THE EFFECT OF EXCESS VITAMIN A ON THE EMBRYONIC RAT OESOPHAGUS IN CULTURE

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PLATES 1 AND 2

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It has been shown that vitamin A profoundly modifies the differentiation of many squamous keratinising epithelia. Excess vitamin A suppresses keratinisation and provokes a mucous transformation of chick embryonic epidermis (Fell, 1957; Fell and Mellanby, 1953; Weiss and James, 1955) and inhibits keratinisation in foetal human skin *in vitro* (Lasnitzki, unpublished observations). It also delays or inhibits the oestrogen-induced keratinisation in cultures of rat and mouse vagina (Kahn, 1954; Lasnitzki, 1961). The effect on skin seems to be species-dependent; thus topical application of the vitamin induced a hypertrophy of mouse and rat epidermis but failed to influence keratinisation (Bern *et al.*, 1955; Lawrence and Bern, 1958).

Recently, Lawrence and Bern (1960) and Lawrence *et al.* (1960) obtained a mucous metaplasia of hamster cheek pouch epithelium exposed to high doses of the vitamin *in vivo*. It is not known whether this response is characteristic of the hamster only or whether epithelia of the digestive tract of other rodents have retained the capacity to modulate from a squamous to a mucous epithelium.

The present study is an attempt to decide this question and for this purpose the effect of excess vitamin A on organ cultures of rat oesophageal epithelium has been examined.

Material and Methods

The organs, derived from 20-day rat embryos, were divided into two parts, one to serve as a control, the other as an experimental culture. They were explanted as tubes by a modification of the watch glass technique of Fell and Robison (1929) on strips of rayon acetate fabric (Shaffer, 1956) which were placed on clots consisting of cock plasma, horse serum, and chick embryo extract. During incubation they were kept in a chamber which was perfused daily for 30 minutes with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide at a flow rate of 100 ml/minute.

Prior to cultivation the experimental cultures were incubated with 150 I. U./ml of crystalline vitamin A alcohol (Roche Products Ltd., London) for 3 hours; the controls were treated with the solvent for the same time. This method was preferred to that of adding smaller

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quantities of the vitamin to the medium at the start of the experiment since in earlier work it had proved more effective (Lasnitzki, 1961). However, although sufficient amounts of the vitamin are taken up by the tissue during incubation with high concentrations of the vitamin it was found that the level of vitamin A in the tissue diminishes slowly during subsequent incubation in a medium without the vitamin and reaches almost zero on the 3rd day; it was therefore, necessary to add a booster dose of 20 I.U. of vitamin A per ml medium at each transfer to fresh medium. One series of cultures was fixed after 7, 10, and 14 days' growth respectively in the presence of the vitamin; a fourth group was treated for 10 days and maintained without the vitamin for a further 4 days. In all, 62 explants were studied.

RESULTS

The oesophagus of a 20 day rat embryo is a folded tube of epithelium embedded in cellular connective tissue and surrounded by concentric bundles of connective tissue and muscle fibres (Fig. 1). The epithelium is thin and consists of a layer of basal cells, a row of intermediate cells, and a few precornified elements at the lumen (Fig. 7). In the 13 day rat (Fig. 2) the epithelium has thickened and formed keratin; there are two to three rows of intermediate cells beneath the basal layer and a thin stratum granulosum is covered by layers of keratin at the lumen (Fig. 8).

Control explants grown for 7 days also show cornification but this process has been enhanced *in vitro* (Fig. 3). The epithelium is considerably higher than that of the organ *in vivo* and is composed of a layer of cuboidal basal cells followed by two or four rows of intermediate and the same number of precornified elements. This hypertrophy is more marked on the upper side of the explant. The lumen is filled with fibres of keratin (Fig. 9) but a stratum granulosum is missing. The acceleration of the squamous changes relative to cell replacement, leads to a thinning of the epithelium at the later stages of growth and after 2 weeks it is reduced to one or two rows of cells and the lumen is filled with concentric sheets of keratin in which pycnotic nuclei are still recognizable (Figs. 5 and 13).

In cultures exposed to vitamin A the proliferation of epithelial cells is increased to an even greater extent than in the controls but there is no cornification. The cells remain undifferentiated and neither become stratified nor form keratin. Moreover, in two-thirds of the cultures grown for 7 days many superficial cells lining the lumen contain secretory matter which stains strongly with PAS after diastase digestion and with mucicarmine (Figs. 4 and 11). Usually the epithelium is higher and the mucous changes are more pronounced at the side of the explants that is furthest from the clot region; this difference in degree of growth and differentiation in both control and experimental cultures may be attributed to a better supply of oxygen to the free surface of the explant as compared with that resting on the clot.

At 10 days' and 2 weeks' growth all vitamin A–treated cultures show mucin-

filled goblet cells along the whole length of their lumen (Figs. 6 and 10). In explants exposed to the vitamin for 10 days and then maintained for 4 days in normal medium, the squamous epithelium is not restored and the mucous transformation is identical with that observed in cultures treated for 10 or 14 days (Fig. 12). In contrast to the thinning of the epithelium in the control cultures during the later stages of growth, the epithelium in the vitamin A–treated explants remains high and viable.

DISCUSSION

Cultivation of rat oesophageal epithelium leads to an increase in the thickness of the epithelium and in the amount of keratin formed as compared with the organ *in vivo*. The enhancement of keratinisation *in vitro* is not confined to the rat oesophagus; it resembles the precocious keratinisation of chick ectoderm observed by Fell and Mellanby (1953) and also the spontaneous cornification of rat and mouse vaginal epithelium in culture (Kahn, 1954; Lasnitzki, 1961). In the rat oesophagus the acceleration of cornification is progressive and leads to an extreme thinning of the epithelium at the later stages of growth.

Exposure to excess vitamin A drastically modifies the differentiation of the oesophageal epithelium. Keratinisation is inhibited; instead the superficial cells become transformed into mucin-secreting elements. After 10 days' growth all treated culture show this mucous change. The metaplasia involves the whole length of the lining epithelium which is seen to consist of a continuous row of goblet cells. It is interesting to note that the degree of cell proliferation, the size of the goblet cells, and the amount of secretion are greater at the free surface of the explant as compared with the regions resting on the clot. This may be due to the greater ease of diffusion and with it a better supply of oxygen and carbon dioxide to the cells at the free surface and suggests that adequate concentrations of the two gases are necessary for cell proliferation as well as for the metaplastic action of the vitamin.

The mucous metaplasia resembles that induced by the vitamin in chick ectoderm *in vitro* (Fell and Mellanby, 1953) and in the hamster cheek pouch *in vivo* (Lawrence and Bern, 1960). In these tissues, however, the metaplastic epithelium was, usually, reduced to two layers, a row of basal cells covered by one of goblet cells, while in the rat oesophagus the epithelium beneath the goblet cells remained relatively high during the whole period of cultivation. It is not clear whether this effect is secondary to the inhibition of keratinisation or is due to a growth-promoting action of the vitamin on the epithelial cells. Whatever the mechanism, the response of the oesophagus to the vitamin combines the features seen in the hamster cheek pouch with those described for mouse and rat skin after topical application of the vitamin. Thus the rat oesophagus can be added as yet another example of a squamous keratinising

tissue which has retained the capacity to modulate to a mucous type of epithelium under the influence of vitamin A.

SUMMARY

The effect of excess vitamin A on the oesophageal epithelium of late foetal rats has been studied in organ culture.

In explants kept in normal medium the epithelium is, at first, higher and the keratinisation increased as compared with the development of the organ *in vivo*. At the later stages of growth, the acceleration of keratinisation leads to an extreme thinning of the epithelium.

Excess vitamin A completely inhibits keratinisation and induces a transformation of the cells lining the oesophageal lumen into mucin-secreting elements. In vitamin A–treated cultures the epithelium remains high throughout the whole period of cultivation.

The amount of secretory matter and the height of the epithelium seem to depend on an adequate supply of oxygen and carbon dioxide to the cells.

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EXPLANATION OF PLATES

PLATE 1

FIG. 1. Oesophagus of a 20 day rat embryo before explantation consisting of a folded epithelial tube embedded in cellular connective tissue. PAS, after diastase digestion. × 82.

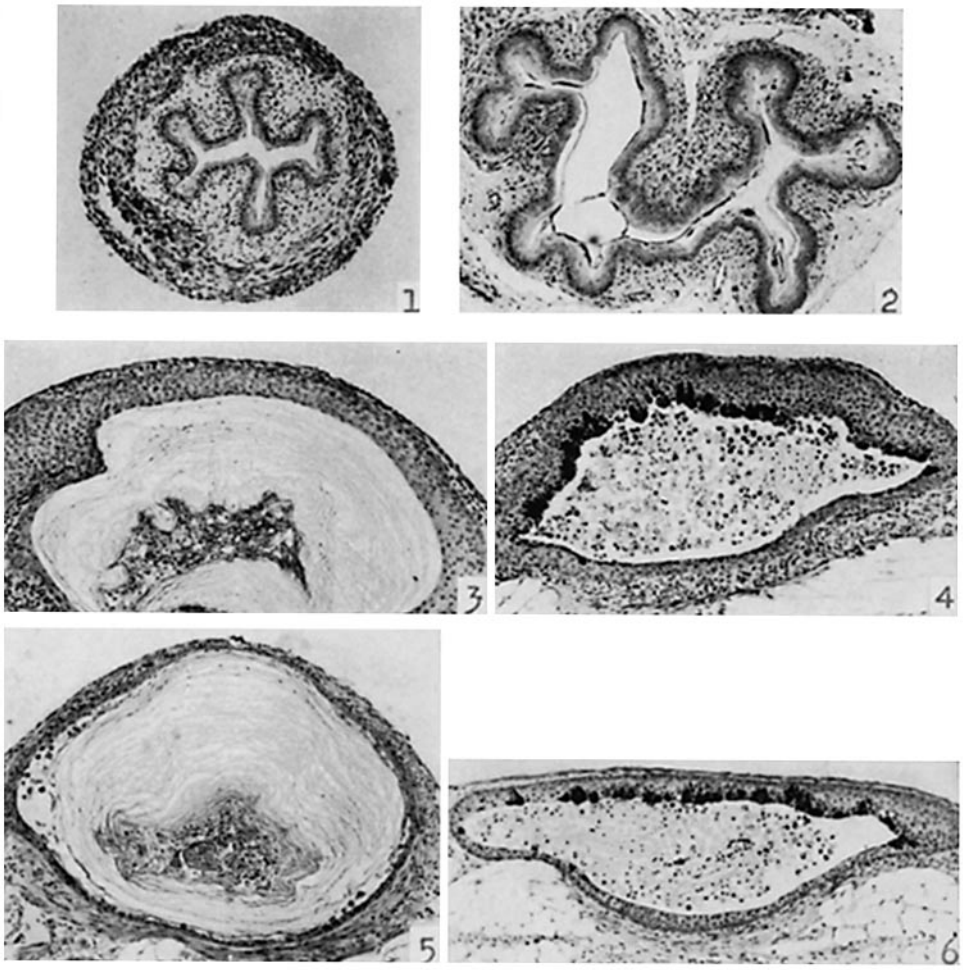
FIG. 2. Normal oesophagus of a 13 day (postembryonic) rat. Note increased thickness of epithelium and stratum corneum. PAS, after diastase digestion. × 82.

FIG. 3. Explant of embryonic rat oesophagus grown for 7 days in control medium, showing a high epithelium and layers of keratin in the lumen. The height of the epithelium and the amount of keratin is greater than in Fig. 2. × 82.

FIG. 4. Explant of rat oesophagus incubated with vitamin A prior to explantation and grown in medium with added vitamin A for 7 days. Note the actively secreting superficial cells. PAS, after diastase digestion. × 82.

FIG. 5. Explant of rat oesophagus grown for 2 weeks in control medium. Note low epithelium and layers of keratin filling the lumen. PAS, after diastase digestion. × 82.

FIG. 6. Explant of rat oesophagus incubated with vitamin A prior to explantation and grown in medium with added vitamin A for 2 weeks. The epithelium is much higher than in the control and the superficial cells on the upper side of the explant are filled with secretion. Mucicarmine. × 82.



(Lasnitzki: Effect of excess vitamin A)

PLATE 2

FIG. 7. Oesophagus of a 20 day rat embryo before explantation, showing one row of intermediate cells and a thin stratum corneum. PAS after diastase digestion, × 370.

FIG. 8. Oesophagus of a 13 day rat *in vivo*. Note the thicker stratum corneum as compared with Fig. 7. PAS, after diastase digestion. × 370.

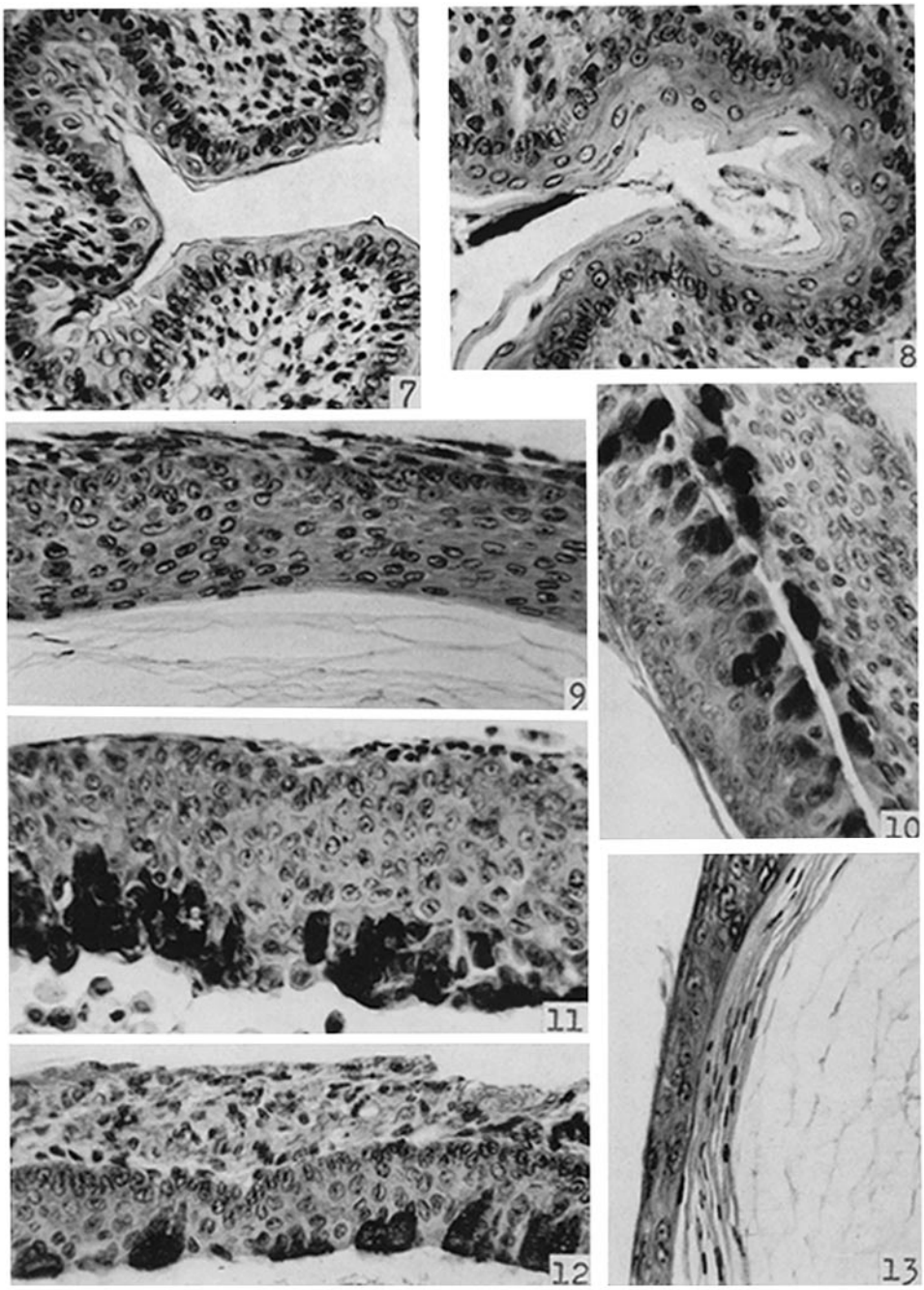
FIG. 9. Explant of rat oesophagus grown for 1 week in control medium. Note thickness of epithelium and layers of keratin. PAS, after diastase digestion. × 370.

FIG. 10. Explant of rat oesophagus incubated with vitamin A prior to explantation and grown in medium with added vitamin A for 1 week. Note increased thickness of epithelium, absence of keratin, and secretory matter in superficial cells. PAS, after diastase digestion. × 370.

FIG. 11. Explant of rat oesophagus incubated with vitamin A and grown in medium with added vitamin A for 10 days. Note absence of keratin and secretory cells at lumen. Mucicarmine. × 370.

FIG. 12. Explant of rat oesophagus incubated with vitamin A and grown for 10 days in medium with added vitamin A, and for 4 days in control medium. Note absence of keratin, and actively secreting cells at lumen. Mucicarmine. × 370.

FIG. 13. Explant of rat oesophagus grown for 2 weeks in control medium. Note low epithelium and layers of keratin in which pycnotic nuclei are still recognisable. PAS, after diastase digestion. × 370.



(Lasnitzki: Effect of excess vitamin A)