until maximum firmness is developed, at which time the lines become parallel. If lysis ensues, the lines begin to converge and merge into a single line when lysis is essentially complete. The pattern of lysis following nicotinic acid in this patient resembles that seen with lysis induced by material extracted from swine plasma(5) in contrast to the more gradual lysis seen with streptokinase activation. Specimen (L), which represents control plasma to which 50 mg/L of nicotinic acid had been added *in vitro,* shows no evidence of lysis. In other experiments, *in vitro* addition of nicotinic acid in concentrations ranging from 0.05 to 500 mg/liter plasma failed to influence fibrinolytic activity or clot firmness. In contrast, intravenous administration of nicotinic acid to 10 human subjects in doses of 1 to 3 mg/K induced fibrinolytic activity in all 10 instances. In this respect, the development of fibrinolytic activity by nicotinic acid *in vivo,* but not *in vitro,* resembles the activation of clearing factor by heparin.

Conclusion. It is concluded that a relatively simple non-enzymatic substance, nicotinic acid, can induce fibrinolytic activity *in vivo.*

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Keratinization *in vitro* of chorionic Epithelium of the Chick Embryo.* (24175)

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The chorionic epithelium (CE) of the chick embryo forms the outer surface of the chorio-allantoic membrane (CAM). It consists of a single layer of small, attenuated cells of ectodermal origin, distally apposed to the shell-membrane and closely associated with blood vessels in the underlying mesenchyme in a manner characteristic of a respiratory epithelium (Fig. 1). Its basic structure is essentially retained also throughout later phases of embryonic development (Fig. 2), when capillaries penetrate through the CE to form a dense network on its surface. This chorio-vascular respiratory system continues to evolve until time of hatching when this structure, together with the rest of the CAM, is discarded with the shell. In its normal course of development, the CE shows no overt indications of morphogenetic propensities other than those which it displays in connection with its role as a respiratory epithelium; the possibility that its cells might possess additional potentialities which they would display under appropriate conditions, has not been adequately explored. In connection with another study, an attempt was made to maintain CE in organ culture in its original attenuated form. It was found, however, that the explants rapidly underwent striking metaplastic changes, during which they acquired new structural and functional characteristics.

Material and methods. Small fragments of CAM were excised from 8-day embryos, rinsed thoroughly in Tyrode's solution, then stretched on small squares of rayon-acetate net(1), the CE facing up, and cultured by

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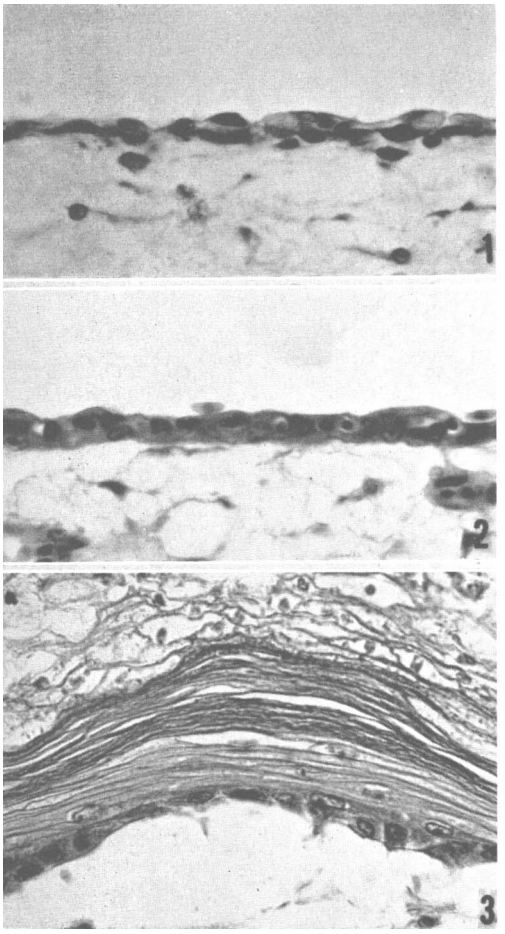

FIG. 1. Chorionic epithelium of 8-day chick embryo. Section through a control fragment of material at time of explantation, showing light staining elongated chorionic cells and dark, smaller endothelial and connective tissue cells. Ehrlich's hematoxylin and Biebrich's scarlet; × 400.

FIG. 2. Chorionic epithelium of an 18-day embryo. Chorionic cells and interspersed capillaries, endothelial and blood cells. E. h. and B. s. × 400.

FIG. 3. An 8-day culture of chorionic epithelium, showing keratogenic metaplasia as indicated by outward proliferation of cells, squamous changes and layers of keratin. E. h. and B. s. × 400.

Fell's watch-glass method(2). The clot was made up of 3 parts of fowl plasma and one part of chick embryo extract (11-day embryos). The culture dishes were kept in sealed containers, under conditions of saturation humidity, with ample supply of air. The explants were transferred to a fresh medium every 2 days and maintained in culture for 8 days. They were then fixed in Zenker's fixative, processed according to Shaffer's procedure(1), sectioned and, either stained with hematoxylin and Biebrich's scarlet, or examined in polarized light for birefringence.

Results. During the first 4 days of cultivation the explants, examined in the living state, showed few noticeable changes. From the fifth day on they became progressively more opaque, due to appearance on their surface of delicate sheets of light-reflecting material, wrinkled and parchment-like in texture. Scraped off fragments of this material were examined in polarized light and found intensely birefringent. In histological sections the CE changed from a membrane one-cell in thickness into a multilayered epithelial structure in an advanced stage of keratinization (Fig. 3). The *basal layer* of this epithelium corresponded in position to that of the original CE and consisted of one row of cuboidal or somewhat elongated cells. The *intermediate layer* comprised several rows of flattened cells, showing distally progressive cytoplasmic and nuclear changes characteristic of squamous keratinizing epithelia; accordingly, there was also an outwardly increasing affinity for Biebrich's scarlet. In polarized light, the upper strata of this layer showed scattered birefringence. The *outer layer* consisted of rows of keratin which stained intensely with Biebrich's scarlet and were strongly birefringent. This keratinized material was evidently largely responsible for the characteristic sheen and parchment-like appearance of the surface of living cultures. On top of the keratin there was usually a mass of deformed cellular fragments and hydrated or lysed cells or fibers.

In addition to the outward proliferation and keratinization of the CE cells, there was also an inward directed formative activity. Keratinized cysts, vesicles and epithelial pearls were occasionally present beneath the basal layer, within the underlying mesenchyme. The frequency of appearance of these formations could be markedly increased by lightly injuring the freshly explanted CE with a fine needle and pushing the dislodged cells into the mesenchyme.

Discussion. The recorded observations have shown that the CE of the chick em-

bryo, when grown *in vitro* for several days, became altered from a simple respiratory membrane into a multilayered keratogenic tissue. The precise identity of the stimuli directly involved in causing this metaplasia remains to be examined. One possible assumption is presence in the culture environment of metabolic requirements, not available to the CE *in ovo* but essential for manifestation of the keratogenic properties of the CE cells. Alternatively, the existence *in ovo* of inhibitory conditions, affecting the CE so as to prevent it from expressing its keratogenic potentialities, should be considered.

Clarification of these and other possibilities, might also relate to the general problem of keratinization. The CE, in being a normally non-keratogenic tissue, appears to be a particularly suitable material for studying processes and events by which cells may acquire capacities for rapid and voluminous production of keratin; it thus provides a useful source of comparison with the properties of skin. In view of the mucogenic metaplasia of embryonic skin demonstrated by Fell and Mellanby(2) and the role of Vit. A in this process(3), the reaction of the CE to this vitamin might be of interest. In this connection, one should also like to know if conditions might be found which would cause the CE to undergo *in ovo* keratogenic changes similar to those displayed in culture. These questions are being presently investigated.

Summary. A keratogenic metaplasia *in vitro* of chorionic epithelium of the chick embryo is described. Explants of chorionic epithelium from 8-day embryos, cultured for 8 days on a plasma clot, became altered from an attenuated, one-cell-thick respiratory membrane into a multilayered, squamous epithelium in advanced phases of keratinization.

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Osteolathyrogenic Action of Mercaptoethylamine and of Cystamine.* (24176)

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Beta-mercaptoethylamine, when fed to weanling rats, was found to produce gross osteolathyric lesions similar to those produced by diets containing sweet peas or β-aminopropionitrile (BAPN)(1). Ponseti *et al.*(2) were unable to confirm this finding. Results of studies on wound healing(3) and of studies using pregnant rats(4) appear to be in agreement with the negative report of Ponseti *et al.* We have now repeatedly produced osteolathyric-like lesions not only with different lots of mercaptoethylamine but with closely-related cystamine as well.

Methods. Beta-mercaptoethylamine was fed as the hydrochloride; cystamine as the dihydrochloride. Three different lots of β-mercaptoethylamine · HCl were purchased from California Fn. for Biochemical Research over a period of 18 months and were stored in vacuum desiccator. Iodometric titrations for –SH as well as chloride determinations indicated a purity of 96% or more for each of the lots. Cystamine 2HCl was purchased from Nutritional Biochemicals Corp. Iodometric determination of –S–S– following reduction(5) and chloride determination indicated a purity of 99%. These supplements were incorporated into finely milled Rockland Rat Stock

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