

not describe any trematode life-cycle in its entirety. According to Reinhard, the German parasitologist, Davis F. Weinland was the first person to suspect that the larval stages of the liver-fluke occur in *L. truncatula*. In 1875, he "told of finding cercaria-sacs" in the digestive gland of this snail, and he also noted that the cercaria tended to leave water, which prompted him to suggest that they encysted on herbage and were the young of *Fasciola hepatica*. Thomas was apparently unaware of Weinland's discovery and conjectures, and his well-deserved success was the result of a methodical search for possible snail hosts in localities where sheep were liable to contract liver-rot. The quest was probably simplified by the knowledge that only fishes and molluscs were to be regarded with suspicion because the only animals known to harbour larval trematodes, but success called for all the resources of a most resourceful zoologist.

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¹ Steenstrup, J. J. M., "On the Alternation of Generations; . . ." (The Ray Society, London, 1845) (first published 1842 in Copenhagen).

² Dawes, Ben, "The Trematoda" (Camb. Univ. Press, 1946, 1956).

³ Reinhard, E. G., *Exp. Parasitol.*, 8, 208 (1957).

Differentiation induced as Season Advances in the Embryo-Gametophyte Complex of *Pinus nigra* var. *austriaca*, using Indole Acetic Acid

MAINTENANCE of growth in very young excised gymnosperm embryos in culture medium has been attempted by one of us with limited success. Indefinite growth was never achieved, and at death, differentiation was lacking other than that suggesting initiation of symmetry or polarity.

The present work on *Pinus austriaca* arises out of attempts to find qualitative evidence of physiological relationship in the region of the parental-embryonic threshold. Indole acetic acid, because of its acceptance as a growth-controlling agent, was added to the culture medium in the hope that it would stimulate differentiation during post-zygotic *in vitro* life.

Seeds were gathered at intervals during the year. Their testas were removed under aseptic conditions, and the naked gametophytes were placed on a sterile culture medium buffered to pH 6.0, and containing a known concentration of indole acetic acid. Various concentrations of indole acetic acid were sampled in the course of the experiments.

The gametophytes were harvested, some after two, others after four weeks *in vitro*.

Examination followed sectioning and staining in borax carmine and fast green.

Comparison of several gametophytes grown on an indole acetic acid medium in July immediately following fertilization showed in each case the presence of a meristematic layer crossing the gametophytic tissue. Position of the meristem varied from one example to another. It consisted of contiguous thin-walled cells resembling the fusiform initials of a vascular cambium.

Cells between the periphery of the gametophyte and the meristematic layer had undergone enlargement which produced local swelling. These cells contained little cytoplasm and only a few granules. Cells on

the other side of the layer were much smaller, irregularly shaped, and contained a strikingly large number of granules which differed markedly in form from those normally present at corresponding positions in controls.

In many of these gametophytes, the parenchyma cells surrounding the archegonial jackets developed secondary thickenings on all their walls. Thus in these cells pits appeared on all the walls; they were large and circular.

Experiments performed in August, long after fertilization, showed neither the development of meristematic layers in gametophytic tissue nor the development of thickenings and pitting outside the archegonial jackets. Therefore, none of the phenomena arising in July examples occurred in the August ones.

In the older examples a different feature arose not seen in the July ones. At several scattered locations, cells one to three times the volume of neighbouring ones arose. These were thick-walled, lignified, pitted and often anisotropic in polarized light. Cytoplasm and nuclei were not visible in them.

Proembryo ontogeny for indole acetic acid *in vitro* cases differed from that for the controls in both the July and August examples. One cultured proembryo developed reticulate pitting in the walls of the suspensor cells. Others showed inhibited growth. Generally, on the basis that incidence of anomaly and differentiation was less frequent for embryo than for gametophytic tissue, the effect of the indole acetic acid *in vitro* conditions was judged to be less marked for the embryo system.

Experiments performed at the end of March with material collected at that time (nine months after fertilization) showed none of the previously described phenomena.

It may be concluded that: (1) In *Pinus austriaca*, as one example of an embryophyte, parental-embryonic environment shows a differential response to a factor stimulating differentiation. This differential suggests presence of a pattern of organization in which time and morphological events are significant and related. (2) In parental tissue, visual differentiation may be extensive; for example, meristem and massive activity near archegonia, or confined to single isolated cells, for example, lignified structures. In embryonic tissue the effect is always confined to single cells. (3) Indole acetic acid appears to be influential in initiating the differentiation and in effecting its course.

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Collection of Urine and Faeces from the Chicken

IN order to examine some factors which influence the intestinal absorption and urinary excretion of calcium and phosphorus in the chicken, a surgical method for the separation of urine and faeces has been developed which depends upon the successful 'exteriorization' of the distal end of the large intestine. This method, which has been employed principally in adult Rhode Island Red females, is equally suitable for adult capons and cockerels. Females operated on one year ago and fed practical