatant fraction was added to the complete control system a further increase in activity was obtained (experiments No. 5 and 6).

Data plotted in Fig. 1 indicate that the stimulatory effect of the nephrotic supernatant fraction was not owing to use of inadequate amounts of con-

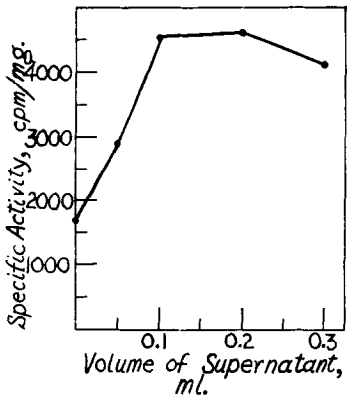

Figure 1

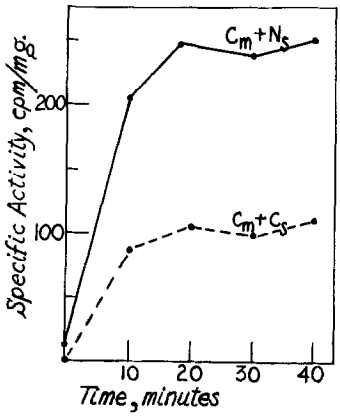

Figure 2

FIG. 1. In vitro incorporation of leucine-U-C^{14} into microsomal protein from liver of control rats in presence of varying amounts of the supernatant fraction.

The incubation mixture was that described in Table 1, except that leucine-U-C^{14} was used. The incubation time in all cases was 20 minutes at 37°C.

FIG. 2. In vitro incorporation of isoleucine-U-C^{14} into microsomal protein from liver of control rats in the presence of control and nephrotic supernatant fractions.

Broken line, control microsomes, C_m, with control supernatant fraction, C_s; solid line, control microsomes, with nephrotic supernatant fraction, N_s. The conditions were the same as those described in Table 1, except that isoleucine-U-C^{14} was used.

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trol supernatant fluid. Fig. 2 shows that the increased incorporation in the presence of nephrotic supernatant fraction occurred in the early stage of the incubation and was not due to a prolongation of the activity of the system.

In these experiments the radioactivity of the trichloroacetic acid insoluble protein was measured. Since in nephrosis metabolism is largely channeled into the synthesis of plasma proteins (1, 2), we have isolated albumin from the microsomes in a complete control and complete nephrotic system containing C^{14}-leucine of high specific activity. The microsomes were treated with 0.4 per cent sodium deoxycholate and the amount of albumin pre

sent in each system measured by an immunochemical method (cf. 3). A known amount of carrier rat albumin (in whole rat plasma) was then added and albumin isolated (cf. 7). The dialyzed aqueous albumin solution was then precipitated with plasma albumin antiserum and the albumin extracted from the immune precipitate by the same method (cf. 7), re-dialyzed and its specific activity determined. The original, control albumin and that from the nephrotic system had respective specific activities of 12,800 and 29,800 cpm/mg.

The cell-free system prepared from nephrotic liver appears to be one of the most promising for the study of the regulatory mechanisms involved in mammalian protein biosynthesis. Our studies are now directed toward an identification of the stimulatory factor or factors present in the supernatant fluid from nephrotic liver.

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