were noted extensively within the stratum corneum and there was a noticeable increase in the occurrence of local blood vessels. The number of melanocytes in the papillary layer of the dermis increased proportionately to the applied DMBA concentration (figs 2b and 3a).

Skin patches from hibernators demonstrated a thin cellular epidermis and a relatively thick stratum corneum. Both epidermal and dermal pigmentation were minimal and appeared histologically identical to untreated patches from nonhibernating animals. Melanocytes with large coarse cytoplasmic granules were absent and local blood vessels appeared similar to those from untreated patches (figs 2c and 3b).

Following sacrifice gross inspection indicated that internal organs were unaffected by treatment of the integument with DMBA in both groups of animals.

Discussion. Although the literature contains reports of tumor induction in various species of mammals exposed to topical applications of DMBA[8-13] this is the first study involving exposure of hibernating and nonhibernating mammals. As such, it was not the intent of this preliminary work to discern why but rather *if* naturally occurring physiological changes can protect an organism from carcinogen-associated gross and microscopic alterations. Indeed, the data obtained from this study confirm the speculation that the altered physiology of hibernation can confer such protection. Early, rather dramatic skin changes were consistently noted in nonhibernators in proportion to DMBA concentration. These included blistering, peeling, drying, hyperpigmentation, hair loss and peripheral vasodilation. Notably, hibernating animals remained unaffected by all treatments.

The reasons for this dichotomous reactivity between hibernators and nonhibernators remain unclear. It is possible that the noticeably thicker stratum corneum in hibernator skin may serve as a natural mechanical barrier to protect the underlying dermis during the protracted period of hibernation. That this mechanical barrier could have reduced the amount of DMBA entering the cellular strata and dermis cannot be discounted. However, this may be somewhat minimized by the fact that DMSO as a solvent has a proven ability to rapidly penetrate skin[15].

In addition, hibernating animals exhibit both a lower mitotic index and severe metabolic depression[16]. These factors may partially account for the observed difference in skin responses between these 2 groups of animals. With fewer cells undergoing mitosis less likelihood exists of incorporating DMBA-damaged DNA[17] into the genome. Insofar as metabolism is concerned, damaged DNA can be repaired more efficiently in cells having to perform at low rather than high levels of routine maintenance[5]. Equally plausible is the possibility that DMBA is not sufficiently catabolized to its active constituents in hibernators due to alterations in the microsomal enzymes[18] responsible for its breakdown.

Apparently physiological factors can render animals sensitive or resistant to a chemical carcinogen. This may provide insight into the stimulation or inhibition or the body's own mechanisms to arrest and control endogenously as well as exogenously-generated aberrant processes. Further study of skin reactivity in hibernating and nonhibernating animals following topical application of DMBA is in progress.

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A scanning electron microscopic study of the air and blood capillaries of the lung of the domestic fowl *(Gallus domesticus)*

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Summary. Air and blood capillaries of the lung of the domestic fowl constitute the functional gas exchange units. They anastomose profusely and interlace with each other in 3 dimensions. Air capillaries are not blind-ending tubules as has occasionally been suggested.

A cross-current relationship between bulk parabronchial gas and pulmonary blood flow has been experimentally demonstrated[2] and anatomically confirmed[3]. There is no doubt that this relationship is of prime importance in the superior arterialisation of blood in the avian lung, compared with that of mammals[4]. Nevertheless the actual gas exchange in the avian lung takes place in the exchange tissue mantle which surrounds the parabronchial lumina, the ultimate functional units being the air capillaries and blood capillaries.

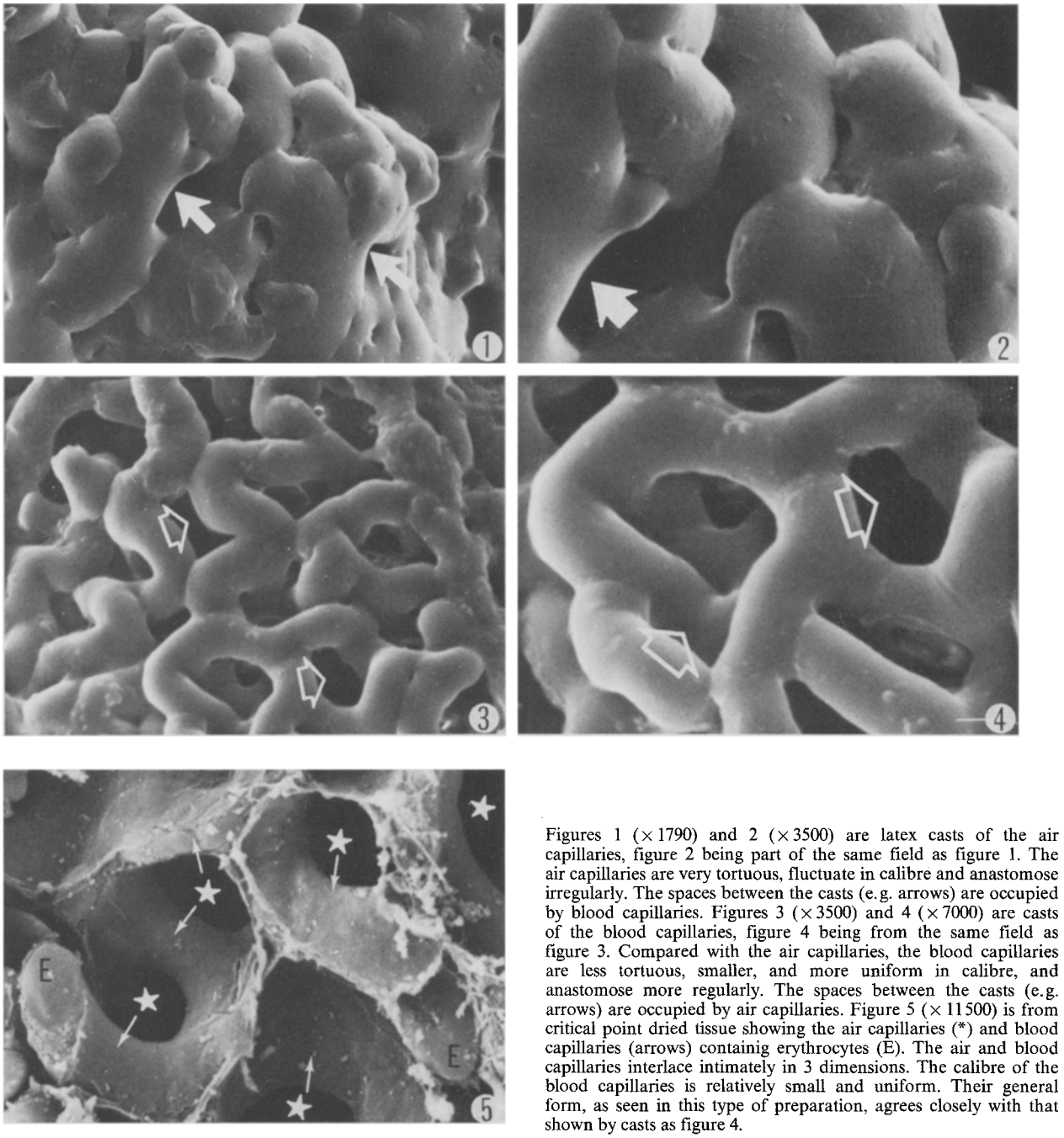
The size, shape and spatial relationships of these structures are undoubtedly of great functional significance, but very little is known about their anatomy. Even the question of the extent to which the air capillaries anastomose with each

other remains unsettled. Whilst it has long been generally assumed that they do anastomose extensively, there have always been some doubts (see King[5] for a review of earlier work) which have been repeated more recently[6,7].

The scanning EM (SEM) should solve such problems about the general arrangement of the air and blood capillaries, either by direct observations on critical point dried tissue or better still on casts. In general the form and arrangement of the pulmonary blood capillaries of birds are relatively well understood; this is because it is comparatively easy to fill these vessels with casts[8,9], and on critical point dried materials[10] the erythrocyte contents facilitate the identification of the blood capillaries. On the other hand the air capillaries are relatively difficult to cast, and furthermore they lack distinctive features which would enable them to be reliably identified in critical point dried tissue. The only convincing photographic evidence of successful casting of the air capillaries seems to be by Fujii et al.[11]. It has been assumed that in casts of the blood capillaries the spaces which they do not occupy represent the air capillaries[9]; this may well be true, but it is very difficult to gain a clear 3-dimensional impression of the configuration of the air capillaries by this approach.

This paper reports observations with SEM on the arrangement of the air capillaries and blood capillaries in the exchange tissue of the domestic fowl, using critical point dried tissue and casts. Observations on the dimensions of the air capillaries using resin sections are also given.

Materials and methods. The lungs of 9 mature female domestic fowls killed by an i.v. barbiturate injection were used. From 2 birds critical point dried materials of glutaraldehyde fixed lungs were prepared. In 2 birds heparin was



Figures 1 (×1790) and 2 (×3500) are latex casts of the air capillaries, figure 2 being part of the same field as figure 1. The air capillaries are very tortuous, fluctuate in calibre and anastomose irregularly. The spaces between the casts (e.g. arrows) are occupied by blood capillaries. Figures 3 (×3500) and 4 (×7000) are casts of the blood capillaries, figure 4 being from the same field as figure 3. Compared with the air capillaries, the blood capillaries are less tortuous, smaller, and more uniform in calibre, and anastomose more regularly. The spaces between the casts (e.g. arrows) are occupied by air capillaries. Figure 5 (×11500) is from critical point dried tissue showing the air capillaries (*) and blood capillaries (arrows) containig erythrocytes (E). The air and blood capillaries interlace intimately in 3 dimensions. The calibre of the blood capillaries is relatively small and uniform. Their general form, as seen in this type of preparation, agrees closely with that shown by casts as figure 4.

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