injected i.v. before the lethal dose of anesthetic, and then the pulmonary vasculature was irrigated with physiological saline; latex was injected manually from a 10 ml syringe into the pulmonary vasculature and airways, but filling varied regionally so that in some areas the resulting casts amounted to single injections. In 1 bird only the airways were cast. The lungs were left in situ for about 12 h for the latex to set before removal and immersion in concentrated hydrochloric acid. All tissues for SEM were coated with gold-palladium complex before viewing.

From the lungs of the remaining 4 birds resin blocks were prepared after initial fixation with glutaraldehyde, post-fixation in osmium tetroxide, and dehydration in ethanol. Semithin sections were then cut and stained with methylene blue; the minimum diameters of the air capillaries were determined using a calibrated graticule.

Results. The network of air and blood capillaries exhibits a 3-dimensional distribution. The calibre of the air capillaries fluctuates very greatly and with remarkable abruptness (figs 1 and 2), while that of the blood capillaries tends to be relatively uniform (figs 3 and 4). The air capillaries are also much more tortuous, and anastomose far more irregularly than the blood capillaries. The mean minimum diameter of the air capillaries as estimated on resin sections was 8 μm. In critical point dried materials red blood cells overlapped each other about half way and were slightly folded longitudinally.

Discussion. The observations confirm that the air capillaries are not blind ending and that they do anastomose repeatedly as they intimately interlace with the blood capillaries.

The number of air capillaries in the duck lung was estimated by Scheid et al.[12] for physiological purposes; however, it would be impossible to quantify this number realistically, even in the domestic fowl, since the individual air capillary is so very ill defined and is by no means a discrete structure like a mammalian alveolus.

Measurements of the dimensions of the air capillaries were not attempted on the casts because of the shrinkage of the latex, the possibility of over- or underfilling, the abrupt fluctuations in calibre, and the frequent anastomoses of these small airways. Minimum diameters were determined (on resin sections), as this measurement is more representative because most air capillaries in profile are cut obliquely and thus their greater diameter is much more variable than their lesser diameter; the value of 8 μm is comparable to that reported by Brackenbury and Akester[9] of 6 μm for the same species. The minimum diameters of blood and air capillaries of the Common Starling *(Sturnus vulgaris)* are 3 and 4 μm[13] respectively; the diameters of air capillaries in birds in general has been estimated to range from 3 to 10 μm[14]. The reported value of 2 μm[11] for the minimum diameter of the air capillaries in the domestic fowl would therefore appear to be an underestimate, possibly due to shrinkage of the cast, or underfilling of the air capillaries, or both.

The observed overlap of erythrocytes in the blood capillaries corroborates a similar finding by Akester[10]; folding may increase the area of contact between the erythrocytes and the capillary endothelium.

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Glycosaminoglycan synthesis by embryonic fibroblasts is age-dependent and modulated by environmental factors[1]

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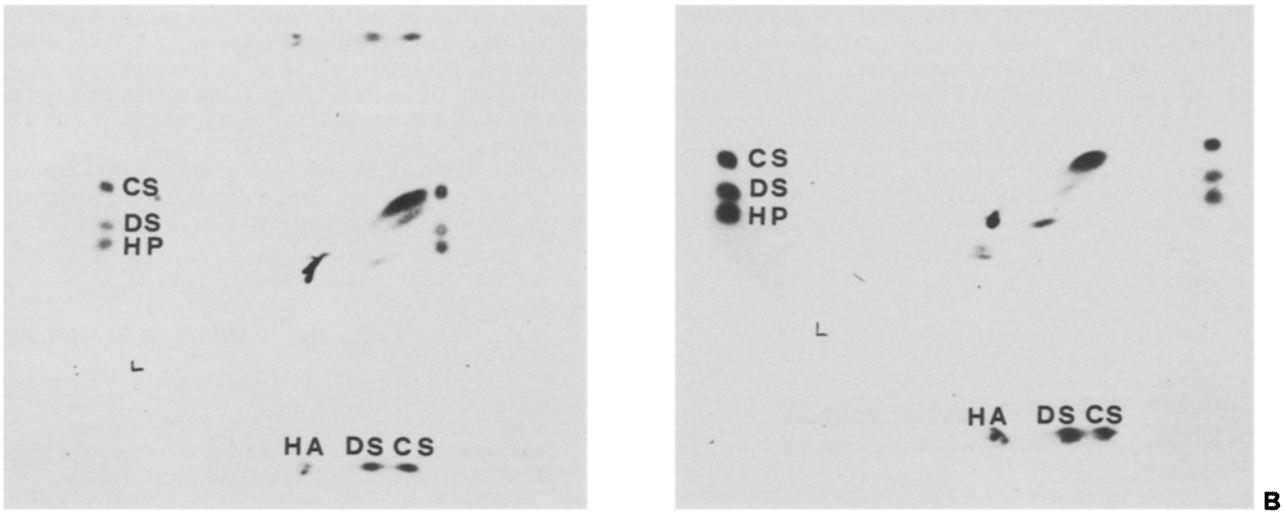
Summary. The glycosaminoglycans (GAG) secreted by primary fibroblasts cultures removed from chick embryo skin after 7 and 14 days of incubation have been investigated. Differences in GAG composition have been detected, depending on age and on the composition of the nutrient medium.

Much histochemical and biochemical evidence indicates that the mesenchymal ground substance of several organ rudiments (for example; skin, lung, alimentary tract) undergoes qualitative and quantitative changes in its patterns of glycoproteins (GP) and glycosaminoglycans (GAG) in the course of development[3-5]. The factors responsible for these effects are poorly understood.

A central role, however, is likely to be played by fibroblasts; e.g. fibroblast-like cells derived from 3 tissues (heart, skin and cornea) of 14-day-old embryonic chick produce different amounts of GAG[6]. The ability of fibroblasts to synthesize different patterns of GAG and GP may be dependent on specific cell differentiation and/or modulation by environmental factors.

We have previously shown that chick embryonic skin in vitro develops in different ways according to its nutrition. Epidermis undergo keratinization in chicken serum-containing medium, it does not in chick embryonic extract-containing medium. In the differentiating explants, dermal ground substance changes its GAG composition in a way which correlates with keratinization[7,8]. Such a system provides a suitable model for studying the factors involved in



2-Dimensional electrophoresis patterns of the glycosaminoglycans secreted by primary fibroblasts cultures derived from 7 day chick embryo skin *a* plated with extract and *b* plated with serum. HA, hyaluronic acid; CS, chondroitin sulfate; DS, dermatan sulfate; HS, heparan sulfate; HP, heparin.

the control of the cellular production of GAG. We have therefore examined the GAG synthesis by in vitro fibroblasts at various developmental stages and in different nutrients.

Material and methods. Back skin fragments were carefully removed from 7- and 14-day-old chick embryos, cut into small pieces, washed in Tyrode's solution and dissociated in 0.25% trypsin (Difco, 1:300; room temperature, 25–30 min). Cells were recovered by centrifugation and suspended in the following nutrients; medium 199 plus 20% calf serum or medium 199 plus 20% homologous chick embryo extract. 5 ml of cell suspension ($5–6 \times 10^6$ cell/ml) were placed in Falcon flasks in a humidity-saturated atmosphere of 5% CO_2 at 37 °C for 24–48 h to obtain confluent and stationary cultures. Since the extract was unable to support the maintenance and growth of 14-day-old fibroblasts, in 1 set of experiments we used cell cultures plated with serum for 36 h and then incubated with chick extracts for another 48 h. The nutrient was then replaced with medium 199 plus 5 µg/ml insulin and the cultures were incubated for an additional 48 h, after which the cells and media were recovered separately. The EDTA-disaggegrated cells were counted with a Bürker chamber. Parallel cultures were used for histological and histochemical analyses (PAS and methylene blue).

From pooled media (4 cultures for each experiment) GAG were isolated according to the method of Breen et al.[9]. Total GAG content was measured as hexosamine according to Cessi and Piliego's procedure[10]. Individual GAG were separated by means of 2-dimensional electrophoresis[11]. They were identified by comparing them to standard GAG run at the same time and by their susceptibility to testicular hyaluronidase. They were quantified using a micro-colorimetric method[12].

Results and discussion. Skin fibroblasts from 7- and 14-day-old chick embryos plated with serum for 24–36 h produced a continuous monolayer which was formed of narrow, elongated and fuse-shaped cells. When plated with extract, 7-day-old fibroblasts reached the confluence stage more slowly (36–48 h), but exhibited little sign of suffering, whereas 14-day-old fibroblasts were unable to adhere to the substratum and died within 18–24 h.

7-day-old skin embryonic fibroblasts produced greater amounts of GAG when plated with serum compared to when they were supplemented with extract (ratio 10:1, see table). Significantly smaller quantities of GAG were produced by 14 day skin fibroblasts plated with serum.

Toxic activity by the extract-containing medium was ruled out by the following experiments; 7 day fibroblast cultures supplemented with 14 day extract grew normally; 14 day fibroblast cultures plated with serum, to which the extract was added subsequently, produced and secreted noticeable amounts of GAG, although less than those produced by 7 day fibroblasts under the same conditions (see table).

The amount of GAG synthesised by fibroblasts is age dependent. A dependence of cell surface GP synthesis on the fibroblast's age has previously been shown[13]. Since protein-carbohydrate complexes play a key role in enabling the cultured cells to adhere to the substratum[14–16], the inability of the embryo extract to support the maintenance of 14-day-old fibroblasts in vitro may be due to reduced GAG synthesis.

Glycosaminoglycans secreted by primary fibroblasts cultures

Culture medium[a]	7-day incubation			14-day incubation	
	Serum	Extract	Serum + extract	Serum	Serum + extract
Total GAGs content[b]	30.9 ± 0.4	3.1 ± 0.3	17.0 ± 2.2	9.1 ± 0.6	4.2 ± 1.9
Hyaluronic acid[c]	28.8 ± 3.9	32.6 ± 4.0	26.5 ± 2.8	52.1 ± 1.4	43.8 ± 7.8
Chondroitin 4/6 sulfate	49.1 ± 4.9	38.6 ± 5.1	54.1 ± 2.0	37.3 ± 2.4	40.9 ± 4.5
Dermatan sulfate	11.5 ± 5.0	18.1 ± 5.4	9.9 ± 0.4	6.9 ± 1.7	9.1 ± 4.6
Heparan sulfate	14.1 ± 1.8	10.6 ± 2.7	9.5 ± 0.4	3.8 ± 2.1	6.2 ± 2.1

3 independent experiments, each in duplicate.
[a]see methods; [b]expressed as µg hexosamine/10^6 cells/culture; mean ± σ; [c]expressed as percentage of total GAGs content; mean ± σ.

Skin fibroblasts secreted the following types of GAG: hyaluronic acid (HA), chondroitin 4/6 sulfate (4/6-CS), dermatan sulfate (DS), heparan sulfate (HS) (fig.). The relative concentration of individual GAG in 7 day fibroblasts cultures reveals a prevalence of CS under the 3 experimental conditions (with serum, extract, serum + extract), but a HA prevalence in 14 day fibroblasts cultures. It was interesting to note that an accumulation of HA was detected in embryonic skin explants when serum was added[7], thus suggesting that the nutrient acts directly at the cellular level. Our experiments demonstrate that the pattern of GAG synthesis by embryonic fibroblasts is a result of a complex system of regulatory factors; on the one side by an age-dependent cell differentiation, and on the other by a modulatory effect of environmental factors.

The demonstration that environment acts on GAG production further supports the possible role of mesenchymal ground substance in regulating epithelial differentiation.

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Receptors for thymosin fraction V on rat thymic lymphocytes[1]

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Summary. Binding by rat thymus lymphocytes of thymosin V, labeled with colloidal gold, was studied. Under the experimental conditions employed at least 2.8% cells exhibited thymosin binding sites.

Peptides of fraction V of thymosin induce expression of T cell surface antigens (Thy, Tl, Ly)[4-6] and differentiation of presursor cells into T_1, and then T_2 immunologically competent cells[7].

Although the mechanism of action of these peptides is still controversial, data from several laboratories indicate that interaction of a target cell population with thymic peptides is mediated by cyclic AMP[8]. Until now, specific surface receptors for thymic peptides have been demonstrated on the following target cell populations; receptor on T cell lymphoblastoid lines for serum thymic factor (FTS)[9], receptors for thymus factor X (TFX, peptides from calf thymus) on rat thymocytes[10].

In this report we present evidence for surface receptors of rat thymic lymphocytes for colloidal gold-labeled peptides of thymosin fraction V.

Material and methods. Thymic lymphocytes were teased from thymuses of Wistar rats, aged 36 days. The suspension of lymphocytes was centrifuged at 2000 × g and washed in PBS. The cell suspension contained approximately 99% lymphocytes, of which 98% were viable, as evidenced by the trypan blue dye exclusion test. Washed cells were fixed with 2.5% glutaraldehyde in 0.5 M phosphate buffer, pH 7.3 for 15 min at 4 °C.

Colloidal gold was prepared by reducing chlorauric acid ($HAuCl_4ICN$, Merck) with trisodium citrate, according to

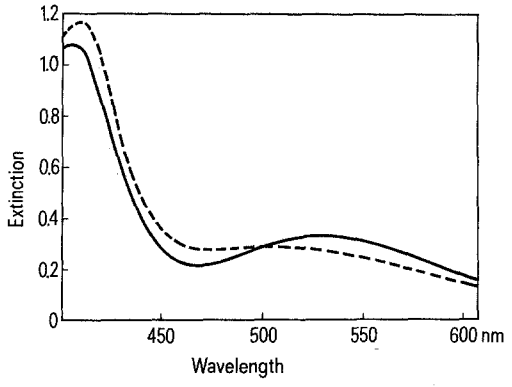

Figure 1. Demonstration of the thymosin-gold-binding sites at the plasma membrane of the thymic lymphocytes. × 33,800.

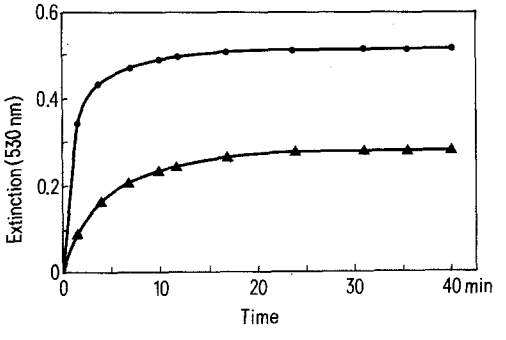

Figure 2. Thymosin-binding lymphocytes. T-Au complex covered some region of cell surface. × 10,000.