COLLAGEN METABOLISM IN THE NORMAL AND LATHYRITIC CHICK*

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Recent investigations into the pathogenesis of experimental lathyrism in chick embryos (1), guinea pigs (2), and rats (3–5) have shown that the lathyrogen, β-aminopropionitrile, produces a marked increase in cold saline-extractable collagen, in addition to causing gross connective tissue deformities and loss of tensile strength. Physical-chemical studies of the extractable collagen indicated normal molecular dimensions, helical conformation and stability to thermal denaturation (6). However, two abnormal properties of lathyritic collagen have been found. First, lathyritic collagen fibrils, whether formed *in vivo* or *in vitro* are temperature sensitive, going back into solution upon cooling (6). Second, intramolecular cross-linking (β-subunits), a characteristic feature of acid-extractable normal collagen (7, 8), is markedly diminished in acid-extractable lathyritic collagen.

It was originally concluded from balance studies and morphologic examination that the increased extractable collagen in lathyritic chick embryo tissues derived mainly from preexisting insoluble fibrils (1, 9). A contrary view suggests the source of the large pool of extractable collagen in lathyritic animals may be ascribed to impaired fibril formation, resulting in the accumulation of newly synthesized, dispersed collagen molecules (10–12).

In this study several different types of radioisotope experiments were performed, each one attempting to localize the source of extractable lathyritic collagen.

Experimental Procedure

Materials.—Uniformly labeled L-proline-C^{14} (5.7 mc per mmole), tritiated L-proline-3,4-H^3 (321 mc per mmole), and L-glycine-1-C^{14} (5.7 mc per mmole) were obtained commercially

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and were found to be radiochemically pure by chromatographic methods. β-Aminopropionitrile (βAPN) fumarate was generously provided by Abbott Laboratories, North Chicago and puromycin dihydrochloride was obtained commercially.

Preparation of Embryos and Tissues.—White leghorn chicken eggs were incubated at 38°C at constant humidity. Twenty mg of β-aminopropionitrile fumarate in 0.1 ml of sterile water was injected through a pin hole onto the chorioallantoic membrane (1). Ten embryos used for each experimental point, were injected during a 5 minute interval and were returned to the incubator as a group. Ten control embryos used for each point, received only isotope.

After the desired period of incubation, the embryos were removed and weighed; the long bones of the legs and wings were dissected free of soft tissues and the cartilagenous condyles. The bones were pooled, minced fine with scissors, and weighed. It is probable that this preparation contained some cartilage and periosteum. One to 2 gm of minced bones (150 to 160 bones per 10 embryos) was suspended in three volumes of cold 1 M NaCl (v/w) and agitated at 0°C for 16 hours. Six sequential extractions were performed after which no more collagen could be dissolved. The final extract contained less than 3 per cent of the radioactivity of the initial extract.

Isolation and Purification of Extractable Collagen.—The mixture of bones and saline was centrifuged at 60,000 *g* for 60 minutes at 0°C and the supernatant fluid was filtered through medium and fine sintered glass. A fraction of the filtrate was monitored for radioactivity. The first and second extracts were pooled and the remaining extracts, containing little collagen, were discarded. All subsequent steps were carried out at 0–5°C.

An equal volume of cold 5 M NaCl was added slowly, with stirring, to the filtered extract and this mixture was stored for 48 hours. The resultant collagen precipitate was collected by centrifugation at 15,000 *g* for 30 minutes; it dissolved after shaking for 16 hours in potassium phosphate buffer, pH 7.6, $\Gamma/2$ 0.4. This solution was centrifuged at 60,000 *g* for 60 minutes; the clear supernatant was dialyzed three times (24 hours each) *versus* 100 volumes of 0.01 M Na_2HPO_4. The resultant precipitate was collected by centrifugation at 15,000 *g* for 30 minutes; it dissolved in 0.1 M acetic acid after shaking for 16 hours. This solution was dialyzed 3 times (24 hours each) *versus* large volumes of 0.1 M acetic acid. It was centrifuged at 30,000 *g* for 30 minutes; the clear supernatant fluid was lyophilized and stored at −10°C. The yield of extractable collagen at the end of this purification procedure was approximately 60 per cent for all samples.

Preparation of Non-Extractable Collagen.—After the final saline extract the bones were dehydrated in 10 volumes (v/w) of absolute methanol for 24 hours. Lipid extraction of the bones was carried out overnight using 3 volumes (v/w) of chloroform–methanol (2:1). This step was repeated; the bones were washed twice with absolute methanol; they were then washed twice with distilled water. Three volumes (v/w) of 0.2 M ethylenediaminetetraacetate acid (EDTA), pH 7.5, were added to the wet bones and the mixture dialyzed for 1 week *versus* large volumes of 0.2 M EDTA, pH 7.5. Dialysis overnight against distilled water was done to free the bone residues of salts.

Hydrolysis was performed in sealed tubes at 138°C for 3 hours using 6 N HCl and the hydrolysate was dried under reduced pressure.

Separation of the Free Amino Acids.—The supernatant obtained after collection of the initial collagen precipitate was dialyzed against 20 volumes of distilled water. The dialysate was brought to pH 2 with concentrated HCl, and mixed in a sintered glass funnel with dowex 50 (H^+) resin. The fluid was removed by suction and the resin was washed with distilled water; the amino acids were eluted with 2 N NH_4OH. The eluate was dried, dissolved in dilute HCl, and clarified with charcoal (norit A). The charcoal was removed by filtration and the filtrate dried under reduced pressure at 65°C. Quantitative recovery of the imino acids was obtained. They were isolated by the chromatographic procedures described below.

Analyses.—One portion of the sample was used for proline and hydroxyproline determina-

tions; another portion was streaked on No. 3 MM chromatography paper. Chromatographic development was performed using phenol–ethanol–water (60:20:20). Proline and hydroxyproline reference markers on each side of the paper were stained with isatin and *p*-dimethylaminobenzaldehyde respectively. The marker imino acids were widely separated. Upon examining the sample regions with a strip radioactivity counter only two radioactive areas corresponding to the two marker imino acids were found.

After elution, hydroxyproline was chromatographed in butanol–water–acetic acid (4:5:1) and its specific activity remained constant. Proline specific activity diminished after this step but was unchanged by a third chromatographic separation in ethanol–water (77:23). In more recent experiments the second proline chromatograph was performed on glass plates, using silica gel G as adsorbent and dioxane–water (60:40) as solvent.

Imino acid radioactivity was measured in a liquid scintillation counter, using an aqueous phosphor solution (13). Glycine was eluted from the initial chromatograph and its specific activity, as the dinitrophenol (DNP) derivative (14), was determined in a gas-flow counter. Hydroxyproline was determined by the method of Neumann and Logan (15), but in a total reaction volume of 1.0 ml. Similarly, proline was measured as described by Troll and Lindsley (16) but in a total volume of 1.0 ml. Standard curves for both assays performed at this volume were linear from 0.1 to 1.5 μg.

RESULTS AND DISCUSSION

Extractable Collagen.—The amount of salt-extractable collagen in normal chick embryo bone did not exceed 1 per cent of the total collagen. In lathyritic bone it rose precipitously immediately following βAPN administration; the amount more than doubled in 2 hours (Fig. 1). There was no change in the rate of accumulation during this 12 hour period; the rate did decrease about 48 hours after a single dose (1). (The amount of extractable collagen produced is dosage dependent, (17), the 20 mg of βAPN administered in these experiments produce a maximum response.) Coinciding with the increase in the extractable fraction, the insoluble collagen concentration fell progressively (Fig. 2). The *total* collagen concentration remained unchanged and approximately equal to the normal, after correcting for purification losses.

If we assume all the lathyritic extractable collagen represents new or recently synthesized protein, the rate of increase shown in Fig. 1 would be a direct measure of collagen formation. This value is approximately 50 μg (corrected for recovery) of hydroxyproline per hour per gram of bone, or about 1 per cent of the total bone collagen per hour.

Isotope Incorporation into Collagen Fractions.—It is generally agreed, concerning the normal collagen pathway, the most recently synthesized molecules are extractable in cold neutral solutions. These molecules aggregate in the tissues, producing fibrils which become progressively insoluble with time (18–21). The specific activity relationships of the collagen fractions in normal embryo bone (Fig. 3 *a*) are in accordance with this concept. There is a typical precursor-product relationship between free proline and extractable collagen hydroxyproline. Furthermore, the non-extractable collagen specific activity rose progressively as the radioactivity of the extractable fraction fell.

The free hydroxyproline specific activity was much closer to the specific activity of the insoluble collagen than to either that of the free proline or extractable collagen implicating the insoluble collagen as its source.

The free proline pool was approximately the same size in normal and lathyritic bone (Table I); there was considerable fluctuation in free proline total activity, corresponding to the specific activity fluctuations. The meaning of the elevated lathyritic free proline specific activity (Fig. 3 *b*) and total activity (Table I) is not clear.

There are unique, repetitive, synchronous fluctuations in specific activity in

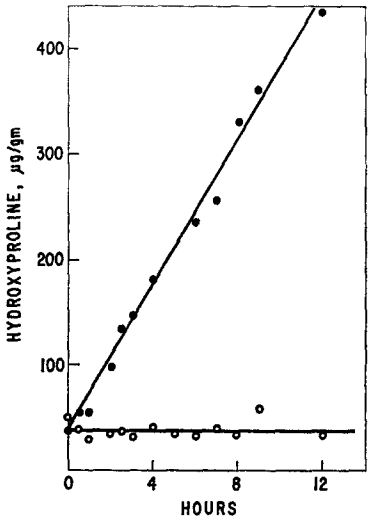

FIG. 1. Effect of βAPN on extractable collagen concentration. Experimental conditions described in Fig. 5. ○, control; ●, lathyritic.

all four components in Fig. 3. Further observations concerning this phenomenon will be described later.

The qualitative pattern of isotope incorporation in the collagen fractions and free hydroxyproline was quite similar, comparing control and lathyritic animals (Fig. 3). The pool sizes and total incorporated radioactivity of the components of Fig. 3 are listed in Table I. Despite the fluctuations, one can compare the distribution of radioactivity (total counts) within the two collagen fractions with respect to time. Radioactivity accumulated in the extractable lathyritic collagen, in contrast with the progressive fall of radioactivity in the extractable normal collagen. However, as in the control, radioactivity rose in the non-extractable lathyritic collagen. We can follow the movement of radioactivity from soluble to insoluble collagen by examining the relationship of total counts (tabulated as a ratio in Table I) in the two pools with respect to time. Such

comparison is not affected by changing pool sizes, since it only reflects flow from one step to another. A *block* in flow will be revealed by an increasing ratio of counts between precursor and product, while, in the *free flowing state*, this ratio will approach zero as the counts leave the precursor pool. Disregarding the initial value, we could see a diminishing ratio of counts between extractable and non-extractable collagen in the control animals (Table I). Again, disregarding the initial value, we found a fluctuating ratio in the lathyritic animals. The ratio was *not* progressively rising, as would be expected if a block in fibril formation occurs. The relative constancy of this ratio between precursor and product

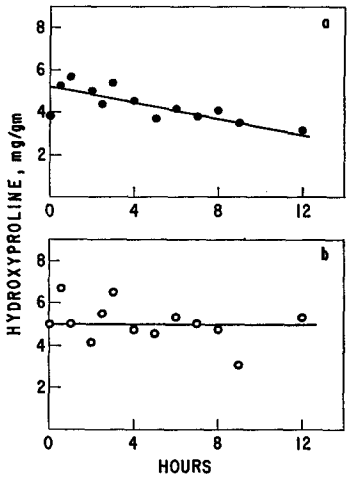

FIG. 2. Effect of βAPN on non-extractable collagen concentration. ○, control; ●, lathyritic.

implies the product is mixing with the precursor *via* another pathway. In this case, we infer that the lathyritic fibrils became dispersed in saline and were extracted with the molecules of the precursor pool.

These results indicate a significant portion of the newly formed collagen aggregates into insoluble fibrils in lathyritic bone, while the remainder forms into fibrils which are soluble in cold saline.

Effect of βAPN on Previously Labeled Collagen.—The above experiment, while demonstrating a portion of lathyritic collagen to be recently synthesized, does not specify at which point in fibrogenesis βAPN is active. In order to determine if older fibrils are affected by lathyrism, the collagen was prelabeled with H^3 proline 24 hours prior to injection of βAPN. In this case, accumulation of labeled extractable collagen in the lathyritic animals would suggest some older collagen fibrils are contributing to the extracted fraction. Fig. 4 shows progressive accumulation of labeled collagen in the lathyritic extracts.

While the data shown in Fig. 4 are consistent with the hypothesis that a

portion of lathyritic extractable collagen derives from fibrils labeled before βAPN was administered, any free H^3 proline remaining after 24 hours will be incorporated into newly synthesized collagen and it is this which may be accumulating. To explore this point one can use the information obtained from introducing a second isotope. If the accumulating soluble collagen comes only from one source, new synthesis, the incorporation of C^{14} glycine should be uniformly related to the incorporation of H^3 proline, because the precursor amino acids are almost simultaneously incorporated during protein synthesis

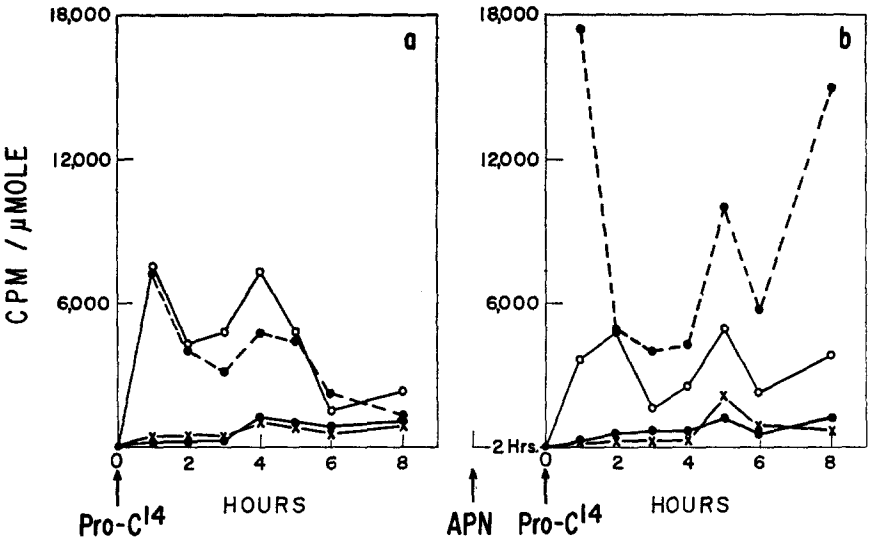

FIG. 3. Incorporation of proline-C^{14} into the collagen and free imino acids of: *a*, control; *b*, lathyritic embryos; ●-----●, free proline; O——O, soluble collagen; x——x, insoluble collagen; ●——●, free hydroxyproline. Experimental conditions described in Table I.

(22). Fig. 5 shows, in normal extractable collagen, a uniform relationship exists between C^{14} glycine and H^3 hydroxyproline; *i.e.*, a nearly constant ratio between specific activities. (The progressive diminution of this ratio with time reflects the normal decrease of specific activity of the recently administered glycine divided by the relatively constant, asymptotic, specific activity of the proline.) By contrast, there appears to be a ***random*** relationship between these same amino acids in lathyritic extractable collagen. Identical results were obtained comparing glycine and proline from these same samples, confirming it as a real finding and providing a check on the analytic methods.

The results of this double labeling experiment suggest extracted lathyritic collagen may originate from at least two separate sources. One cannot locate the exact sites from this type of experiment. However, the incorporation of C^{14} glycine into lathyritic collagen confirms the previous experiment, indicating

TABLE I

Total Activity Relationships of Collagen and Free Imino Acids in Normal and Lathyritic Bone

Ten embryos per point; 2 μc proline-U-C^{14} given 2 hours after βAPN fumarate.

Time after APN	Free imino acids				Extractable collagen		Non-extractable collagen		(a)/(b)
	Proline		Hydroxyproline		Hydroxyproline		Hydroxyproline		
hrs.	*μmole/gm**	CPM/*gm*	*μmole/gm*	CPM/*gm*	*μmole/gm*	CPM/*gm* × 10^{-2} (*a*)	*μmole/gm*	CPM/*gm* × 10^{-2} (*b*)	
Control									
2	0.19	471	0.12	8	0.21	0.1	39.6	78	—
3	0.21	1524	0.19	38	0.32	25.0	25.0	98	0.25
4	0.18	707	0.15	36	0.31	12.4	28.5	126	0.10
5	0.21	666	0.19	59	0.39	18.4	36.7	135	0.14
6	0.28	1344	0.22	278	0.34	25.2	32.4	357	0.07
7	0.19	858	0.19	192	0.33	15.6	30.9	265	0.06
8	0.20	454	0.22	178	0.37	5.8	32.1	188	0.03
10	0.22	282	0.19	213	0.38	8.8	32.4	306	0.03
Lathyritic									
2	0.23	1136	0.17	7	1.08	0.1	31.1	51	—
3	0.22	3922	0.16	47	1.41	51.6	30.2	51	1.01
4	0.21	1030	0.17	113	1.68	82.3	28.4	79	1.04
5	0.23	925	0.16	113	2.42	41.5	28.5	81	0.51
6	0.22	940	0.14	89	2.44	62.1	28.6	84	0.74
7	0.29	2880	0.16	200	2.21	110.0	27.1	589	0.19
8	0.26	1468	0.12	82	3.01	67.8	25.0	201	0.34
10	0.24	3589	0.13	146	3.16	119.0	22.6	153	0.78

* Wet weight, bone.

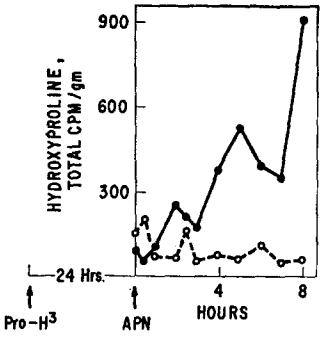

FIG. 4. Effect of βAPN on radioactive extractable collagen in prelabeled embryos. Experimental conditions described in Fig. 5; O-----O, control; ●——●, lathyritic.

a portion of the lathyritic collagen is newly synthesized. Perhaps the lathyritic process affects collagen in various physical states including the newly formed dispersed molecules (a small and very transient phase) *and* the newly formed

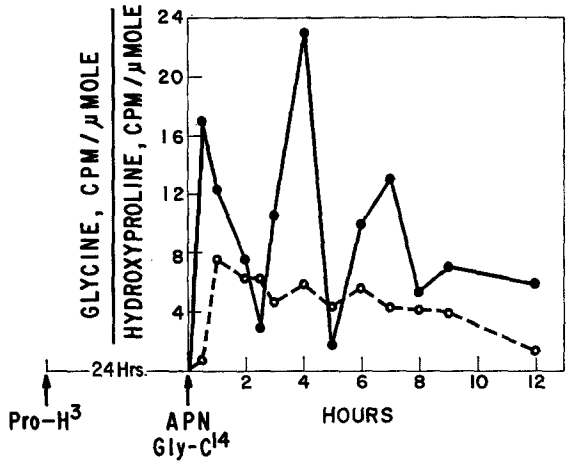

FIG. 5. Effect of βAPN on ratio of amino acid specific activities in extractable collagen. O-----O, control; ●——●, lathyritic. 10 animals per point, 20 μc proline-3,4-H^3 administered 24 hours prior to βAPN (20 mg), and 20 μc glycine-1-C^{14}. Soluble collagen isolated and purified per Experimental Procedure.

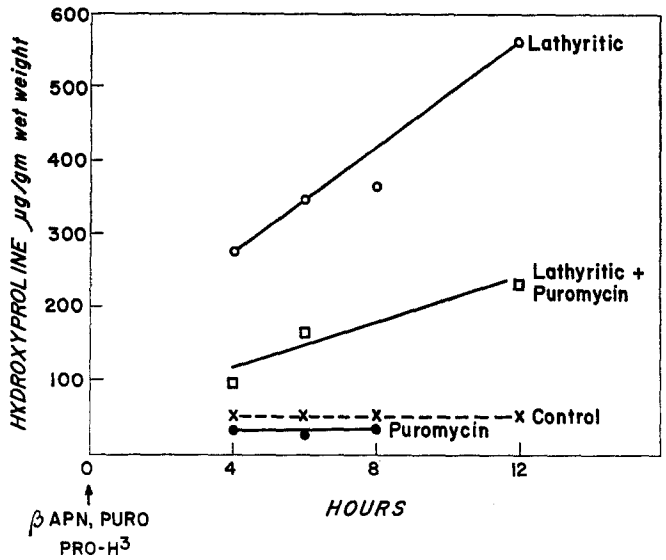

FIG. 6. Effect of puromycin on normal and lathyritic extractable collagen. Experimental conditions described in Table II.

fibrils, including some which have become insoluble. It appears from autoradiographic studies, carried out under the conditions of the present experiment (23) that no large proportion of *old* insoluble fibrils, in bone, are extractable.

Effect of Puromycin on Lathyrism.—In order to further localize the site of

βAPN effect, collagen synthesis was partially blocked by the administration of puromycin (24). Puromycin impaired the production of normal extractable collagen and decreased the amount and rate of accumulation of lathyritic

TABLE II

Effect of Puromycin upon Incorporation of Proline-3,4-H^3 into Normal and Lathyritic Collagen

Three embryos per point, 20 mg βAPN fumarate, 5.0 mg puromycin dihydrochloride, 10 μc proline-3,4-H^3 (5mc/mmole). Drugs and isotope simultaneously injected intravenously (37). Collagen extracted in 1 M NaCl and precipitated by dialysis *versus* distilled water. Insoluble collagen obtained as described in Experimental Procedure.

Time	Extractable collagen $\times 10^{-2}$		Non-extractable collagen $\times 10^{-2}$		Total collagen $\times 10^{-2}$
hrs.	CPM/*gm**	CPM/*μmole*	CPM/*gm*	CPM/*μmole*	CPM/*gm*
Control					
4	13	33	242	9	255
6	14	37	511	20	525
8	9	23	701	24	711
12	17	42	1386	51	1400
Puromycin					
4	12	46	28	1	39
6	25	131	52	2	76
8	27	69	52	2	79
12	—	—	—	—	—
Lathyritic					
4	274	178	110	4	484
6	367	128	139	5	475
8	301	109	255	12	556
12	287	67	163	7	449
Lathyritic and Puromycin					
4	66	92	111	5	179
6	100	80	76	3	176
8	—	—	—	—	—
12	125	71	60	2	185

* Wet weight, bone.

collagen (Fig. 6). The incorporation of radioactivity into total bone collagen was diminished in the two groups which received puromycin (Table II).

Comparing the control and puromycin groups we note that relatively little radioactivity accumulated in the insoluble collagen of puromycin groups, although the soluble collagen radioactivity rose above the control values. As indicated by Tables I and II and Fig. 3 *a*, almost all the neutral extractable bone collagen is normally transformed into insoluble fibrils within 4 to 6 hours. In

the puromycin groups, synthesis was apparently inhibited immediately; subsequently inhibition decreased as shown by the increasing radioactivity in the extractable pool at 6 and 8 hours. Probably the smaller size of the extractable pool in the puromycin groups is due to the rapid conversion of extractable collagen (synthesized just prior to giving puromycin) into insoluble fibrils. As the puromycin effect diminished, radioactivity appeared first in the extractable

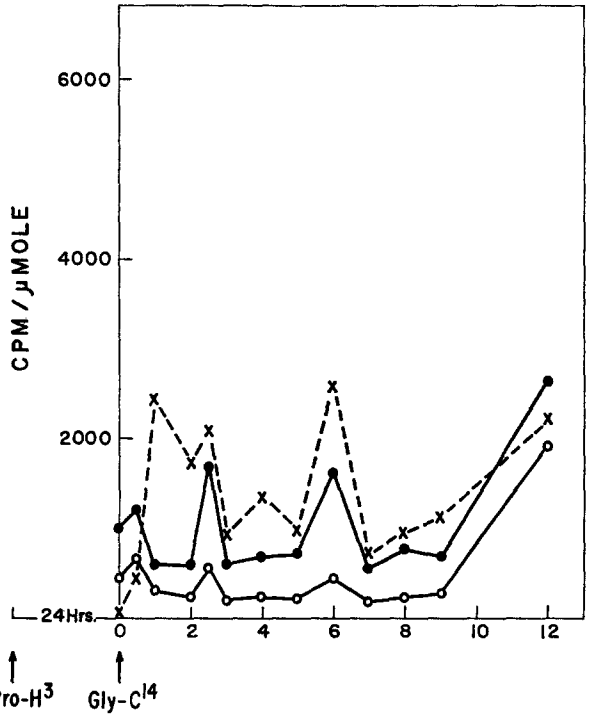

FIG. 7. Incorporation of proline-3,4-H^3 and glycine-1-C^{14} into normal extractable collagen Experimental conditions described in Fig. 5. x-----x, glycine-1-C^{14}; ●——●, proline-3,4-H^3 O——O, hydroxyproline-3-H^3.

collagen. An increase in this pool size and accumulation of radioactivity in the insoluble collagen was not seen and probably requires prolonged incubation to be detected. Also, puromycin may cause a delay in the process of "insolubilization." The greater incorporation of label in the group given βAPN plus puromycin is unexplained.

Once again, the results suggest a significant portion of the lathyritic extractable collagen originates from new synthesis. However, it is also possible that puromycin, by generally depressing formation of all proteins, may interfere indirectly with the lathyritic mechanism, particularly if enzyme production is required (see general discussion).

In these data we again see the significant incorporation of radioactivity in the insoluble lathyritic fibrils, and also see the relatively constant distribution of radioactivity between the two lathyritic collagen fractions.

Fluctuations in Radioactivity of Collagen.—Fluctuations in specific activity have been a constant feature of all these radioisotope experiments and of several other experiments (unpublished). Their origin is unknown, but they seem to be an intrinsic feature of the biologic system. Fig. 7 demonstrates the fluctuations in specific activity of three amino acids isolated from normal

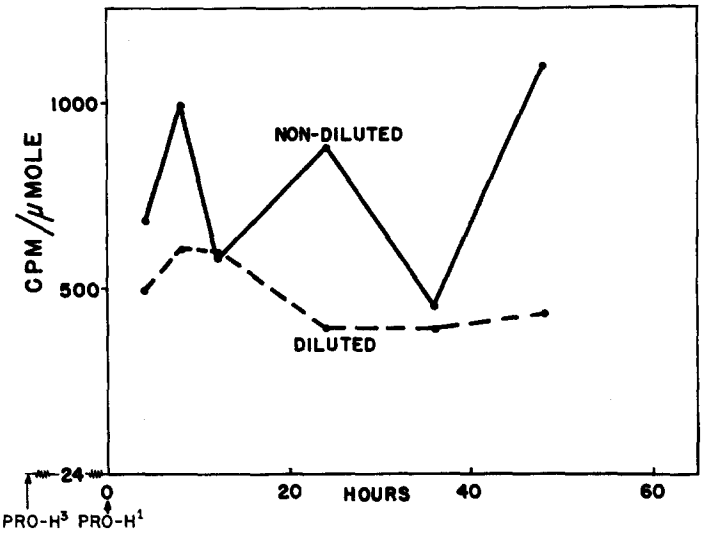

FIG. 8. Effect of non-radioactive proline on hydroxyproline specific activity of lathyritic extractable collagen. 10 animals per point. 10 μc proline-3,4-H^3 administered 24 hours prior to βAPN (20 mg), and 150 mg L-proline. Soluble collagen extracted in 1 M NaCl and precipitated by dialysis *versus* distilled water.

extractable collagen, obtained from the double labeling experiment described above. While synchrony is apparent, there is no obvious periodicity. True periodicity may possibly be masked by variation among the individuals which make up each group. Bentley and Jackson (25) have reported similar fluctuations of isotope incorporation into guinea pig skin collagen and, more recently Jackson (26) has observed large periodic, hourly, fluctuations in the specific activity of proline in collagen and other proteins from the chick embryo.

As suggested by Goodwin (27), the rise and fall of radioactivity may reflect changes in availability of intracellular free proline, directly affecting the rate of collagen synthesis. If this is the case, saturation of the intracellular proline pool might damp the oscillations of radioactivity. Fig. 8 illustrates damping of the oscillations does occur when the animal is loaded with cold proline. Thus, the fluctuation in radioactivity may actually represent pulses or waves at some

point in the collagen metabolism, either on the synthetic or degradative side. As has been previously suggested (28), discontinuous synthesis may be necessary for the organization of collagen fibrils into stacked arrays, forming a laminated connective tissue.

GENERAL DISCUSSION

The pathway of collagen metabolism in the normal animal seems, from present evidence (20, 21), to begin with intracellular synthesis of the complete three stranded molecules, tropocollagen, followed by their secretion into the extracellular space where they rapidly aggregate into fibrils. Whether molecules aggregate at the cell surface (29), at a distance from the cell, or both, is not relevant to the present discussion.

The degree to which the individual molecules and newly formed fibrils are extractable in cold neutral solutions varies greatly with the tissue, species, and stage of development. In the skin of rapidly growing guinea pigs as much as 10 per cent of the collagen is soluble in cold 0.45 M saline, whereas in the very rapidly growing chick embryo bone less than 1 per cent is soluble. Thus, the rate at which collagen fibrils become insoluble varies greatly. The factors responsible for such differences in solubility are unknown.

The marked change in collagen solubility induced by lathyrogenic agents may reflect a subtle alteration of the collagen molecule, manifested as a lack of intramolecular crosslinking and impairment of firm intermolecular bonding. The mechanisms producing such an alteration might be:

(*a*) A change in primary structure either as a result of a substituted amino acid, or of altered sequence, or due to incomplete assembly of the molecule. Although quantitative amino acid composition of lathyritic collagen appears normal (8), this analysis would not detect very small changes.

(*b*) A postsynthetic modification of normal primary structure, by deletion of a portion of the molecule, or by binding of a low molecular weight substance. These changes could occur either inside or outside the cell. β-Aminopropionitrile or its products are apparently not bound to lathyritic collagen nor is their continued presence required to maintain increased extractability (30).

Attributing increased collagen solubility in lathyrism to a failure of fibril formation (10–12, 31, 32) seems untenable for the following reasons:

(*a*) Very little collagen is extractable from normal or lathyritic tissues at physiologic temperature and pH (1).

(*b*) There appears to be no deficiency of fibrils in lathyritic skin, whereas after extraction the bulk of them are gone (9).

(*c*) Lathyritic collagen forms striated fibrils *in vitro* at about the same rate and extent as the normal (6).

(*d*) There is a significant flow of radioactivity into the insoluble collagen in

lathyritic tissues (Tables I and II), also shown by the data of others (11, 12, 32) but not emphasized to any extent.

In attempting to define the lathyritic process in terms of collagen metabolism, we have devised a tentative scheme. Assuming the synthesis of complete, normal collagen molecules, the lathyritic aberration would occur after the collagen leaves the synthesizing site. The subsequent alteration of the molecule could be accomplished either within the cell or in the extracellular space. We propose that the individual molecules remain dispersed for only a brief interval, and then rapidly aggregate into fibrils. Two maturation processes occur, perhaps independently, one being formation of intramolecular crosslinks (8, 33), the other being a progressive decrease in solubility of the fibrils (20, 21). Both processes seem to be disturbed by lathyrism.

The present studies suggest that lathyrism affects all phases of the newly produced collagen, the transient, dispersed molecules as well as the newly deposited fibrils. Possibly, some of the older insoluble fibrils are returned to an extractable state. In bone the mineral may be a complicating factor in that it may encase the lathyritic fibrils, preventing their extraction, and may also prevent encrusted, normal fibrils from becoming lathyritic.

In as much as a variety of proteolytic enzymes, pepsin (34), chymotrypsin (35), and trypsin (36), can remove intramolecular cross-links from the β-components of soluble collagen, lathyritic collagen may be produced by the attack of a tissue protease, possibly released by connective tissue cells which are exposed to lathyrogenic agents.

We thank Miss Barbara McKee for her invaluable technical assistance.

SUMMARY

1. Radioisotope incorporation studies of normal and lathyritic chick embryo bone collagen do not demonstrate any interference by lathyrism with collagen synthesis or fibril formation.

2. The results indicate that a portion of the extractable collagen from lathyritic chick embryo bone represents newly synthesized protein. Evidence from a double labeling experiment and from analysis of isotope flow between the extractable and non-extractable pools suggests the extractable lathyritic collagen is heterogeneous. We propose that the lathyritic process affects collagen in all states of aggregation, probably in varying degree.

3. Puromycin, administered intravenously, reduces the amount of extractable collagen in both normal and lathyritic chick embryo bone, and diminishes the incorporation of labeled proline into collagen.

4. Marked fluctuations in incorporation of labeled amino acids into chick embryo bone collagen suggests the occurrence of wide fluctuations in metabolism of this protein.

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