possible keratinization were observed in the fourth. The fifth was unhealthy and showed no sign of stimulation other than the prominent layer of superficial cells. Four of the cultures which received the high dose of oestradiol-3:17 β were also examined. All were healthy; two were keratinized, one became stratified and possibly keratinized, and one failed to respond. The histological appearance of the epithelium in control and in treated explants, respectively, is shown in Figs. 1 and 2.

In more recent work, the above experiments have been repeated with some modifications of technique, with oestrone, oestriol, oestriol glucuronide, oestradiol-3:17 β , equilin and diethylstilboestrol. Similar results have been obtained; a full description will be given elsewhere.

The results reported above indicate that oestrogens are capable of producing vaginal cornification *in vitro*. This finding is important in discussions of the mode of action of these hormones. By means of the double-vaginal pouch technique, Robson and Adler⁴ and Emmens⁵ showed that the intravaginal action of true oestrogens is purely local. Our results directly corroborate these earlier findings and render untenable the recent thesis of Szego and Roberts⁶ that oestrogens have to be 'activated' by the liver before they are capable of exerting their action.

Hechter, Lev and Soskin⁷ believe that the development of hyperaemia is essential for cornification of the vagina. Our results do not support this view, but suggest that the oestrogens act primarily on the responding cells.

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Acetocarmine as a Nuclear Stain in Rhodophyceae

IN recent years, the earlier acetocarmine technique of Belling has been modified by Godward¹ and also Cave and Pocock² for use in staining the nuclei of various Conjugales and Volvocales respectively. So far as is known, the technique has not been applied previously to the Rhodophyceae, and at the suggestion of Dr. K. M. Drew, therefore, an attempt has been made to stain some members of this group by this method.

Of those available for use, the following have stained successfully: *Porphyridium cruentum* (Ag.) Naeg., *Porphyra umbilicalis* (L.) J. Ag., *P. umbilicalis*

var. *laciniata* (Lightf.) Ag., *P. linearis* Grev., *Batrachospermum fruticosum* Drew, *Antithamnion spirographidis* Schiffner, *Antithamnionella sarniensis* Lyle, and *Griffithsia setacea* C. Ag. (= *G. flocculosa* Batt.). However, *Polyides caprinus* (Gunn.) Papenf., *Furcellaria fastigiata* Lamour. and *Dumontia incrassata* Lamour. have not stained satisfactorily, and this may be due to the thick mucilaginous cell walls and large amount of floridean starch that surrounds the nucleus in some of the cells of these species.

The method which has been employed is a modification of those previously tried and is as follows: (1) Fix material in 3:1 absolute alcohol-acetic acid for ten minutes to a few hours. Best results are obtained when the material is stained soon after fixation. (2) Bring material down to water through 90, 70, 50 and 30 per cent alcohols. (3) Wash (two or three changes) with distilled water. (4) Mordant in freshly prepared 4 per cent iron-alum for 5-20 minutes. (5) Wash (two or three changes) with distilled water. (6) Place material on slide and add acetocarmine; cover, and heat to boil (repeat boiling twice or thrice). (7) If material requires spreading, squash with cloth-covered finger. (8) Add fresh acetocarmine to margin of cover-glass, heat again and keep slide warm for ten minutes on hot plate. (9) Make permanent according to (McClintock's) method recommended by Manton³. (Temporary preparations invariably look more elegant than permanent ones.)

As the method is a rapid one, it has been found very useful in determining times of nuclear divisions, for example, in tetrasporangia and germinating tetraspores. In illustration of the results, a photograph of the first mitotic division in a germinating tetraspore of *Antithamnion spirographidis* Schiffner is reproduced herewith.

Prophase of first mitotic division in germinating tetraspore of *Antithamnion spirographidis* Schiff. Permanent acetocarmine. ($\times 1,000$)

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