Effect of Cortisol Injection upon Amino Acid Incorporating Activity Of Rat Spleen Microsomes.* (31297)

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Feigelson(1) has reported that cortisone administration to adrenalectomized rats depressed radioactive glycine incorporation into acid-soluble adenine, RNA and protein of spleen to 68%, 84% and 83%, respectively, of control values. Pena *et al*(2) observed a marked decrease of leucine-C^{14} incorporation into the total protein of cell-free preparations of thymic tissue due to a single injection of cortisol. Gabourel and Comstock(3) showed a dose-response relationship between cortisol administration and reduced amino acid incorporation by rat thymus microsomes. Thus, it appears that glucocorticoid administration results in a depression of the protein synthesizing capacity of lymphoid tissues. This report describes a microsomal leucine-C^{14} incorporating system derived from rat spleen and the effects of daily cortisol administration on this system.

Material and methods. Injections and homogenization. Male Sprague-Dawley rats, weighing 250 to 300 g at sacrifice, were injected intraperitoneally with 5 mg of cortisol in 0.2 ml of 0.9% NaCl or with the same amount of saline every day for 3 days and were sacrificed on the fourth day. Medium A of Wust and Novelli(4) (0.35 M sucrose, 0.004 M $MgCl_2$, 0.0125 M KCl, 0.01 M mercaptoethanol and 0.05 M Tris buffer at pH 8.0) was used to prepare 20% spleen and 25% liver homogenates. The spleens from each group of rats (10 per group) were pooled prior to homogenization in a Teflon pestle-glass homogenizer having a clearance of 0.006 to 0.009 inch.

Liver pH 5 fraction. Smith and Koeppe (5) found that the spleen was not a good source of active pH 5 fraction in studies with cell-free amino acid incorporating systems from lymphoid tissues. Wust and Novelli(4) also found that the pH 5 fraction from spleen was rather inactive, likely due to the high soluble ribonuclease activity described by Eichely and Roth(6). For these reasons, pH 5 fractions from normal rat livers were used in the present studies. The 25% liver homogenate was centrifuged sequentially at 10,000 *g* for 10 minutes, 32,000 *g* for 20 minutes and 105,000 *g* for 1 hour. The pH of the 105,000 *g* supernatant was adjusted to 5.0 with 1 N acetic acid, and the mixture was centrifuged at 10,000 *g* for 10 minutes. The pellet was dissolved in Medium A for use.

Spleen microsomes. The spleen homogenates were centrifuged like the liver homogenates except the 32,000 *g* supernatant was carefully layered over 5 ml of Medium B (same as Medium A except the sucrose was 0.9 M) prior to centrifugation at 105,000 *g*. The resulting microsomal pellets were resuspended in Medium A, and the protein in the microsomal and pH 5 preparations was determined by a turbidimetric method(7).

Incorporating system. Incubations were carried out at 37° for 45 minutes. The complete system contained 0.1 ml each of 0.5 M Tris buffer at pH 7.6, 0.2 M $MgCl_2$, 0.1 M AMP, 0.01 M ATP, 0.002 M GTP, pyruvate kinase (200 μg/ml), and 0.0001 M L-leucine-UL-C^{14} (240 μc/μmole), and 0.25 ml of 0.08 M phosphoenolpyruvate (PEP). It also contained about 1.5 mg of microsomal protein, 2.2 mg of pH 5 fraction protein and water to bring the volume to 1.3 ml.

The reactions were stopped by addition of 5 ml of 5% trichloroacetic acid (TCA). The precipitated protein was washed(8) and dissolved in 98% formic acid. Aliquots were removed for protein determination(9) to insure that no protein had been lost during washing, and separate aliquots were dried on sample pans for counting in a gas-flow counter. In zero-time controls the 5 ml of 5% TCA was added to the reaction mixture prior to addition of microsomes and pH 5 fraction. The

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TABLE I. Requirements for Leucine-C^{14} Incorporation into Protein by Rat Spleen Microsomes.

System	CPM per mg microsome protein added	% of complete
Complete	10,650	100%
Minus microsomes	1,080	10
" pH 5 fraction	510	5
" Mg^{+2} ions	350	3
" pyruvic kinase	6,080	57
" ATP	6,480	61
" GTP	7,580	71
" AMP	9,400	89

radioactivity data were expressed as counts per minute (CPM) per mg of microsomal protein added to the incubation mixture.

Results. Requirements of the incorporating system. The results shown in Table I indicate the requirements of the system for leucine-C^{14} incorporation into TCA-precipitable protein. The AMP requirement was small but consistent. Earlier results demonstrated a requirement of 15 to 20 μmoles of PEP per incubation mixture for optimum incorporation.

Effect of pH 5 fraction. The effects of increasing amounts of pH 5 fraction upon leucine-C^{14} incorporation are shown in Fig. 1. The ratio of liver pH 5 fraction to spleen microsomes had to be at least one (on a protein basis) for optimum incorporation, therefore it was maintained at somewhat above this ratio in all subsequent experiments.

Effects of cortisol. Experiments were conducted to compare the leucine-C^{14} incorporating activity of microsomes from spleens of cortisol-injected rats with that of microsomes from spleens of control rats. The results of 3

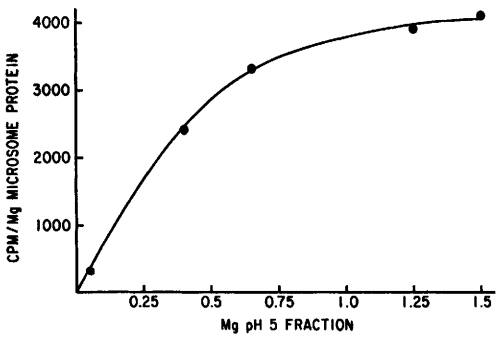

FIG. 1. Effect of pH 5 fraction upon incorporation of L-leucine-C^{14} by rat spleen microsomes. A constant 1.53 mg of microsomal protein was present in each reaction mixture.

such experiments are shown in Table II and indicate that daily 5 mg cortisol injections decreased incorporation to an average of about 60% of that of controls. As can be seen, the absolute counts varied from experiment to experiment, but the causes of these variations were not determined.

Discussion. The results demonstrating a decrease in leucine-C^{14} incorporating activity of rat spleen microsomes resulting from cortisol injections are consistent with those reported by Pena *et al*(2) and Gabourel and Comstock(3) for rat thymus. These workers were able to show significant decreases in incorporating activity of thymus microsomes 3 hours(2) and 12 hours(3) after a single injection of 5 mg/kg(3) to 5 mg/100 g(2) of cortisol. In the present work, a single injec-

TABLE II. Leucine-C^{14} Incorporation by Microsomes from Spleens of Cortisol- and Saline-Injected Rats.

Exp No.	Microsome preparation	CPM per mg microsome protein added*	% of saline
1	Saline	10,560	
	Cortisol	7,480	71
2	Saline	7,630	
	Cortisol	3,570	47
3	Saline	9,290	
	Cortisol	5,650	61

* Each value represents microsomes prepared from 10 pooled spleens and incubated in triplicate with a deviation of less than 5%.

tion of 2 mg of cortisol/100 g at 2.5 and 13 hours before sacrifice had no significant effect on the spleen microsome incorporating system. It should be noted that the levels of cortisol injected here and by others(2,3) are as much as 100 times that required to elicit an increase in rat liver tryptophan pyrrolase activity (Gray, unpublished observations). Further, in the present work, the results of preliminary experiments showed no difference in the incorporating activity of spleen microsomes from control and adrenalectomized rats. These observations raise questions as to the physiological significance of these effects of cortisol injections on microsomes from lymphoid tissue.

The decrease in incorporating activity resulting from cortisol injection must have been

associated with changes in the microsomes, because normal rat liver pH 5 fraction was used throughout. An attempt was made to detect the presence of possible inhibitory factors associated with spleen microsomes from cortisol-injected rats by adding such microsomes to a reaction mixture containing spleen microsomes from control rats. This approach seemed reasonable since Macleod *et al*(10) reported that steroid administration to mice resulted in increased ribonuclease activity in lymphosarcoma tumor and Wust and Novelli (4) observed a ribonuclease activity associated with rat spleen ribosomes which was increased by storage. However, addition of microsomes from spleens of cortisol-injected rats caused no inhibition of leucine-C^{14} incorporation by spleen microsomes from control rats.

Summary. L-leucine-C^{14} incorporation has been studied in cell-free systems containing microsomes from spleens of control and cortisol-injected rats and pH 5 fraction from the liver of a control rat. Daily injections of 5 mg of cortisol for three days followed by sacrifice on the fourth day resulted in spleen microsomes that had about 60% of the incorporating activity of microsomes from spleens of control rats.

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Effects of Chronic Excess Salt Ingestion: Lack of Gross Salt Retention in Salt-Hypertension.* (31298)

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Several years ago we reported that in a small series of patients with fixed NaCl intake, most of those with essential hypertension had a longer biological half-life ($T_{½}$) of ^{22}Na than did normotensive subjects on the same regimen(1,2). Indirect calculations suggested that the hypertensives had a larger mass of sodium with which the isotope exchanged, thereby providing an apparent explanation for the prolonged $T_{½}$ observed. This work was in conflict with the overwhelming body of evidence pertaining to essential hypertension in man: from the earliest observations by Dole *et al*(3) and ourselves(4) to the latest by Hollander, Chobanian, and Burrows(5) and Gifford *et al*(6) there had been agreement that patients with uncomplicated hypertension did *not* have an increase in total body sodium, as measured after equilibration with the radioactive isotopes of sodium.

The present report is concerned with our explorations of this subject experimentally. Because of evidence that potassium influences the development of hypertension(7-9), the behavior of potassium as well as sodium has been explored. To this end we have used 2 selectively inbred strains of rats of which one strain (the Sensitive or S strain) is genetically predisposed to develop rapidly severe hypertension from any one of several standard techniques, including a high salt diet, DOCA plus salt, unilateral renal artery compression without salt, cortisone, and ad-

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