

Appearance of Enzyme Activity During Metaplastic Changes of the Chick Embryo Chorioallantoic Membrane¹ (34831)

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(Introduced by B. R. Bhussry)

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When the chorioallantoic membrane (CAM) of the chick embryo is exposed to air, squamous metaplasia and keratinization ensue within a few days (1). Since we have found that chick embryonic epidermis normally develops strong esterase and acid phosphatase activities just prior to cornification (2), we decided to examine another keratinizing system. Normally, there is little histochemically demonstrable esterase or acid phosphatase activity in the developing CAM (3, 4). If the appearance of these enzymes is a requisite for keratinization, then we would expect to find activity in chorionic epithelium about to undergo keratogenous metaplasia.

In addition, the appearance of esterase and acid phosphatase activities as well as keratinization in the epidermis of hypophysectomized chick embryos is markedly delayed (2, 5). We, therefore, examined keratinizing CAM in pituitaryless embryos for similar changes. If the process of keratinization is dependent upon normal pituitary function, then we would expect the appearance of enzyme activity associated with metaplastic changes in the CAM of hypophysectomized embryos to be delayed or absent.

Materials and Methods. Fertile White Leghorn eggs were used. The CAM was "dropped" and a window opened in the shell as described by Moscona (1). After the windows were sealed with cellophane tape and paraffin, the eggs were incubated until either 8, 12, or 13 days. The seals were then removed from the windows, and incubation of the fenestrated eggs was continued in an at-

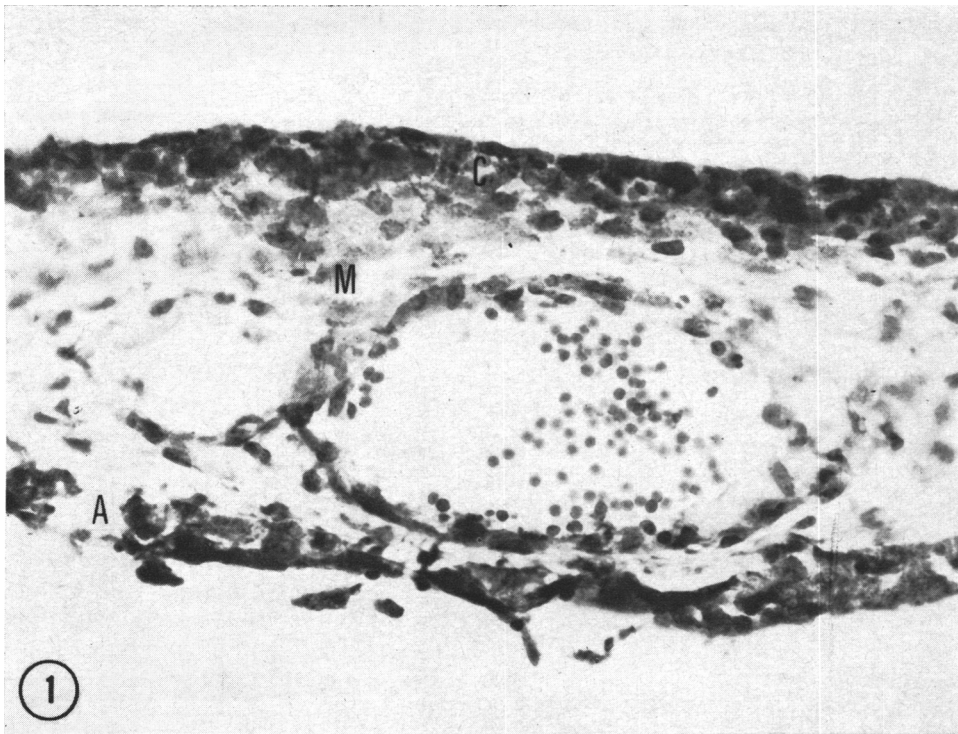
mosphere saturated with water vapor. Thirty-three normal embryos were so prepared.

Twelve embryos hypophysectomized by Fugo's (6) method of partial decapitation were similarly processed. Normal controls were prepared in the same manner except that the seals were left intact until harvesting. Membranes from normal experimental and control embryos were harvested at 1, 2, 3, 4, 6, and 7 days after fenestration. Those from pituitaryless embryos were taken at 3 and 4 days after exposure to air. Samples from each membrane were quick-frozen and sectioned at 12 μ on a cryostat. After air-drying, sections were fixed in cold, buffered (pH 7.2) formalin for 20 min. Esterase was demonstrated by using α -naphthyl acetate as substrate and fast blue B salt as indicator (7). Acid phosphatase activity was shown by using α -naphthyl phosphate as substrate and fast garnet GBC salt as indicator (7). Reference sections were stained routinely with hematoxylin and eosin.

Results and Discussion. Normally, the chorionic epithelium of the early CAM consists of a single layer of small cells. From about Day 13 the respiratory functions of this organ become more pronounced. The epithelium is progressively invaded by capillaries from the underlying mesoderm. Cells in adjacent areas of epithelium assume a cuboidal appearance and tend to form two layers. Neither esterase nor acid phosphatase activities have been demonstrated in appreciable amounts during the development of non-fenestrated eggs with either normal or hypophysectomized embryos (Fig. 1).

The histological and histochemical picture in the CAM of fenestrated eggs contrasts

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FIGS. 1 and 2 show $12\ \mu$ frozen sections of CAM from 11-day normal embryos. Both are stained to demonstrate α -naphthyl acetate esterase activity (represented only by intense black coloration). C = chorionic epithelium; M = mesoderm; A = allantoic endoderm. ($\times 300$).

FIG. 1. Chorioallantoic membrane of 11-day normal embryo. Note almost complete absence of α -naphthyl acetate esterase activity.

sharply to that seen in the normal condition. After only 1 day of exposure to air, hypertrophic and hyperplastic changes can be seen in the chorionic epithelium. These changes are restricted to small patches of epithelium scattered through the area of exposed membrane. After a second day of exposure, some keratin is evident in the form of macroscopically visible plaques. After successive days of exposure, the plaques are larger and thicker. In each instance, esterase (Fig. 2) and acid phosphatase are demonstrable in the epithelial cells beneath the layers of forming keratin.

At the plaque edges enzyme activity can be seen in the epithelium although the outermost layer of cells has not yet cornified. Where keratinization seems greatest, enzyme activity seems most intense.

We found no consistent differences in membranes exposed for the same period of time whether the eggs were fenestrated on

Day 8, 12, or 13. Hence, the epithelial cells are capable of responding to the stimulus of this type of environmental change at Day 8 just as readily as chorionic cells from older embryos.

Finally, prior hypophysectomy has no obvious effect on membrane metaplasia. That is, CAM from pituitaryless and normal embryos undergoes comparable metaplastic changes. It seems, then, that the response of embryonic epidermis to hormone deprivation (2, 5) must involve some process or aspect of differentiation that occurs prior to the appearance of esterase and acid phosphatase activities and keratinization.

In view of the facts that the CAM is morphologically uncomplicated, that it will also undergo keratogenous metaplasia *in vitro* (1), and that this process is apparently not affected by hormonal deprivation, we are dealing with a relatively simple system. We

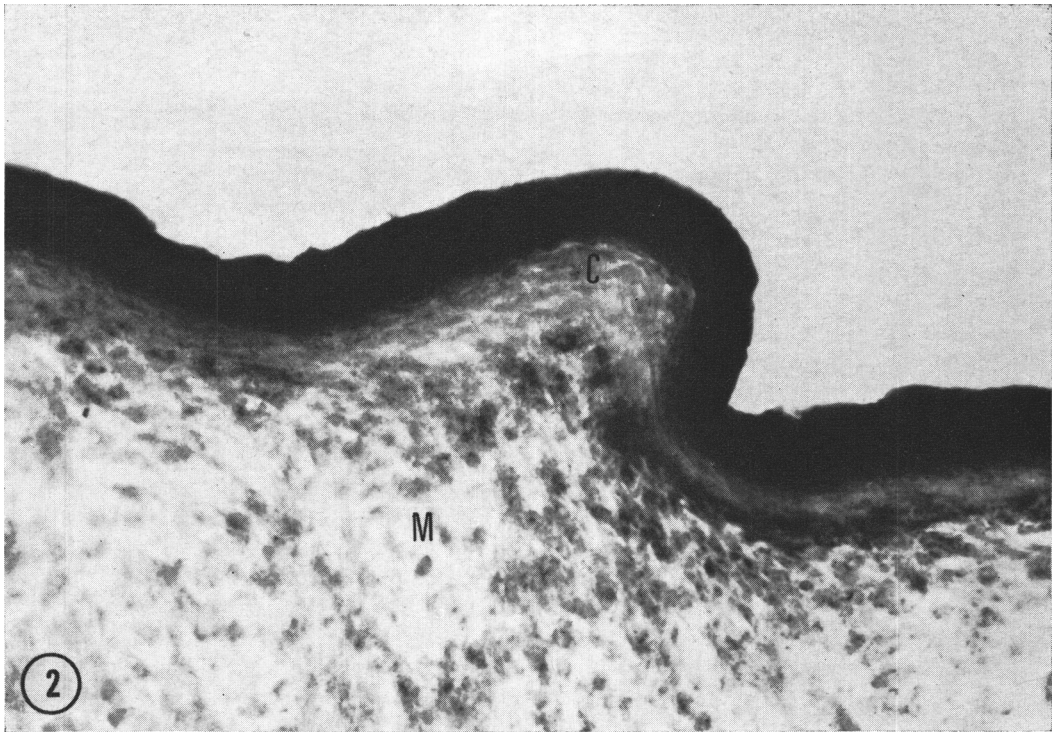


FIG. 2. CAM of 11-day embryo. Chorion was exposed to air for 3 days. The chorion has thickened considerably and is composed of stratified squamous epithelium. There is intense esterase activity in the flattened surface layer and considerable activity in some areas of deeper layers of the chorion.

hope that it will prove useful in the study of metaplastic changes at the cellular and molecular levels.

Summary. Chick embryo chorion undergoes a keratogenous metaplasia when the egg is fenestrated and the chorioallantois exposed to air. Normal chorion contains little histochemically demonstrable esterase or acid phosphatase. By contrast, the chorion of fenestrated eggs undergoes a squamous metaplasia and keratinization which is accompanied by a dramatic increase in activity of both enzymes. In this respect, the chorion is similar to epidermis in which esterase and acid

phosphatase also increase strikingly prior to keratinization. Unlike the epidermis, these chorionic enzymes are not affected by prior hypophysectomy.

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