were found to be polypeptide in nature, and the amino-acid compositions were determined by the use of an automatic amino-acid analyzer.

From rat heart, an active polypeptide of molecular weight approximately 700 was isolated, with an amino-acid composition as follows, the figures in parenthesis indicating the number of amino-acid residues per molecule of polypeptide: – alanine (1), aspartic acid (1), glutamic acid (1), glycine (3), lysine (1). From ox plasma, an active polypeptide was isolated of molecular weight approximately 5000, with an amino-acid composition as follows: – alanine (3), arginine (2), aspartic acid (3), cystine (2), glutamic acid (4), glycine (2), histidine (2), isoleucine (1), leucine (3), lysine (5), phenylalanine (2), proline (2), serine (2), threonine (2), tyrosine (1), valine (2).

The polypeptide from blood proved to be a much better complexing agent than the smaller cardiac polypeptide, and results indicate that it is capable of complexing approximately 50 sodium ions per molecule of polypeptide in 0.15 *M* sodium chloride solution. This is an order of magnitude greater than would be in accord with the number of acidic side chains available, which seem to be the most obvious source of complexing ability, but a possible mechanism for the complexing of relatively large numbers of alkali metal cations was proposed by MUELLER and RUDIN[9]. This involves the replacement of water molecules in the cation hydration shell by carbonyl oxygen atoms, which, of course, are plentiful in polypeptide structures.

It was felt desirable to confirm the existence of sodium ion complex formation with an independent method. The studies of PALATY[10] indicated that the presence, even in relatively small amounts, of a cation with a high surface charge density inhibits the close approach of sodium ions to binding sites, and that lanthanum is the best available ion for this purpose. We therefore investigated the effect of low concentrations of lanthanum ion on the apparent depression of sodium ion activity by the isolated plasma polypeptide and found that the presence of lanthanum ions did indeed prevent the plasma polypeptide from lowering sodium ion activity.

It seems possible that one function of the polypeptides isolated by us and reported here could be that of a sodium ion buffer. The concentration of sodium ions in plasma is known to be precisely controlled. According to PITTS[11], for mammalian plasma the sodium ion concentration lies in the range 138–146 m*M*. The presence of an effective sodium ion buffer would lead to an even narrower range for the sodium ion activity. The precision of control which this represents may be appreciated from the calculation that this sodium ion concentration range, when expressed on a logarithmic scale, corresponds to a range of only 0.02. By comparison, the normal range of hydrogen ion concentration, expressed on the pH scale, is 0.10 (i.e. 7.35–7.45, cf. PITTS[12]). Thus the precision of control exerted on the sodium ion concentration is remarkable; by implication, the presence of a sodium ion buffer must exert an additional control with a precision even more remarkable.

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Is there a Recycling of Hydroxyproline?

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Summary. Rats can produce glycine from hydroxyproline and vice versa. After the injection, hydroxyproline is rapidly converted into glycine and then incorporated into collagen. Later the labelled amino acids in collagen are hydroxyproline, proline, serine, threonine, alanine, and glutamic acid. We suppose that all these labelled amino acids come from glycine.

It is generally held that hydroxyproline, in contrast to the large amounts of proline, cannot be recycled. This means that hydroxyproline liberated in collagen catabolism would have to be eliminated[1,2]. On the other hand, we have shown in previous experiments that remarkable quantities of labelled glycine can be found in collagen after the application of ^{14}C-proline[3]. The best explanation for this is the assumption that this glycine was derived from hydroxyproline directly and not from glutamic acid. Therefore hydroxyproline must be catabolized in the rat's organism.

Methods. L-4-hydroxyproline-^{3}H from NEN (5 mCi/0,148 mg) with a purity grade of > 98% (scan of thin layer chromatography in phenol (75 g): H_2O (25 g): NaCN (20 mg)) was injected into the peritoneum of male Sprague-Dawley rats weighing ca. 100 g each (ca. 400 µCi/animal). The animals were sacrificed 1 or 24 h later. Tail skin, and tendon collagen was extracted, dialyzed, and isolated in fibres by dialysis against phosphate buffer (0.02 *M* Na_2HPO_4)[5]. The fibres were also washed many times. Purification was stopped when there was no longer any radioactivity in the washing water. The collagen was then hydrolyzed and its amino acids isolated by column chromatography as described previously[6] (Figure). All the isolated ^{3}H-amino acids again underwent chromatography and were identified separately.

Results. 1 h after the injection of L-4-hydroxyproline-^{3}H radioactivity can be detected in the collagen of skin. This radioactivity comes almost exclusively from glycine (Table).24 h after application, the radioactivity of skin

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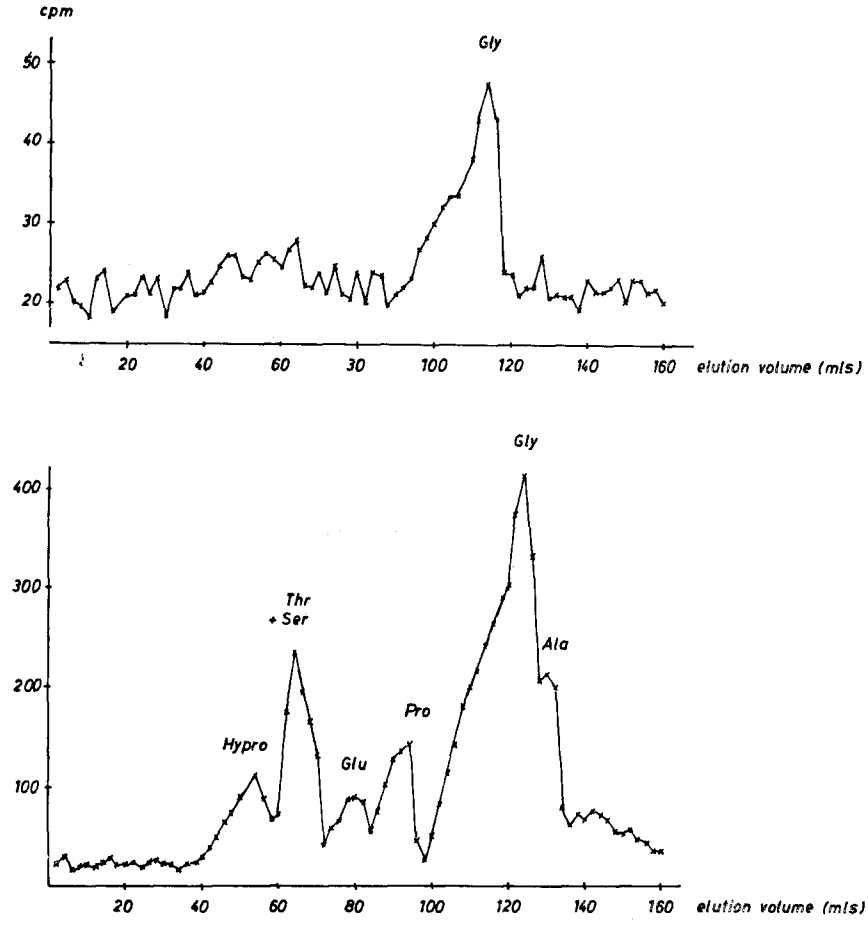
Labelled amino acids in collagen of tail skin, and tendon after ^{3}H-hydroxyproline injection to rats

Amino acids	Skin Total radioactivity (%)		cpm/mg		Tendon Total radioactivity (%)		cpm/mg	
	1 h	24 h	1 h	24 h	1 h	24 h	1 h	24 h
Hy-proline	—	8.84	—	269	n.i.	13.48	n.i.	192
Threonine	—	8.65	—	2.035	n.i.	8.78	n.i.	1.141
Serine	—	5.37	—	843	n.i.	7.02	n.i.	536
Glut. ac.	—	5.30	—	230	n.i.	4.49	n.i.	118
Proline	—	8.95	—	353	n.i.	13.68	n.i.	248
Glycine	80%	53.55	106	956	n.i.	45.93	n.i.	390
Alanine	—	9.34	—	713	n.i.	6.62	n.i.	169

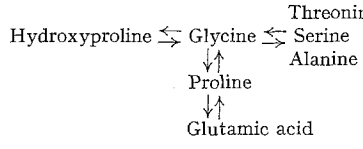
n.i., not investigated



Profile of eluted radioactivity from chromatography of hydrolysates of skin collagen 1 h (above) and 24 h (bottom) after ^{3}H-hydroxyproline injection.

collagen is distributed among 7 amino acids (Table). Here again glycine is predominant, accounting for more than 50% of radioactivity. Tail tendon collagen did not differ remarkably from this. Glycine also has the highest specific radioactivity, except for threonine. At 24 h the amount of radioactive hydroxyproline is approximately equal to that of radioactive proline.

Discussion. Our results have shown, in accordance with other investigations[4,7], that hydroxyproline cannot be incorporated into collagen directly, but that it is metabolized into glycine and then rapidly incorporated into collagen. The distribution of radioactivity in collagen amino acids 24 h after ^{3}H-hydroxyproline injection suggests the following assumptions concerning the metabolic pathway of hydroxyproline:



Our previous experiments[3] with ^{14}C(U)-proline injection into rats have shown that the distribution of radioactivity in collagen amino acids is similar to the present results 24 h after ^{3}H-hydroxyproline application. The recycling of hydroxyproline via the glycine pathway is probable in plants or microorganisms, but is not yet determined in animals.

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