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INFLUENCE OF EXCESS VITAMIN A ON THE SULPHATE METABOLISM OF CHICK ECTODERM GROWN IN VITRO

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[WITH SPECIAL PLATE]

In previous experiments (Fell and Mellanby, 1953) it was shown that when the presumptive epidermis from 6–7-day chick embryos is grown in medium containing excess vitamin A (+A medium) it differentiates, not into the squamous, keratinizing epithelium of the skin as in normal medium but into a mucus-secreting, often ciliated, membrane resembling that of the nose. Recently an attempt has been made to investigate some of the biochemical changes which accompany this metaplasia, by studying the uptake of radioactive sulphur given in the form of the sulphate ($Na_2{}^{35}SO_4$) in ectoderm grown in normal and in +A medium.

Singher and Marinelli (1945) found that when labelled sulphate was injected into rats it was incorporated into chondroitin sulphate and related compounds; its incorporation into amino-acids was negligible in animal though considerable in plant tissues (Howard and Pelc, 1951). Dziewiatkowski *et al.* (1949) confirmed these findings in cartilage. Boström *et al.* (1952) demonstrated the uptake of sulphate in cartilage and also in mucous epithelium by means of autoradiographs, though the resolution was not sufficient to allow detailed investigation of the distribution and movement of the

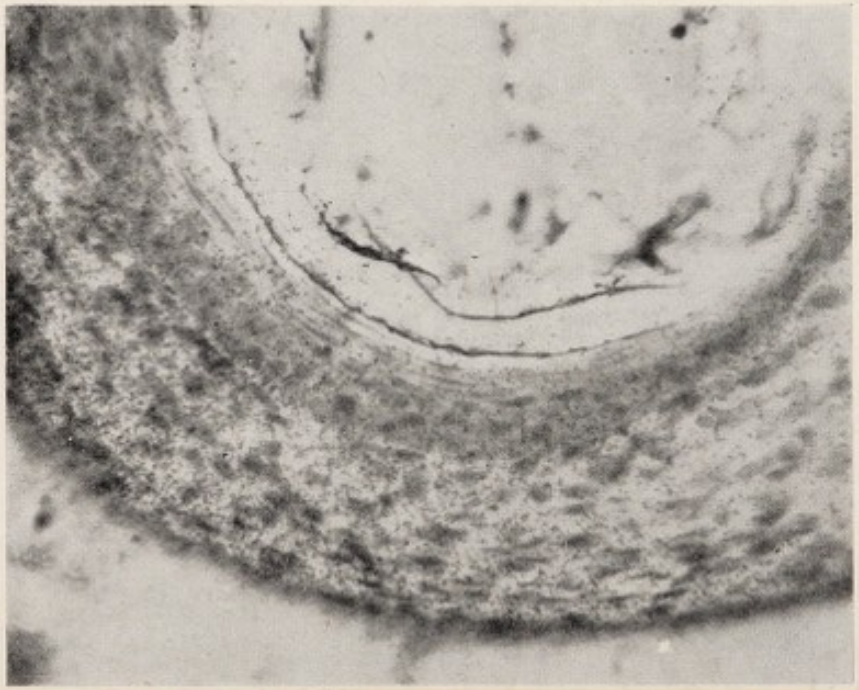

FIG. 1*a*.—Control culture; 11 days in normal medium. Stained section (Delafield's haematoxylin) with autoradiograph. Note cyst of squamous, keratinizing epithelium.

FIG. 1*b*.—Adjacent section (unstained) to Fig. 1*a* by ordinary lighting to show overlying autoradiograph. The blackening of the autoradiograph, due to $^{35}SO_4$, is considerable over the connective tissue but very scanty over the squamous epithelium.

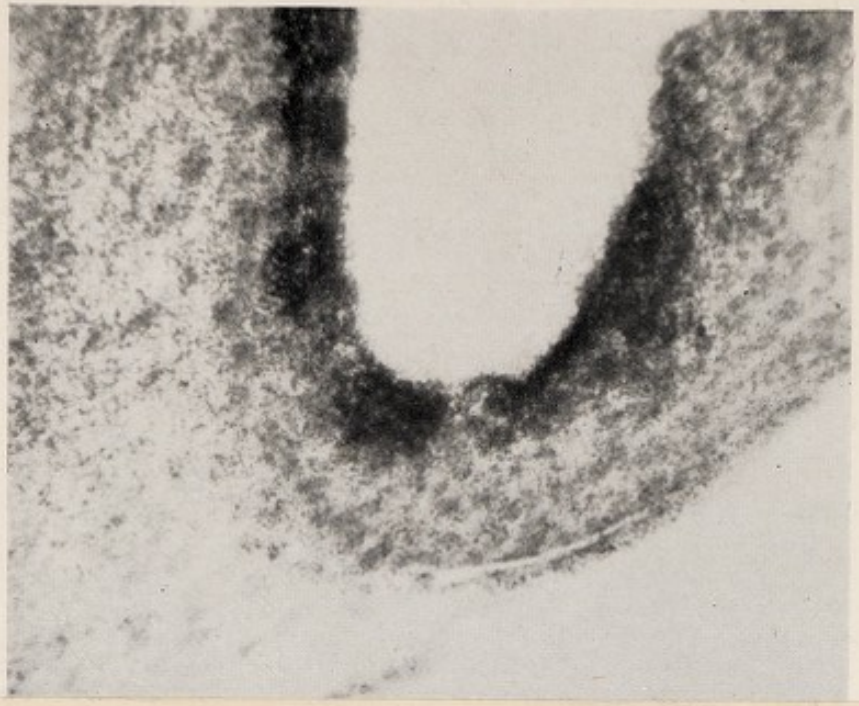

FIG. 2*a*.—+A culture; 11 days in medium containing 1,500 i.u. vitamin A per 100 ml. Stained section (Delafield's haematoxylin) with autoradiograph. Note cyst of mucous epithelium.

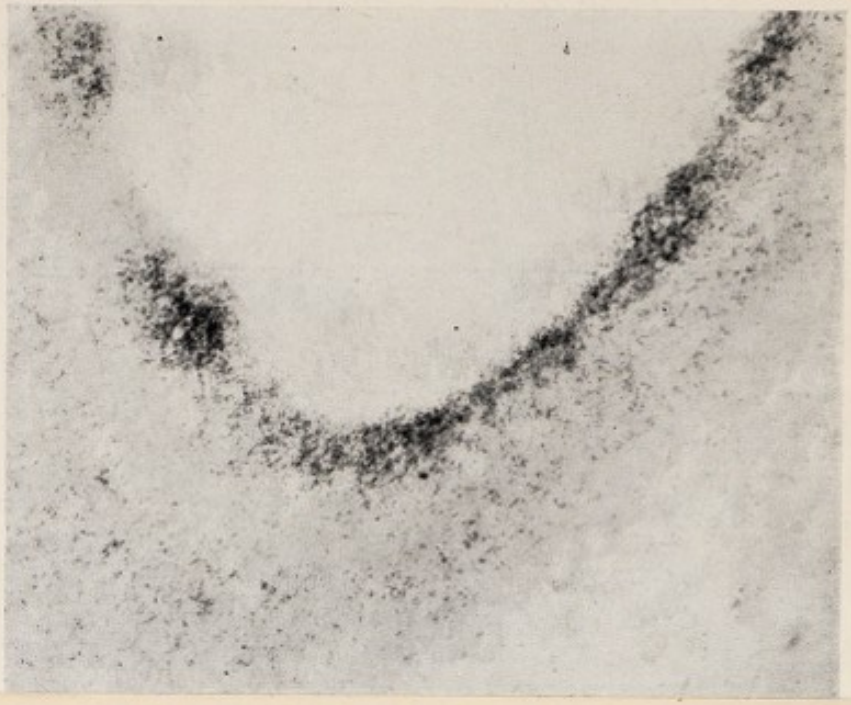

FIG. 2*b*.—Adjacent section (unstained) to Fig. 2*a* by ordinary lighting to show overlying autoradiograph. Note intense blackening over mucus-secreting epithelium due to $^{35}SO_4$.

sulphate within the tissues. Using a different autoradiographic technique, Glücksmann and Pelc (1954) showed that in the tracheal cartilage sulphate is taken up by the cells and then diffuses into the matrix.

In unpublished experiments, Glücksmann *et al.* (1954) have found that sulphate is taken up readily by mucus-secreting cells, but only to a small extent by squamous, keratinizing epithelium. The object of the present investigation was to see whether cultures of ectoderm in normal and in +A medium, respectively, would show a similar difference in the uptake of sulphate corresponding with their histological development in the two media.

Present Investigation

The method was as follows. Fragments of ectoderm and underlying connective tissue were removed from the trunk and limbs of 7-day chick embryos and cultivated by the hanging-drop method (see Fell and Mellanby, 1953). Explants taken from one side of each chick were grown in normal medium (3 parts plasma and 1 part embryo extract) and those from the opposite side in the same medium to which had been added 1,500 I.U. of vitamin A alcohol per 100 ml. The tissue was transplanted every 48 hours. The results confirmed those previously described. In both the control and the +A series the ectoderm usually formed a closed cyst surrounded by connective tissue, but after seven to nine days' cultivation the epithelium in normal medium had assumed a squamous, keratinizing structure, while that in +A medium had formed a mucus-secreting membrane which sometimes was ciliated.

After seven days (two pairs of explants) or ten days (12 pairs) the coverslips bearing the cultures were detached from the hollow ground slides, and a drop of Tyrode's solution containing 200 μC. per ml. of $^{35}SO_4$ was deposited on each explant with a fine pipette. The drop was allowed to stand for about 30 seconds, after which it was sucked off, leaving only a thin layer of fluid over the tissue; the coverslip was replaced and re-sealed, and the preparation was returned to the incubator for about twenty-four hours. The cultures were then fixed for fifteen minutes in acetic alcohol, followed by thirty minutes in formol-saline; they were transferred from the fixative to 70% alcohol and the tissue was removed from the coverslip with a Bard–Parker knife. After being embedded in paraffin wax the cultures were serially sectioned. Autoradiographs were made by the stripping film technique (Doniach and Pelc, 1950).

There was a striking difference between the autoradiographs of the controls and those of the +A cultures. In the former (Plate, Figs. 1*a* and 1*b*), although the connective tissue and basement membrane were labelled, there was very

little deposit over the epithelium and none over the keratin. On the other hand, in the +A explants (Figs. 2*a* and 2*b*) the connective tissue was labelled as before, but the basal cells of the epithelium had a higher content of sulphate than in the controls, and there was a dense autoradiograph over the inner secretory cells.

Summary

These results show that the sulphur metabolism of the presumptive epidermis of the chick converted into mucus-secreting epithelium *in vitro* by the presence of excess vitamin A in the culture medium is profoundly modified. In the controls, little sulphate is taken up by the squamous epithelium, which thus behaves like normal epidermis *in vivo*, whereas in the +A explants the utilization of sulphate is comparable to that of a normal mucous membrane in the body.

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