# EMBRYONIC SKIN COLLAGEN. REPLACEMENT OF THE TYPE OF ALDIMINE CROSSLINKS DURING THE EARLY GROWTH PERIOD

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## 1. Introduction

Recent studies have clearly demonstrated that the collagen fibres of mammalian skin are stabilized by at least two different intermolecular crosslinks of the aldimine bond type. One of these crosslinks has been isolated in the reduced form from borohydride-reduced collagen and identified as hydroxylysinonorleucine [1, 2]. The crosslink must therefore exist in the native fibre as an aldimine bond derived from the condensation of the aldehyde produced by oxidative deamination of peptide bound lysine and the $\epsilon$-$NH_2$ group of a peptide bound hydroxylysine residue. The structure of the second intermolecular crosslink of mammalian skin collagen has not yet been elucidated but has the properties of an aldimine type structure [3].

Because of their labile nature these intermolecular aldimine crosslinks cannot account for the observed decrease in solubility with increasing age; for this the presence of bonds of higher thermal stability would be required. Analysis of the skin collagen from older mammals revealed that the proportion of these reducible crosslinks decreased with age and that they were virtually absent at maturity [4]. The mechanism by which the crosslinks lose their reducing capacity and become thermally stable with age has not yet been established but *in vivo* reduction to hydroxylysinonorleucine does not occur [5].

In continuation of these age studies we have analysed embryonic skin collagen to determine the nature of the crosslinks present in this tissue, particularly in view of its surprisingly low solubility. In this paper we report that the major crosslink present in embryonic chick skin and mammalian foetal skin (peak 1, figs. 1a and 2a) is identical to that previously shown to be present in insoluble bone collagen [6]. The structure of this compound suggests that it may be derived from a collagen molecule of a chemically different type. Since this crosslink is not present in adult skin the embryonic collagen is probably rapidly replaced during the latter part of pregnancy and the initial growth stage after birth.

## 2. Methods and results

### 2.1. *Reduction and analysis of the reducible components*

Samples of skin were obtained from chicks at various ages from 11 day embryos to 12 wk pullets. Skin samples were also obtained from foetal and young calf, and from foetal and human skin. The samples were analysed for the presence of reducible cross-links and their precursors by amino acid analysis after reduction with potassium borohydride as previously described in detail [3]. The identification of the radioactive peaks as the intermolecular crosslinks previously characterised was confirmed by analysis against authentic samples using the amino acid autoanalyser and by high voltage electrophoresis.

Typical elution patterns of reduced skin of 15 day embryonic, 3 wk and 12 wk old chick skin, are shown in fig. 1. There is a dramatic disappearance of the major embryonic component (peak 1, fig. 1a) to be replaced after hatching of the egg, by the usual elution pattern obtained for young skin which contains only peaks 2 and 3 (fig. 1b and 1c). Similar changes were observed with 12 wk bovine foetus and a 3 mon old calf (fig. 2) and again with a 16 wk human foetus and a 1 day old baby. Analysis of the components under peaks 1 and



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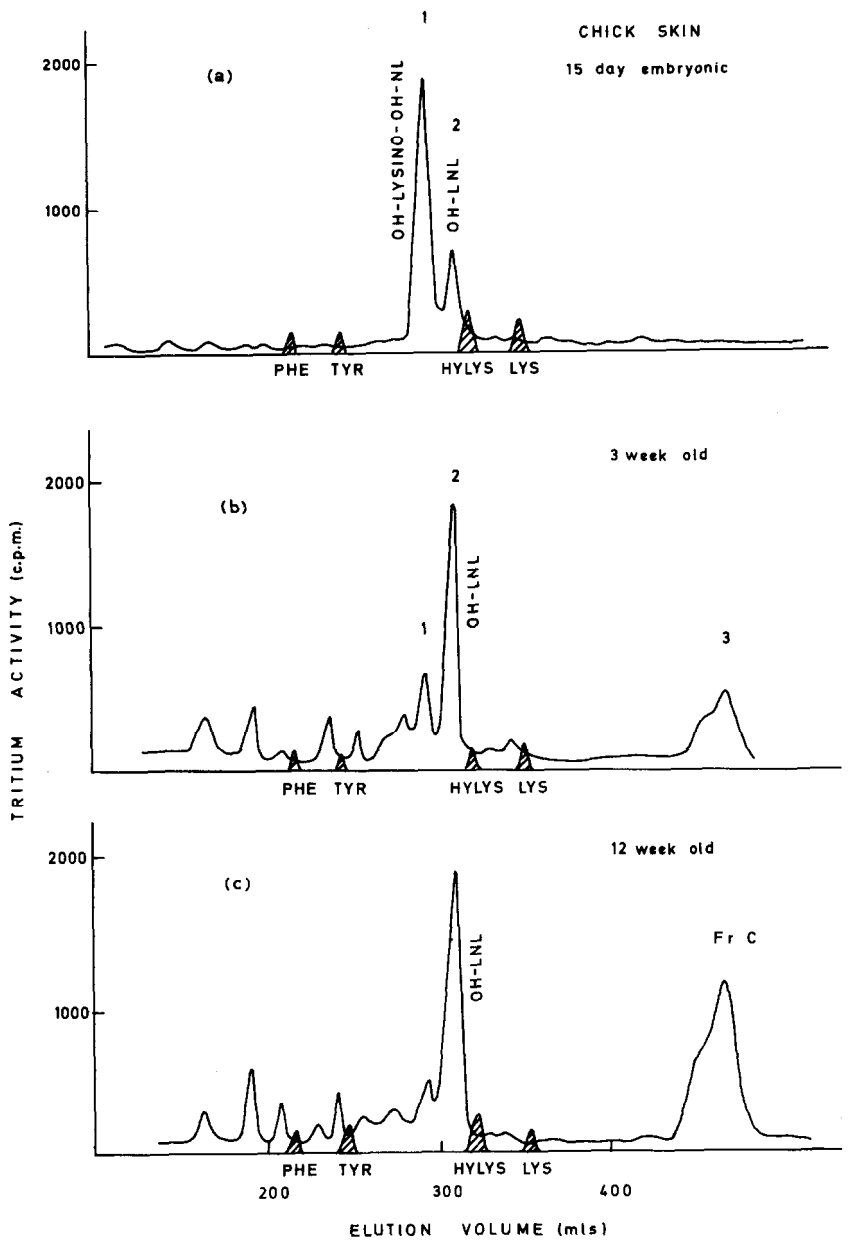


Fig. 1. Distribution of radioactive components from an acid hydrolysate of $KB^3H_4$ reduced intact collagen from chick skin. The components were separated by ion-exchange chromatography using volatile buffers. a) 15 day embryonic chick; b) 3 wk old chick; c) 12 wk old chick. (Peak 1: hydroxylysino hydroxynorleucine; peak 2: hydroxylysinonorleucine; peak 3: unknown frac. C).

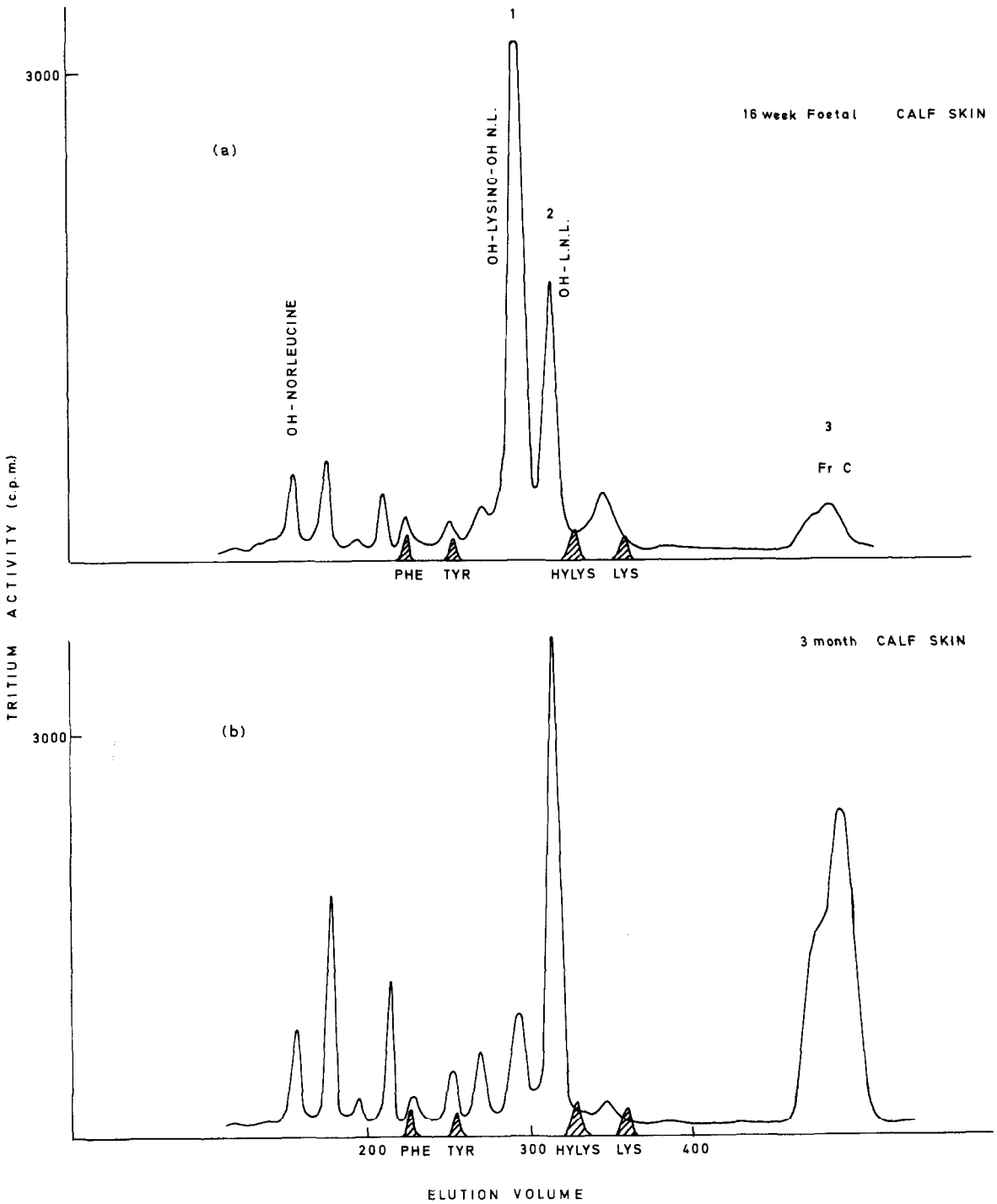


Fig. 2. Elution pattern of $^{3}H$-labelled reducible components obtained from the acid hydrolysate of intact collagen from mamalian skin. a) 3 mon foetal calf; b) 3 mon old calf. Basically similar patterns were obtained for 16 wk human foetus and 1 mon old human baby skin. (Peak 1: hydroxylysino hydroxynorleucine; peak 2: hydroxylysinonorleucine).

2 revealed them to be hydroxylysino hydroxynorleucine and hydroxylysino norleucine, respectively.

### 2.2. *Acrylamide gel electrophoresis*

Denatured, solubilized collagen from the foetal and young skins were analysed by SDS acrylamide gel electrophoresis. The ratio of $\alpha 1$ to $\alpha 2$ was determined on the stained gels using a Joyce Loebl Chromascan. The foetal skin had a slightly higher proportion of $\alpha 1$, the ratios being 2.5:1 and 2.0:1 for the foetal and 3 mon old calf skin, respectively.

## 3. Discussion

In contrast to collagen from young avian and mammalian skin (fig. 1c and 2b), the major reducible component of embryonic skin collagen (fig. 1a and 2a) has been shown to be identical to the crosslink previously observed in bone [6] and tendon [3] collagen. This crosslink, given the trivial name syndesine, was originally thought to be an aldol derived from hydroxylysine-aldehyde but an unambiguous identification has now been achieved by chemical synthesis demonstrating that it is an aldimine bond derived from hydroxylysine-aldehyde [7]. This crosslink has also recently been identified in tendon collagen [8] and must exist in the native fibre as dehydro-hydroxylysino hydroxynorleucine.

The presence of this crosslink indicates that hydroxylysine occurs in the N-terminal telopeptide of embryonic and foetal collagens. However, a number of workers have carried out sequence studies on young chick, rat, calf and human skin collagen [9–11] and demonstrated the absence of hydroxylysine in this region, whereas in postnatal bone collagen this telopeptide lysine residue is hydroxylated [12]. In contrast, the recent findings of Barnes et al. [13, 14] show that in embryonic chick skin this specific lysine is about 50% hydroxylated. These results would be consistent with our present findings that the crosslink is present only in embryonic skin, and our contention that it is derived from the N-terminal telopeptide hydroxylysine. The non-helical nature of this region presumably facilitates accessibility to an amine oxidase thus permitting conversion to the aldehyde and subsequent crosslink formation to an adjacent molecule.

Trelstad et al. [15] have recently suggested the presence of a 'primitive' collagen believed to be comprised of three identical $\alpha$-chains (type III collagen) in embryonic chick skin, which is not present in chicks a few wk old. This collagen may be similar to the collagen consisting of 3 identical $\alpha$-chains observed in cartilage by Miller [16]. However, based on the acrylamide gel analysis the proportion of the type III collagen must be small (about 20%) since the ratio of $\alpha 1$ to $\alpha 2$ is not markedly increased, in contrast to cartilage collagen where very little $\alpha 2$ is detectable. It is interesting to note that cartilage collagen is highly hydroxylated and contains the same reducible crosslink as embryonic skin [4]. Preliminary amino acid analyses indicate that embryonic skin collagen contains twice as many hydroxylysine residues compared to adult skin. This highly hydroxylated embryonic collagen is probably reabsorbed and replaced by normal skin collagen, i.e. type I, $(\alpha 1)_2\alpha_2$ during the rapid growth phase, since the crosslink is absent from 3 wk chicks and 3 mon old calves. This would be the simplest explanation: however, the studies of Barnes et al. [14] showed that the $\alpha 2$ telopeptide, as well as the $\alpha 1$ peptide, of embryonic skin loses hydroxylysine during the initial growth stage. The replacement of the embryonic collagen may be more complex than initially appeared.

The variation in the nature of the crosslinks that we have shown to occur in different tissues is evidently finely controlled, and clearly must be closely related to function. The replacement of one type of relatively stable aldimine bond by 2 labile aldimine bonds and their eventual conversion into a non-reducible, permanently stable system of crosslinkages presents a further problem in the elucidation of the relationship between structure and function in collagen fibre.

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