cases this absorption maximum appears in the Pincus colour of the total ketonic fraction. This maximum has also been described previously by Meschaks and by Mixner and Saunders.

Among these ionone derivatives, which form by far the greater part of the compounds determined by the usual ketosteroid reactions, a few 17-keto-steroids occurred in minute quantities. Three of these could be identified with more or less certainty as dehydro-*epi*-androsterone, etiocholanolone and 11-hydroxyandrosterone. In any event, the large amounts of the testosterone metabolites andro-sterone and etiocholanolone, which are excreted in human urine, were shown to be absent in cattle urine. This is most peculiar, as testosterone is believed to occur in the cattle organism. It was isolated for the first time from bulls' testes by Laqueur in 1935. These findings, however, are in accordance with the very low content of androgens in bulls' urine.

As to the origin of these ionone compounds, it is very likely that they are derived from carotenoids (Prelog *et al.*). Bearing in mind the fact that the ionone derivative 5-hydroxy-*cis*-tetrahydro-ionol, which Prelog and his co-workers isolated from pregnant mares' urine (diol *A*), has also been found in bulls' and pregnant cows' urine by Broadbent, Brooks and Klyne[3], and that horses and cattle mainly feed on the same carotene-rich material, it is not surprising that the same carotenoid derivatives should occur in the urine of both kinds of animals. It seems very probable, therefore, that these compounds will occur also in the urine of other animals whose food is rich in carotenoids, and that they will interfere there with ketosteroid determinations. We found in a preliminary investigation that they are absent from dogs' urine.

Full details of this work, which is still in progress, will be published elsewhere.

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[1] Meschaks, P., *Skand. Vet. Tidskr.*, **38**, 278 (1948); Ciba Foundation Colloquia on Endocrinology, **3**, 61 (1950). Mixner, J. P., *J. Dairy Sci.*, **34**, 495 (1951). Mixner, J. P., and Saunders, H. L., *J. Dairy Sci.*, **35**, 500 (1952).

[2] Prelog, V., *et al.*, *Helv. Chim. Acta*, **31**, 1799 (1948); **32**, 1632 (1949); **32**, 2082 (1949); **33**, 1725 (1950); **35**, 981 (1952).

[3] Broadbent, I. E., Brooks, R. V., and Klyne, W. *Biochem. J.*, **55**, vi (1953).

Effect of Œstrogen and of Vitamin A on Vaginal Cornification in Tissue Culture

Recent interest in the rodent vaginal epithelium as a target organ for studying the interaction of dietary and endocrine factors[1] has led to an investigation of the post-fœtal vaginal epithelium of the rat in tissue culture. Whereas Emmens and Ludford[2] were unsuccessful in a similar attempt, quite recently Hardy, Biggers and Claringbold[3] were able to culture the mouse vaginal epithelium by the hanging-drop method and demonstrated the sensitivity of this tissue to œstrogenic substances.

In the present investigation, 99 explants of the vaginal epithelium of eleven prepuberal rats (3–4 weeks old) have been successfully grown for one to nine days in watch glass cultures[4,5], and keratiniza-tion has been produced precociously by means of œstrone (0·13 mgm./100 ml. media) added to the nutritive medium. It was found, however, that the control vaginal epithelium cultivated in a standard medium also underwent keratinization, although not as rapidly as that treated with the folliculoid.

Before cultivation the vaginal epithelium is in the typical prepuberal state. After only one or two days on the œstrogenized culture medium, the epithelium becomes stratified, squamous and highly keratinized, although not hyperplastic. Up to the second day, the control groups appear much like the tissue before cultivation; but during the third or fourth day the cells beneath the original cuboidal epithelium become squamous, and by the fourth or fifth day have formed a typical stratified squamous epithelium and keratin. On continued cultivation, the epithelium becomes highly cornified and is histologically indistinguishable from that treated with œstrone. Careful examination of the control photograph presented by Hardy *et al.*[3] shows the beginning of a stratified squamous epi-thelium beneath the superficial cuboidal cells in their 96-hr. culture of mouse vagina; in our experiments with rat tissue the cuboidal cells are afterwards sloughed.

These results confirm the observations of Hardy *et al.*[3] that œstrogens have a direct action on the rodent vaginal epithelium *in vitro*, but the local response to a steroid under the simplified conditions of organ culture may not be evoked in the same way as a systemically distributed steroid *in vivo*. These *in vitro* results, therefore, do not exclude the possi-bility of metabolic 'activation' as suggested by Szego and Roberts[6].

The tendency for the control groups to undergo keratinization may be due to a lack of available vitamin A. Recent experiments have shown that synthetic vitamin A added to the control culture medium not only suppresses keratinization but also induces the production of a mucus-secreting epi-thelium (cf. Fell and Mellanby's observations[5] on chick ectoderm *in vitro*). On the other hand, a con-centration of vitamin A which inhibits keratinization in normal medium does not prevent but only delays cornification in the presence of œstrogen. A detailed report of this work will be published in the near future.

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[1] Kahn, R. H., and Bern, H. A., *Science*, **111**, 516 (1950); *Amer. J. Anat.* (in the press).

[2] Emmens, C. W., and Ludford, R. J., *Nature*, **145**, 746 (1940).

[3] Hardy, M. H., Biggers, J. D., and Claringbold, P. J., *Nature*, **172**, 1196 (1953).

[4] Fell, H. B., and Robison, R., *Biochem. J.*, **23**, 767 (1929).

[5] Fell, H. B., and Mellanby, E., *J. Physiol.*, **119**, 470 (1953).

[6] Szego, C. M., and Roberts, S., "Recent Progress in Hormone Research", **8**, 419 (1953).