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Incorporation of S^{35} from L-Methionine in Tumor-bearing and Protein-depleted Rats.* (26601)

ROBERT W. WANNEMACHER, JR. (Introduced by J. B. Allison)
Bureau of Biological Research, Rutgers University, New Brunswick, N. J.

Data have been presented to demonstrate an increase in the specific activity of certain plasma and tissue proteins in protein-depleted or tumor-bearing animals given S^{35} methionine. For example, Garrow found an increased uptake of S^{35} from labelled methionine in plasma proteins of depleted dogs(1) and malnourished children(2). Waterlow (3) reported a greater incorporation of S^{35} in the viscera of protein-depleted rats. Allison *et al.*(4) demonstrated an increased specific activity of serum proteins following an injection of S^{35} methionine into tumor-bearing or protein-depleted dogs. These and other data suggest a shift in the dynamic state of protein metabolism in depleted or tumor-bearing animals that could involve a number of cellular structures. The following experiments were performed to study the incorporation of S^{35} from labelled methionine into cellular proteins of liver and tumor tissues and into serum protein fractions of control, protein-depleted and tumor-bearing rats.

Methods. Male Wistar rats, 100-150 g, were divided into 3 groups of 30 animals each and placed on a diet containing 18% of casein as previously described by Allison *et al.* (5). On the first day of experiment a suspension of a Walker 256 carcinosarcoma was transplanted into one group, while on the seventh day another group was started on a protein-free diet. All animals were sacrificed after 17 days of experimentation. On day 7 of the experiment 10 rats from each group were placed in metal metabolism cages and daily food, urine and fecal collections were made for the next 10 days. The nitrogen concentration of the sample was determined by the micro-kjeldahl method.

At ½, 1, 2, 4, 6 and 12 hours before autopsy, 5 rats in each group were injected intraperitoneally with 0.2 mg of S^{35} L-methionine per kg of body weight. All rats were anesthetized with sodium pentobarbital (50 mg/kg) and sacrificed by exsanguination. The labelled tissue proteins were extracted and purified by the method of Zamecnik *et al.* (6). Liver and tumor tissues were fractionated into various cellular fractions by differential centrifugation. The nuclei were prepared in non-aqueous medium by the method of Carruthers *et al.*(7), and the mitochondria, microsomes, and cell sap were separated by a modification of the method of Hogeboom *et al.*(8). Serum was separated electrophoretically into various fractions by paper electrophoresis and dyed with alcoholic bromphenol blue, while total serum protein was determined by the copper sulfate falling drop method. All radioactive samples were counted in a windowless proportional flow counter and corrected for background, self-absorption, radioactive decay, back-scattering, geometry and body weight of the animals.

Results. The data in Table I demonstrate that the tumor-bearing rats were in positive nitrogen balance. If it is assumed that tumor tissue was essentially a "nitrogen trap", (9) then subtracting the tumor nitrogen from the total balance leaves the carcass (body minus tumor) near nitrogen equilibrium. During the same time period, the protein-depleted animals were in marked negative balance. These data are in agreement with these of Allison *et al.*(4) who reported a cor-

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TABLE I. Nitrogen Balance in Tumor-Bearing, Protein-Depleted and Control Rats Fed a Diet Containing 18% Casein for 10 Days. Each value is the mean for 10 animals.

Diet	Nitrogen intake	Urinary nitrogen	Fecal nitrogen	Nitrogen balance	Tumor nitrogen	Carcass nitrogen bal. (nitrogen bal. – tumor nitrogen)
	g/kg					
18% casein (control)	11.18 ±.26*	2.94 ±.11	1.68 ±.14	+6.53 ±.21		
18% casein (tumor-bearing)	9.00 ±.18	3.51 ±.12	1.12 ±.13	+3.94 ±.22	3.03 ±.66	+.91 ±.88
P.F.D. (depleted)	0	.41 ±.16	.84 ±.04	−2.14 ±.06		

* Stand. error.

relation between decrease in serum albumin concentration and loss in body nitrogen in both tumor-bearing and protein-depleted dogs.

Fig. 1 shows that the specific activity of the serum proteins of the tumor-bearing group (open circles) was initially significantly (P less than 0.01) greater than either the protein-depleted (solid circles) or control animals (crossed circles), and that this activity fell rapidly to control levels by the end of 12 hours. Throughout the experiment, however, the protein-depleted rats had a higher specific activity than the controls. These results confirm the results of Garrow in the dog(1) and in human infants(2) and the conclusion of Allison *et al.*(4) that protein-depletion produces increased uptake of S^{35} methionine which can be correlated with degree of body nitrogen loss. The increased uptake of S^{35} by the tumor-bearing rat, however, could not be related to loss in body ni-

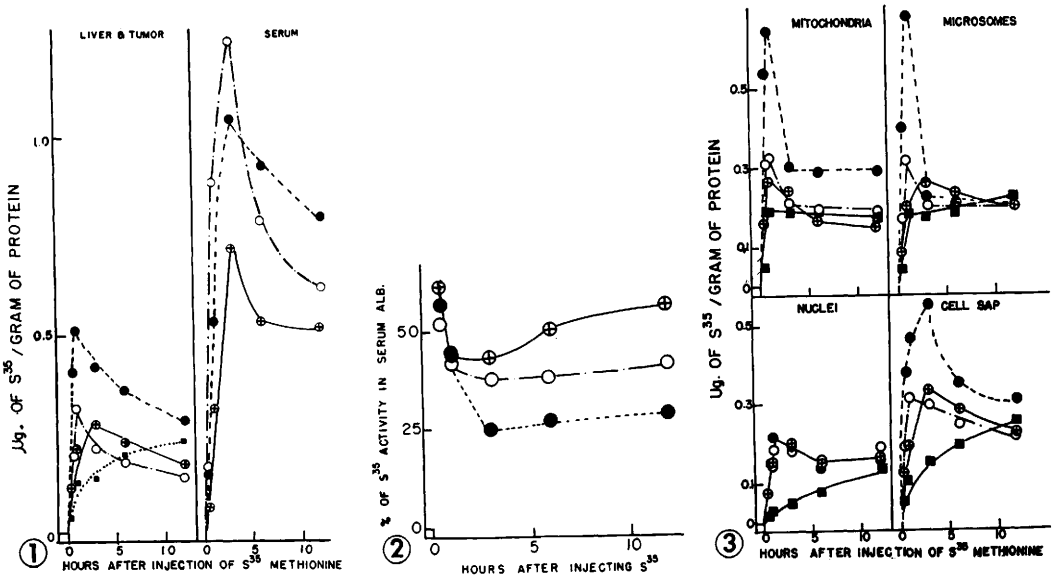

FIG. 1. Specific activity in μg of sulfur per g of protein of liver and tumor tissues and serum at various times after inj. of S^{35}-L-methionine. Each point is mean of 5 animals. Cross circles illustrate data using control animals, open circles tumor-bearing and closed circles protein-depleted rats. A square illustrates data from tumor tissue.

FIG. 2. Percent of total serum S^{35} activity in the albumin fraction at various times after inj. of S^{35}-L-methionine. Each point is mean of 5 animals. For significance of symbols see Fig. 1.

FIG. 3. Specific activity in μg of sulfur per g of protein of liver and tumor tissue of nuclei mitochondria, microsomes, and cell sap at various times after inj. of S^{35}-L-methionine. Each point is mean of 5 animals. For significance of symbols see Fig. 1.

trogen, results which are in agreement with those reported also for tumor-bearing dogs (4).

The average serum albumin concentrations were, control group, 2.72 ± 0.07, tumor-bearing 2.10 ± 0.10 and protein-depleted 1.77 ± 0.05 g per 100 cc. There was a lower percentage of total serum radioactivity in the albumin fraction in depleted rats than in the controls while the tumor-bearing animals fell in between (Fig. 2). These results can be interpreted to mean that a smaller amount of serum albumin was synthesized in depleted animals, and this decreased synthesis could be correlated with serum albumin concentration and body nitrogen loss.

Liver proteins of the depleted rats (Fig. 1 and 3) had a higher specific activity in the total proteins and in the mitochondria, microsomes and cell sap than was found in the control animals. Henderson *et al.*(10) and Thompson *et al.*(11) found that when rats were placed on a protein-free diet there was a decrease in serum and liver non-protein methionine. The concentration of S^{35} methionine would be relatively greater in an amino acid pool which was low in non-labelled methionine, and thus, a higher specific activity would be expected in the proteins synthesized from this pool. This concept, emphasized by Garrow(1,2), can explain the results found in serum and liver of protein-depleted rats. The liver nuclear proteins of the depleted animals, however, had a similar specific activity to that found in the control group. Because of the smaller amino acid pool, one would expect to find the values to be higher in the nuclear fraction of the liver in depleted rats. Therefore, it is possible that synthesis of liver nuclear proteins was decreased in the depleted animals. The specific activity of liver protein in tumor-bearing animals on the other hand, decreased below the values found for control rats 2 hours after injection of isotopic methionine. Subjecting these livers to differential centrifugation revealed (Fig. 3) that protein in nuclei, mitochondria, and microsomes had approximately the same specific activity as found in the control animals, while the cell sap fraction was initially higher in activity in the tumor-bearing animals and then fell below control values. As the specific activity of the liver protein (Fig. 1) of the tumor-bearing animals decreased, there was an increase in serum protein activity which then decreased as the tumor tissue (Squares with dotted line) continued to gain in isotopic activity. The data of several workers(12) indicated that tumor tissue has a preference for serum proteins as a source of amino acid for protein synthesis. It is possible that the tumor stimulated the liver to increase the synthesis of serum proteins which were then used by tumor tissue as source of amino acid for its rapid growth rate.

The nuclear proteins of the tumor tissue (Fig. 3) continued to increase in activity even 12 hours after injection of radioactive methionine. This fraction represented 40% of total tissue activity while in the liver it was 10% of total isotope concentration. Busch *et al.*(13), using 17 different radioactive amino acids, found a greater synthesis of nuclear protein in tumor tissue than in any other tissue studied. From these facts it appears that one of the major metabolic activities of tumor tissue was the production of nuclear proteins.

Summary. The specific activities of total liver protein and of the proteins of mitochondria, microsomes, and cell sap were higher in the protein-depleted rats than in the controls. Specific activities of these proteins in tumor-bearing rats, on the other hand, decreased below those in control animals. As the specific activity of the liver proteins of the tumor-bearing animals decreased, there was an increase in serum protein activity which then decreased as the tumor tissue continued to gain in isotopic activity. The data suggested that the protein-depleted animals had a decreased rate of synthesis of liver nuclear and serum albumin proteins. The data also indicated a rapid synthesis of nuclear protein in the tumors of tumor-bearing animals.

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Connective Tissue III. Dermal Chemical Response to Age.* (26602

J. C. HOUCK, L. DE ANGELO AND R. A. JACOB
Biochemical Research Laboratory, Children's Hospital Research Foundation, and Department of Biochemistry, Georgetown Medical School, Washington, D.C.

Several investigators have shown that the collagen concentration of connective tissue increases with age(1-5) while changes in the hexosamine content of skin have been reported to decrease(2), remain constant(3) and even increase(1) with age. Kao and McGavack(6) have indicated that soluble dermal protein concentration decreased with age while there was a significant increase in the scleroprotein content of the tissue.

We have studied the effect of age upon the chemical anatomy of rat skin as revealed using a sequential extraction technic for dissection of skin into ground substance, neutral and citrate soluble collagens and insoluble collagen and scleroprotein(7). These results are described below.

Material and methods. Six groups of 12 male Sprague-Dawley rats weighing an average of 100, 150, 200, 270, 350 and 500 g each were sacrificed by etherization and exsanguination. About 2 g of abdominal skin were collected from each animal, shaven and dissected clean of adhering fat, fascia and muscle. Tissue from each animal was minced, and aliquots removed. The remaining minced skins were pooled into 2 collections from 6 animals each. Five g aliquots from each tissue pool were extracted sequentially in the cold with 0.15M NaCl, 0.50M NaCl, and 0.50M citrate buffer, pH 3.6 as has been described previously(7).

These extracts, as well as samples of skin from each animal separately (0.5 g) were hydrolysed in 4N HCl for 8 hours, and analyzed in triplicate for their hexosamine(8), hydroxyproline(9) and nitrogen(10) content. The results were expressed in μmoles per mmoles of dermal nitrogen to minimize variations in skin water and fat content. Subtraction of the sum of the soluble components from total dermal concentration permits the calculation of the insoluble dermal content of these tissues. These results were expressed as per cent of total hexosamine, collagen and noncollagenous protein which was insoluble.

Insoluble non-collagenous protein was calculated from the fact that one mg of collagen contains about one μmole of hydroxyproline and 13.3 μmoles of nitrogen(11). Therefore, the contribution of collagen nitrogen to total dermal nitrogen may be determined. The amount of non-collagenous nitrogen solubilized, when subtracted from total non-collagenous nitrogen, permits the amount of insoluble non-collagenous protein to be calcu-

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