THE INCORPORATION OF LYSINE INTO GROWING ELASTIN*

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During a study of the changes in the amino acid analysis of elastins isolated from the bovine nuchal ligament at various ages, it was found (Cleary et.al. 1965) that during fetal development there was a sharp fall in the lysine content accompanied by an increase in the cross-linking compounds, isodesmosine and desmosine (Thomas et. al., 1963), and a similar rise in the content of X4 (Franzblau et.al., 1965), another compound unique to elastin. Although the desmosines had reached a steady value by the last fetal month, the lysine content continued to fall (more slowly) and the X4 content slowly increased. These results were consistent with the postulate that X4, like the desmosines, was derived from lysine, although it was difficult to reconcile this with the formula proposed for X4 by Franzblau et. al. (1965a). An attempt was made to study this relationship during the incorporation of radioactive lysine into aorta elastine in vivo. It has been found that radioactivity derived from lysine is incorporated not only into the desmosines and X4, but also into two other similar, small, ninhydrin-positive peaks in this same

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region of the chromatogram. These too, appear to be unique to elastin. It has been found further, that there are three other zones of significant radioactivity, eluted with the neutral amino acids.

MATERIALS AND METHODS

Five suckling rats (Long-Evans strain) were weighed daily until the 12th post-natal day when they entered the phase of rapid growth. At this time the average weight was 30g. Two animals each received a total of 0.5mc of uniformly labelled C^{14} lysine (Nuclear-Chicago), given intraperitoneally in doses of 16.6 uc according to the following schedule : one injection daily on days 12 through 30, followed by one injection every 8 hours for 4 further days. The three control animals received equivalent amounts of physiological saline. Eight hours after the final injections, all animals were killed and the aorta, including the carotid and iliac arteries, was removed.

The vessels were freed of loose adventitial tissue, minced and then extracted with saline. Collagen was removed by autoclaving in water at 30 psi. for 6 hours, and the alkali-insoluble elastin residue prepared by heating in 0.1 N NaOH at 98°C for 30 minutes. The dried elastin residue was hydrolyzed in 6N HCl at 115° C for 72 hours, in a sealed tube under nitrogen. Portions of the hydrolysate were subjected to amino acid analysis using the standard 22 hour system with the Technicon Autoanalyser, coupled in series with a Packard anthracene scintillation flow cell (Rapkin and Gibbs 1962) using a modification of the system described by Elwyn (1964).

RESULTS

The experimental and control animals showed no significant differences in growth rate, aorta weight or in elastin yield.

Figure I shows a typical chromatogram in the region from ammonia to lysine. It is apparent that there are three well separated ninhydrin-

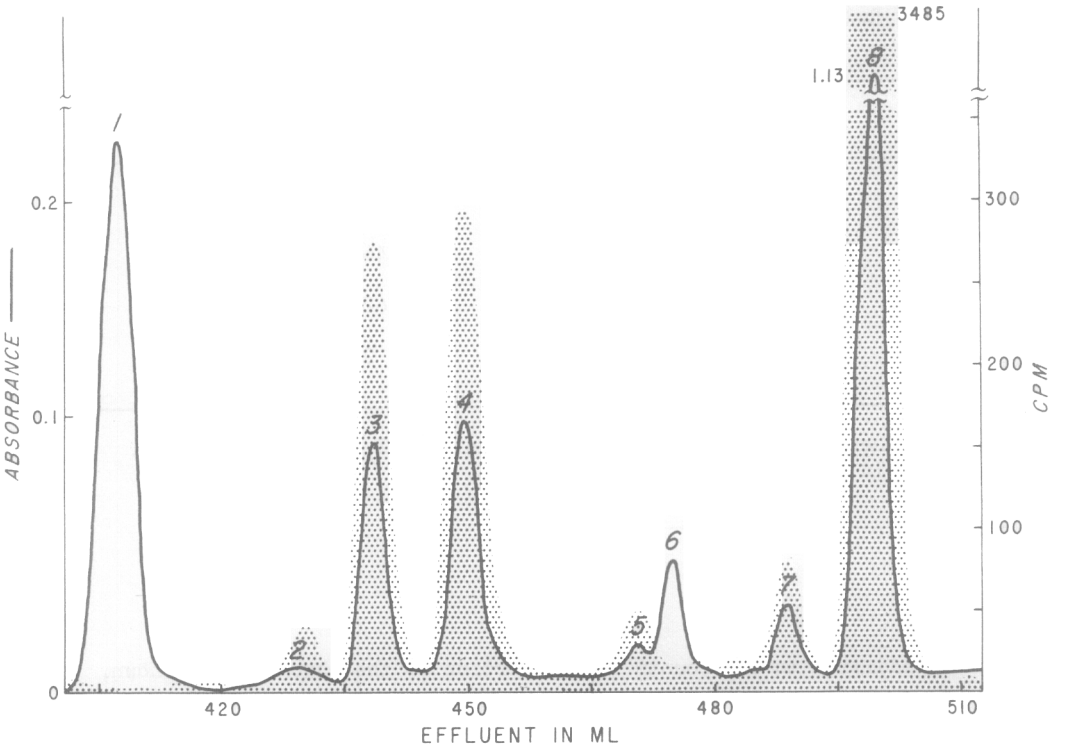

Fig. 1. Portion of a typical elastin chromatogram and radioactivity scan. Peak numbers refer to 1. NH_3 2. X_2 3. Isodesmosine. 4. Desmosine. 5. X_3 6. Ornithine. 7. X_4 8. Lysine.

Radioactivity has been related to the ninhydrin curve by using lysine as reference allowing for a delay of 1.5 ml, due to an isotope effect (Piez 1962). This was confirmed by adding radioactive norleucine as an independent marker. Background was 21 cpm.

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positive peaks in this region, apart from those due to the desmosines and to ornithine. These are usually present in elastin chromatograms. Radioactive counts are present not only in the lysine and in the desmosines, but also in the X4 and X3, peaks and in the small un-named peak immediately preceding isodesmosine (X_2 in Fig.1.). The number of counts, relative to the ninhydrin colour yield is approximately the same for each of these latter three peaks.

The amount of radioactivity in the identified compounds is shown in Table I.

TABLE I

Radioactive lysine incorporation into elastin amino acids.

Amino Acid	DPM/UM of amino acid
Isodesmosine	166,365
Desmosine	146,069
X_4	62,516
Lysine	2,427

For these calculations, it was assumed that X_4 gives a ninhydrin colour yield equivalent to that of leucine.

In addition to the counts in the portion of the chromatogram shown, significant radioactivity was found in three other regions of the chromatogram - under glycine, under tyrosine and associated with the leucine/isoleucine region. The relative distribution of the radioactivity throughout the chromatogram is shown in Table 2.

TABLE 2.

Percentage distribution of DPM

dpm/mg. elastin	23,657
% under glycine	5.0
% in leucine area	3.7
% under tyrosine	4.5
% in desmosines	13.0
% in X_3 and X_4 areas	3.3
% under lysine	65.0
Total % accounted for	94.5

In similar experiments in chicks, a much higher proportion of the radioactivity was associated with the glycine peak. (unpublished data). This fraction of the column effluent was collected, desalted with Bio-Rad AG11A8 ion retardation resin and concentrated. On thin layer chromatography in methanol:water:pyridine, 8:2:4, the radioactivity separated completely from the glycine as a ninhydrin-positive zone with an Rf 0.7. Addition of methanol to chambers 3 and 4 of the Technicon buffer system permitted complete resolution of this radioactive material from the glycine in the amino acid chromatogram. The material appeared as a faintly ninhydrin-positive peak 15 mls. before glycine. In this system also the radioactivity under the tyrosine peak was delayed and completely separated from the tyrosine.

DISCUSSION

The radioisotope dosage schedule limits interpretation of the exact significance of the data, in terms of precursor relationships but clearly establishes certain points. Radioactive lysine is incorporated not only into X_4 but also into X_3 and into the ninhydrin-positive material represented by the small peak preceding isodesmosine. The nature of these two substances is unknown. Since this work was undertaken, Franzblau et. al. (1965b) have revised their data on the structure of X_4, which they have now designated lysinonorleucine, and for which they postulate a possible cross-linking role in elastin. The present results are in agreement with their findings.

The presence of significant radioactivity associated with glycine and leucine has not been reported before although a study similar to this has been made (Partridge et. al. 1964).

As it has been postulated that the aldehyde of lysine is involved as an intermediate in the synthesis of the desmosine (Partridge et. al. 1964) and of lysinonorleucine (Franzblau et. al., 1965b), it is tempting

to suggest that one or other of the unidentified radioactive materials may be related to this aldehyde.

It would seem then, that the role of lysine in elastin biosynthesis will prove to be much more complex than at present envisaged.

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