Connective Tissue IX. Metabolism of Collagen in Bone of Rat.* (30244)

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Previously we reported the metabolism of collagen and elastin in several soft tissues of rat(1,2). The same method of fractionation cannot be used for bone, since the bone salts diminish the extractability of the solvents used in Lowry's method(3). Demineralizing with citrate-formic acid(4) or EDTA(5) is time consuming and involves the loss of soluble collagen. Because of these difficulties, very little has been written about the metabolism of collagen in bone. Gerber and Gerber studied the metabolism of collagen in the femur of young rats(6). Their observations covered 15-day periods. Soluble collagen was not considered. Recently, we published a method for fractionation of proteins in bone and cartilage(7). With use of this method both soluble and insoluble collagens could be separated from other proteins of bone. Therefore, a more nearly complete picture of collagen metabolism in bone could be drawn.

The present study deals with (a) the metabolism of soluble and insoluble collagens in several bones of rats, and (b) a comparison of the metabolism of the soluble and insoluble collagens in rats at different ages.

Methods. Female rats 5 weeks, 6 months and 2 years of age, respectively, were injected intraperitoneally with 4.26 μc glycine-2-^{14}C (specific activity 53 μc/mg) per 100 g body weight in physiological NaCl solution. At 1, 3, 7, 11, 20 and 31 days after injection, the 5 weeks and 6 months rats were sacrificed in groups of 4 and the 2-year-olds, in groups of two. Bones were freed of all soft tissue; whole bones were freeze-dried. Soluble and insoluble collagen were extracted by the method reported previously(7). Methods for determining nitrogen, for the expression of protein concentration and for isotope counting have been detailed elsewhere(1,2).

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The hydroxyproline content of both soluble and insoluble collagens was 12-13%. In preliminary experiments, using ^{14}C-lysine as precursor for the synthesis of bone collagen, radioactive hydroxylysine was recovered from both soluble and insoluble collagen samples. The radioactivity of hydroxylysine and lysine was always proportional to the radioactivity of the collagen sample from which these amino acids were derived. Therefore, in the present study, counting was performed on the collagen samples from both TCA extracts.

The specific activity (SA) has been defined as counts per min per mg of collagen nitrogen. A correction for changes in body weight in the 5 weeks old group was made by multiplying the SA of insoluble collagen by the following ratio: (total collagen weight at time of determination, C_f) $\times$ (% insoluble collagen at time of determination)/(total collagen weight at beginning of experiment, C_i) $\times$ (% insoluble collagen at beginning of experiment). The ratio C_f/C_i was obtained from Sobel's equation(7a), based on data from studies of femoral bone: $\log C_f/C_i = 0.78633 \log {}^{w}f/{}^{w}i$ and used for all bones (^{w}f and ^{w}i are the body weights at time of determination and at beginning of the experiment, respectively). When this correction factor was applied to data from the 5 weeks old animals, it was found that the total collagen of rat femur increased by an average of 34% in 31 days. The soluble collagen was initially 3.5 to 6.0% of the total, but only 2.0 to 3.0% thirty days later, thus showing a decrease of from 30 to 60% in 30 days; no correction was applied to the SA data. No change in body weight was observed in the 6 months and 2-year-old groups during the experimental period.

Results. The SA as a function of time for soluble collagen and insoluble collagen in femur, rib, vertebra and skull of 5 weeks old

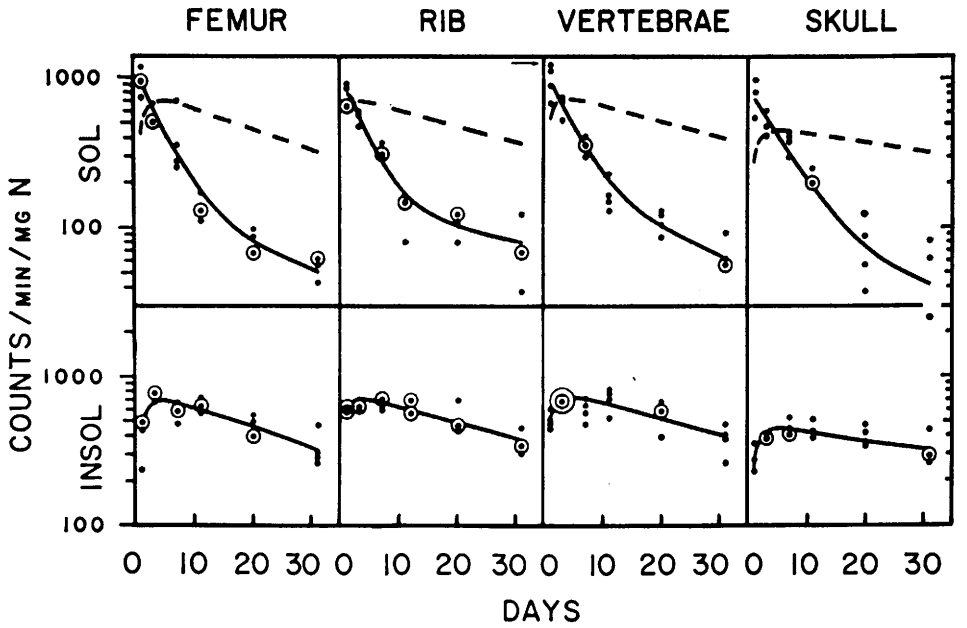

FIG. 1. Specific activities of soluble (upper graphs) and insoluble (lower graphs) collagen as a function of time following injection of glycine-2-^{14}C. Small dots represent individual experiments; larger dots, 2 experiments with identical results. Curves have been fitted by the method of least squares. For convenience in comparison, curves for insoluble collagen have been superimposed as dotted lines upon those for soluble collagen.

rats is shown in Fig. 1. Solid lines on the upper series of graphs refer to soluble collagen and the lower graphs to insoluble collagen. The broken curves in the upper series represent the curves for insoluble collagen superimposed upon the corresponding curves for soluble collagen. As was true with our earlier findings(1), the maximal SA of soluble collagen occurred within a fraction of a day after injection. On the 31st day the SA of soluble collagen of femur had decreased to 0.06, of rib to 0.10, of vertebra to 0.07 and of skull to 0.08 of the 1-day values. The maximum SA of insoluble collagens was found between 3 to 11 days after injection of glycine-2-C^{14}. On the 31st day, the SA of insoluble collagen of skull had decreased to 73% of the maximum SA; of the other bones, to 55% of the respective maxima. The SA curves for soluble and insoluble collagen of femur, rib and vertebrae were very similar. Their insoluble collagen curves are steeper than that for the skull and their maxima are higher.

The comparison of the changes of SA of soluble and insoluble collagen as a function of time in the femur of rats at several ages is summarized in Table I. No statistical differences among the 3 age groups could be found in the maximum SA of soluble colla-

TABLE I. Metabolism of Collagen in Femur of Rats.

Days after injection	Age of animals	Specific activity (cpm/mg nitrogen) 1	3	7	11	20	31
Soluble collagen	5 wk	956 ± 94*	569 ± 54	300 ± 9	138 ± 13	81 ± 9	56 ± 4
	6 mo	1277 ± 133	774 ± 34	306 ± 34	208 ± 10	137 ± 3	85 ± 3
	2 yr	975	426	196	132	106	72
Insoluble "	5 wk	481 ± 62	751 ± 21	590 ± 43	685 ± 39	471 ± 52	360 ± 60
	6 mo	92 ± 10	75 ± 17	65 ± 24	68 ± 3	85 ± 10	58 ± 5
	2 yr	11	14	13	16	27	35

* Mean ± standard error(8).

TABLE II. Metabolism of Collagen in Rib of Rats.

Days after injection	Age of animals	Specific activity (cpm/mg nitrogen) 1	3	7	11	20	31
Soluble collagen	5 wk	775 ± 71*	556 ± 39	325 ± 18	138 ± 19	113 ± 11	75 ± 18
	6 mo	1010 ± 89	611 ± 38	331 ± 17	331 ± 31	222 ± 55	130 ± 14
	2 yr	507	365	192	140	115	106
Insoluble "	5 wk	593 ± 14	620 ± 11	656 ± 12	633 ± 44	538 ± 75	380 ± 37
	6 mo	129 ± 17	120 ± 20	95 ± 20	72 ± 3	99 ± 10	62 ± 7
	2 yr	22	15	19	17	44	31

* Same as in Table I.

TABLE III. Metabolism of Collagen in Vertebra of Rats.

Days after injection	Age of animals	Specific activity (cpm/mg nitrogen) 1	3	7	11	20	31
Soluble collagen	5 wk	988 ± 119*	663 ± 69	363 ± 20	169 ± 22	113 ± 10	69 ± 9
	6 mo	1379 ± 222	597 ± 55	396 ± 14	144 ± 14	191 ± 10	126 ± 7
	2 yr	613	367	170	136	102	81
Insoluble "	5 wk	510 ± 33	684 ± 10	611 ± 62	734 ± 60	587 ± 65	417 ± 66
	6 mo	61 ± 10	41 ± 7	34 ± 10	112 ± 10	89 ± 10	72 ± 7
	2 yr	14	18	14	27	53	43

* Same as in Table I.

TABLE IV. Metabolism of Collagen in Skull of Rats.

Days after injection	Age of animals	Specific activity (cpm/mg nitrogen) 1	3	7	11	20	31
Soluble collagen	5 wk	781 ± 194*	506 ± 56	388 ± 31	219 ± 19	75 ± 19	63 ± 6
	6 mo	1044 ± 96	732 ± 65	365 ± 48	296 ± 7	341 ± 17	123 ± 10
	2 yr	379	183	85	115	64	111
Insoluble "	5 wk	288 ± 37	403 ± 8	459 ± 24	476 ± 37	421 ± 36	348 ± 51
	6 mo	38 ± 7	38 ± 10	30 ± 7	85 ± 3	100 ± 10	68 ± 10
	2 yr	11	14	13	21	21	21

* Same as in Table I.

gen. Relatively slow decay of the soluble collagen was noted in the 6 months and the 2-year-old groups (to approximately 7% in 31 days) as compared to the 5 weeks group (to 6%). The SA for insoluble collagen at any experimental time is highest in the 5 weeks old rats and decreases as the age of the rats increases. Similar findings were seen in rib, vertebrae and skull. The data are summarized in Tables II, III and IV.

Discussion. Collagen metabolism in the femur of young rats has been studied by Gerber *et al*(6). Two components were assumed. The quickly metabolized component had a turnover time of 4 days and the slowly metabolized one, a turnover time of 40 days. In the present study, we separated soluble from insoluble collagen. Our data indicated that even the soluble collagen fraction consisted of more than one component. Collagen is a heterogeneous protein and exists in variously polymerized forms(9-12). The solubility of the several forms of collagen is decreased when the degree of polymerization is increased. The more soluble collagens are believed to be the precursors of insoluble collagen(1,2,9,13,14). The present data tend to confirm such an hypothesis. Furthermore, after correction for growth dilution, the insoluble collagen in bone of young rats continued to be metabolized actively. This is probably the reason that embryonic or young bone can be used for the *in vitro* study of collagen synthesis(15,16). The active metabolism is further supported by the more recent papers on collagenolytic activity found in young and embryonic bone of chicks (17,18). From the present data, it can be

seen that similar metabolic behavior can be seen in femur, rib and vertebrae. Relatively slower metabolism of collagen was observed in skull.

A previous report from this laboratory has shown that collagen metabolism is an inverse function of age in the soft tissues of rats(2). From the present experimental results, it may be concluded that a major portion of bone collagen is synthesized at an early age. The rate of metabolism of collagen in bone is an inverse function of age.

Summary. 1. Collagen metabolism in femur, rib, vertebrae and skull of rats at 5 weeks, 6 months and 2 years of age has been studied following intraperitoneal injection of glycine-2-^{14}C. 2. The SA of soluble collagen reached its maximum within a fraction of a day and decreased rapidly. The multicomponent feature of the soluble collagen is shown by the SA *vs* time curves. 3. No difference could be found in the maximal SA of soluble collagen among 3 different age groups. In contrast, slower decay of the soluble collagen was observed with increasing age of the rats. 4. Very active synthesis of insoluble collagen was observed in rats of 5 weeks of age. The rate of such synthesis was decreased in rats 6 months and 2 years of age. 5. The SA of insoluble collagen reached its maximum in 3-10 days and decreased slowly in 5 weeks old rats. This maximum occurred late in the 6 months old rats. No decay could be observed in the 2-year-old rats. 6. Similar metabolic behavior of soluble collagen and insoluble collagen was shown in femur, rib and vertebrae. A relatively less capacity and slower metabolism was seen in insoluble collagen of skull as compared with the rest of the bones studied.

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