Connective Tissue IV. Synthesis and Turnover of Proteins in Tissues of Rats.* (26257)

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Neuberger *et al.*(1) reported that collagen of rat tendon is metabolically relatively inert. Harkness(2) reported that collagen disappears rapidly during the postpartum involution of rat uterus. Gould(3) found that collagenolysis appears in scorbutic guinea pigs. The present study is undertaken to determine the synthesis and turnover rates of collagen and elastin in various tissues.

Methods. Female rats, Wistar strain, 5 weeks of age were injected, intraperitoneally, with saline containing 7.8 μc of uniformly labeled C^{14}-lysine†/100 g body weight. Groups of 3-6 rats were sacrificed at 1, 4, 8 and 24 hours, 3, 10, 30, 40, 60 and 90 days after C^{14}-lysine had been administered. Tissue samples (0.1-0.5 g) from aorta, uterus, tendon and skin were homogenized with 10 ml normal saline in a VirTis "45"‡ homogenizer for 5 minutes at 50 setting. The homogenate was centrifuged at 27,000 $\times$ g for 30 minutes. The residue was extracted with 16 ml 0.1N NaOH at room temperature overnight and centrifuged at 105,000 $\times$ g in a Spinco Model L centrifuge for 1 hour. The resulting residue was extracted again with 10 ml 0.1N NaOH at room temperature for 2 hours. The supernatants of the saline and the 2 base extractions were combined, dialyzed against distilled water and evaporated to dryness. Soluble collagen (SC) was extracted from the dried specimen with 0.3 M trichloracetic acid (TCA) at 90°C(4). The residue from this extraction was nonscleroprotein (NSP).

The insoluble collagen (IC) was extracted from the basic residues mentioned above by use of 0.1N NaOH at 100°C for 15 minutes. This extraction was repeated. The 2 extracts thus obtained were combined and dialyzed against distilled water. The final residue was elastin (E). Proteins of each fraction, except in the case of E, for which, in tendon, skin and uterus, the amount was too low, were hydrolyzed in 6N HCl in a sealed tube. Nitrogen was determined by Conway microdiffusion method(5). Protein was calculated as 6.25 $\times$ N. Aliquots of the hydrolyzate were evaporated to dryness, neutralized and made up to a concentration of 0.5-1.0 mg protein/ml. One ml of the neutralized hydrolyzate was evaporated to dryness on a stainless steel planchette for radioactivity determination. Isotope counting was done by an automatic gas flow counter with a counting error of 2.5%. Half-lives were calculated according to Radiation Health Handbook and Glasstone(6,7).

Representative samples of soluble and insoluble collagen, respectively, were subjected to treatment by column chromatography according to the method of Moore and Stein (8). Chemical and radioactivity determinations were carried out by the method of Kao *et al.*(9).

Results. The synthesis and turnover of proteins in aorta of rat are shown in Fig. 1. Specific activity (SA) (cpm/mg of protein) of NSP reached its maximum, 220, at 24 hours and decayed with a half-life of 14 days. The SA of SC reached its maximum, 140, at 24 hours and decayed with a half-life of 14 days. The SA of IC reached its maximum, 42, at 10 days. From 10 to 40 days the SA decreased with a calculated half-life of 15 days. Between the 40th and 90th day there was no further decrease in the SA of IC. The SA of E reached its maximum, 33, at 30 days and decayed with a half-life of 53 days. The synthesis and turnover of proteins in uterus is shown in Fig. 2. Uterine protein fractions had highest SA of the tissues studied. The NSP reached its maximum, 400, at 4 hours

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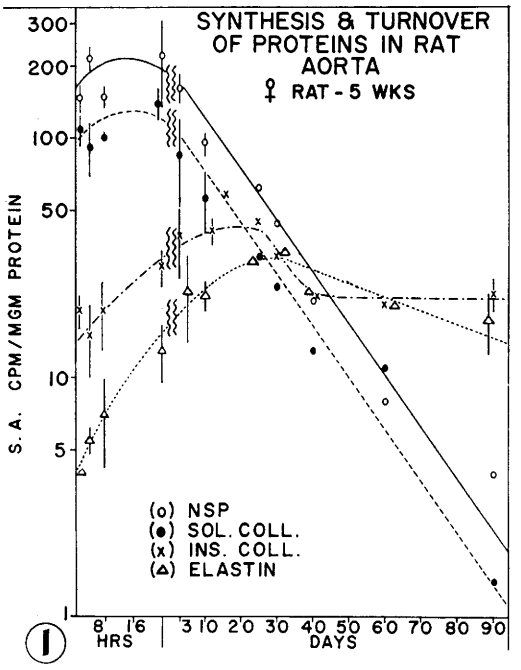
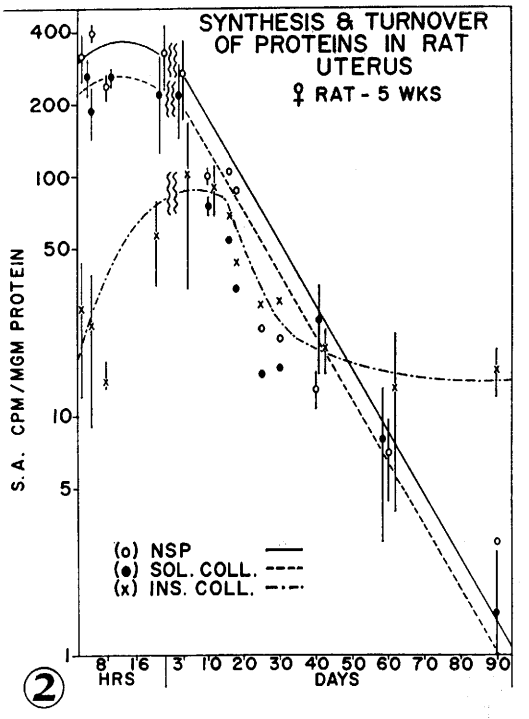
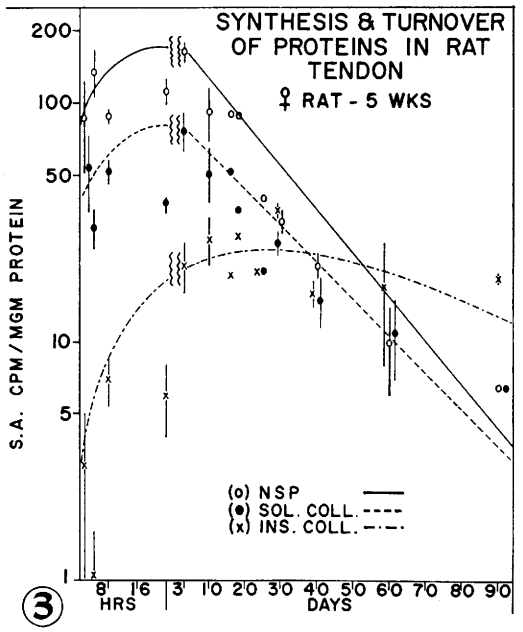
† Purchased from Nuclear Chicago, Inc.

‡ VirTis Co., Yonkers, N. Y.

and decayed with a half-life of 12 days. The SC reached its maximum, 260, at 8 hours and decayed with a half-life of 12 days. The IC reached its maximum, 100, at 3 days and decreased up to 30 days with a half-life of 11 days. There was little decay in activity







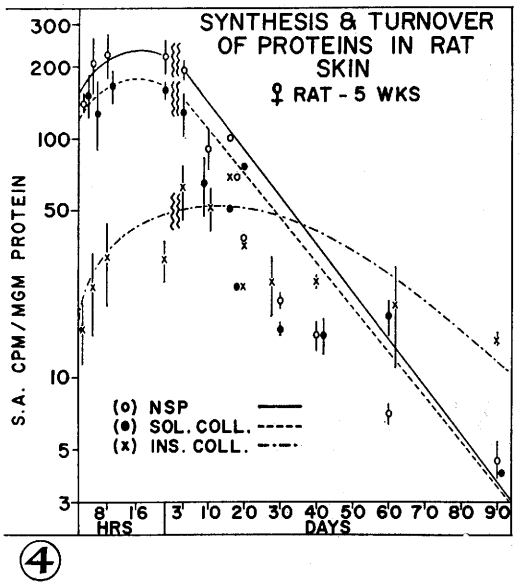


FIG. 1. Synthesis and turnover proteins in rat aorta. All bars represent stand. dev. All symbols represent means.

FIG. 2. Synthesis and turnover proteins in rat uterus (see legend for Fig. 1).

FIG. 3. Synthesis and turnover proteins in rat tendon (see legend for Fig. 1).

FIG. 4. Synthesis and turnover proteins in rat skin (see legend for Fig. 1).

TABLE I. Synthesis and Turnover of Insoluble Collagen and Elastin after Correction for Growth (SA = cpm/mg Protein).

		Days after C^{14}-lysine injection					
		3	10	30	40	60	90
		Actual value of SA					
Insoluble collagen—	Aorta	38	41	32	22	20	22.5
	Uterus	101	89	30	19	13	15.5
	Tendon	21	27	36	16	17	18.5
	Skin	61	51	25	25	20	14.5
Elastin—	Aorta	23	22	33	23	20	17.5
		SA corrected for weight					
Ratio $\frac{\text{Body wt (W)}}{\text{Body wt at max SA (W max)}}$	max		1.51	2.16	2.20	2.65	3.12
			max	1.43	1.46	1.76	2.07
				max	1.01	1.23	1.44
Insoluble collagen—	Aorta		41	46	32	35	46
	Uterus	101	134	65	42	34	48
	Tendon			36	16	21	27
	Skin	61	77	54	55	53	45
Elastin—	Aorta			33	23	24	25

thereafter. The synthesis and turnover of protein in tendon is shown in Fig. 3. The NSP reached its maximum, 160, at 3 days and decayed with a half-life of 16 days. The SC reached its maximum, 80, at 3 days and decayed with a half-life of 20 days. The IC reached its maximum, 25, at 10 days and decayed with a half-life of 65 days. The synthesis and turnover of protein in skin is shown in Fig. 4. The NSP reached its maximum, 220, at 8 hours and decayed with a half-life of 15 days. The SC reached its maximum, 170, at 8 hours and decayed with a half-life of 17 days. The IC reached its maximum, 52, at 3 days and decayed with a half-life of 28 days.

Discussion. These data indicate that soluble collagen has higher specific activity and higher turnover rate than insoluble collagen and elastin. By column chromatography and subsequent counting, it was shown that specific activity of lysine and hydroxylysine is higher in soluble than in insoluble collagen. This agrees with reports of other workers(10, 11,12,13,14) that soluble collagen is the precursor of insoluble collagen.

From the present data it is also shown that there is a difference in rate of synthesis and turnover of insoluble collagen in various organs. In the tissues studied, the collagen of uterus had the highest rate of synthesis, highest specific activity, as well as shortest half-life. By contrast, the collagen of tendon had the lowest rate of synthesis and lowest specific activity. Collagen turned over more slowly in the aorta than in any other tissue, if, indeed, it turned over at all.

Since rats used in this experiment were 5 weeks of age, rate of decay was influenced by new collagen formed after C^{14}-lysine had been administered. Therefore, insoluble collagen and elastin were actually metabolized more slowly than indicated by the data shown in the figures. In Table I, the data have been corrected on the assumption that new formation of collagen and elastin was directly proportional to increase in body weight. From this table, it is clear that there is a turnover of insoluble collagen and elastin in connective tissue and that while the "dilution factor of new tisue" is important, it does not alone account for all of the changes observed.

Summary. 1. The synthesis and turnover of protein in aorta, uterus, tendon and skin were studied in 5-week old rats. 2. The SA of connective tissue proteins in decreasing order of magnitude was nonscleroprotein, soluble collagen, insoluble collagen and elastin. 3. Among the tissues studied, greatest synthesis and turnover of collagen was observed in uterus. As regards synthesis, skin, aorta and tendon followed in descending order, while, for turnover, the order was skin, tendon and aorta.

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1. Neuberger, A., Perrone, J. C., Slack, H. G. B., *Biochem. J.*, 1951, v49, 199.

2. Harkness, R. D., Moralee, B. E., *J. Physiol.*, 1956, v132, 502.

3. Gould, B. S., *Fed. Proc.*, 1960, v19, 149.

4. Fitch, S. M., Harkness, M., Harkness, R. D., *Nature*, 1955, v176, 163.

5. Conway, E. J., *Microdiffusion Analysis and Volumetric Error*, 3rd ed., Van Nostrand Co., London, 1950, p124.

6. Glasstone, S., *The Elements of Physical Chemistry*, D. Van Nostrand Co., Inc., New York, 1946, p588.

7. *Radiation Health Handbook*, U. S. Dept. of H.E.W., P.H.S., Robert Taft Sanitary Engineering Center, Cincinnati, Ohio, 1955, p23.

8. Moore, S., Stein, W. H., *J. Biol. Chem.*, 1951, v192, 663.

9. Kao, K.-Y. T., Boucek, R. J., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 526.

10. Jackson, D., C.I.O.M.S. Connective Tissue Symposium, London, 1956; *Biochem. J.*, 1957, v65, 277.

11. Orekhovitch, V. N., *2nd Inter. Congr. Biochem. Communications*, 1952, p106.

12. ———, *3rd Inter. Congr. Biochem. Communications*, 1955, p50.

13. Harkness, R. D., Marks, A. N., Muir, H. M., Neuberger, A., *Biochem. J.*, 1954, v56, 558.

14. Gross, J. H., Highberger, J. H., Schmitt, F. O., *Proc. Nat. Acad. Sci., U. S.*, 1954, v40, 679.

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Metabolism of Exogenous Sialic Acid in the Rat.* (26258)

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The ubiquitous occurrence of sialic acid in mammalian tissues as a component of glycoproteins, glycolipids and oligosaccharides(1) implicates a role for this acidic carbohydrate in a variety of basic physiological phenomena. Free, unbound, sialic acid has not been detected except in cerebrospinal fluid (2). The liberation of sialic acid by bacterial and viral neuraminidase(3) raises the question of the metabolic fate of this substance. This report is concerned with the metabolism of sialic acid following intra-arterial, intraperitoneal, subcutaneous and oral administration to the rat.

Materials and methods. Sialic acid was prepared from bovine submaxillary mucin by the method of Svennerholm(4). The lyophilized product obtained following chromatography on Dowex-1 was dissolved in 0.9% saline and neutralized in preparation for administration to the animals. Twice recrystallized material (m.p. 185-186°C), which was shown by paper chromatography(5,6) to contain N-acetylneuraminic acid and N-glycolylneuraminic acid in the approximate ratio of 9:1, was used for calculation of extinction coefficients with the thiobarbituric acid assay(7).

Adult male albino rats were anesthetized with chlorolose-urethane and heparinized. The abdominal aorta was cannulated with a T-cannula and sialic acid (approximately 100 mg/kg body wt, in 1.0 ml) was injected through the side arm followed by 1.0 ml of saline. Arterial blood samples were obtained at time intervals indicated and diluted plasma samples were analyzed for free sialic acid using the thiobarbituric acid assay of Warren(7). In several experiments the renal arteries were bilaterally ligated. Data from typical experiments are presented in Table I.

The assay of sialic acid in urine was complicated by the presence of a chromogen resembling that given by deoxyribose. How-

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