controls, these experiments provide further instances of the normal type of differentiation of ventral mesoderm-ectoderm *in vitro*.

Over 600 cultures were isolated in Niu-Twitty medium containing concentrations of $CaCl_2$ ranging from 0.006 to 0.06 *M* for $^1/_2$–2 day periods, and then cultured in Niu-Twitty solution alone. Concentrations of calcium chloride below 0.025 *M* were not effective in initiating new pathways of differentiation, and 0.06 *M* $CaCl_2$ usually led to cytolysis of the explants. At concentrations above 0.02 *M* $CaCl_2$, the curling movements of the surface coat were retarded somewhat. After exposure to calcium chloride levels of 0.025–0.05 *M* for periods of 12–24 h, followed by culture in Niu-Twitty solution alone for 6–11 days, approximately 15% of the explants of ventral mesoderm-ectoderm developed nervous tissue. Muscle frequently was present in such explants, but notochord was usually absent. The presence of nerve and muscle tissue was not only recognized histologically, but also by twitching movements of these explants. An example of the induction of a mass of nervous tissue by 0.05 *M* $CaCl_2$ is illustrated in Figure 1.

Guanidine HCl (0.01, 0.1, 0.5, 0.75%) was added to the normal saline medium in 300 cultures of ventral mesoderm-ectoderm. The two lower concentrations were without effect and 0.75% guanidine HCl caused dissociation of the cells after 4–6 h. Exposure of 100 fragments of ventral mesoderm-ectoderm to 0.5% guanidine HCl stimulated the differentiation of nerve and muscle in approximately 25% of the explants as ascertained by twitching movements or histological examination (Figure 2).

Regarding the mechanism by which $CaCl_2$ and guanidine HCl can elicit dorsal axial differentiation from ventral explants, it has been mentioned that 0.05 *M* $CaCl_2$ lyses isolated ovarian yolk platelets *in vitro*[6], but little is known about the effect of guanidine HCl. Accordingly a suspension of ovarian yolk platelets in distilled water was prepared by homogenization and washing by low speed centrifugation. This washed suspension was divided into three 5 ml aliquots and guanidine HCl was added to two of the aliquots to give final concentrations of 0.5 and 1.0%. After 5 h at room temperature the three suspensions were centrifuged to sediment the intact yolk platelets and trichloroacetic acid was added to each of the three centrifugal supernatants to provide a final concentration of 5% TCA. These samples were then centrifuged in 12 ml graduated centrifuged tubes and the volumes of the TCA precipitates were obtained in order to provide an indication of the amount of yolk protein that was solubilized from the platelets. There was no TCA precipitate from the water suspension of yolk platelets, 0.45 ml of TCA precipitate from the 0.5% guanidine HCl suspension and 0.97 ml from the 1.0% guanidine HCl suspension. This indicates that 0.5% guanidine HCl, which can promote dorsal axial differentiation in ventral explants, can solubilize protein from yolk platelets.

Résumé. La culture de fragments isolés de mésoderme et d'ectoderme ventraux des gastrula de grenouilles (*Rana pipiens*) en présence de $CaCl_2$ 0,025–0.05 *M* pendant un jour ou en présence de HCl guanidine 0,5% pendant un demi jour, et suivi d'une période de culture dans un salin physiologique induit une différentiation de tissus nerveux et musculaires dans 15 à 25% des cas. Des concentrations de $CaCl_2$ et de HCl guanidine pareilles à celle qui induit cette différentiation peuvent solubiliser des plaquettes vitellines isolés.

R. A. Flickinger

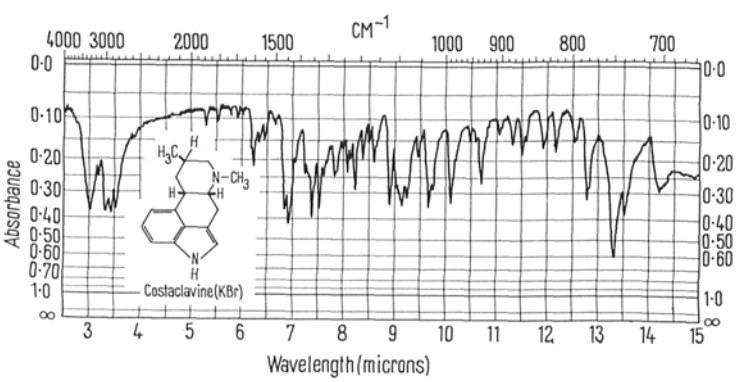
Department of Zoology, University of California, Davis (U.S.A.), August 15, 1963.

Costaclavine from *Penicillium chermesinum*

During a 'screening' of fungi for their ability to convert clavine type ergot alkaloids, a strain of *Penicillium chermesinum* Biourge was found to be capable of synthesizing on its own small amounts of a clavine alkaloid, behaving chromatographically as costaclavine. This latter alkaloid has previously been isolated by Abe[1] from saprophytic cultures of *Agropyrum* type ergot, together with the isomers festuclavine and pyroclavine.

P. chermesinum Biourge PC 106-I was grown in 500 ml Erlenmeyer flasks on 100 ml of a medium containing malt extract 'Difco' 10%, lactose 5% and asparagine 0.5% in distilled water. Addition of tryptophan had no positive effect on the alkaloid yield. The alkaloids (11 mg) were isolated from 60 culture flasks and separated as described earlier[2]. A number of pigments were removed by extraction with chloroform of the aqueous acidic alkaloidal extract.

The major alkaloid was recrystallized from ether-acetone, m.p. 182–184° (costaclavine[1] 182°). It gave the van Urk reaction and showed the ultraviolet absorption at 275, 283 and 293 mμ, typical[2] of the dihydro ergot alkaloids. The infrared spectrum of the isolated alkaloid was identical with that of authentic costaclavine. Further, the paper and thin layer chromatographic[2] behaviour of the isolated costaclavine was the same as that of authentic costaclavine.



The occurrence of ergot alkaloids in nature, previously restricted to *Claviceps* and *Aspergillus* among the fungi, therefore, also has been extended to the genus *Penicillium*. However, it should be noted that ergot alkaloids have been suspected earlier in *P. roqueforti*[3]. Quite interesting is the fact that

[1] M. Abe, S. Yamatodani, T. Yamono, and M. Kusumoto, Bull. Agr. Chem. Soc. (Japan) *20*, 59 (1956).

[2] S. Agurell and E. Ramstad, Lloydia *25*, 67 (1962).

the costaclavine produced here is the only ergot alkaloid which has been assigned[1,4] to have the hydrogens at C-5 and C-10 in *cis*-configuration.

Zusammenfassung. Es wurden in *Penicillium* Mutterkornalkaloide nachgewiesen, die bisher nur unter den Pilzen aus *Aspergillus* und *Claviceps* bekannt waren.

S. L. AGURELL[5]

Royal Pharmaceutical Institute, Stockholm (Sweden), September 23, 1963.

[3] W. A. TABER and L. C. VINING, Canad. J. Microbiol. *4*, 611 (1958)
[4] S. YAMATODANI and M. ABE, Bull. Agr. Chem. Soc. (Japan), *20*, 95 (1956).
[5] For the identification of the species used in this investigation, I am indebted to Dr. J. R. KINSLEY, Purdue University. The technical assistance of Miss S. Roos and the financial support from the Swedish Natural Science Research Council was appreciated. The sample of authentic costaclavine was generously provided by Dr. M. ABE.

Avian Oogenesis and Yolk Deposition

The yolk of the hen's egg is often given as an example of a huge cell containing a food supply for the developing chick embryo. Current views hold that oocytes grow by removing nutrients from the blood and the follicle cells which surround them. Thus yolk deposition is thought of as primarily an intracellular phenomenon[1]. We believe that this viewpoint is inadequate, and wish to propose the following alternative hypothesis.

After they have enclosed an oocyte, the follicle cells of the young chick's ovary begin to participate in the deposition of material into the follicle. The oocyte, in contrast, assumes a relatively passive role in yolk formation. Indeed, the cytoplasm of the oocyte is eventually lost, in a functional sense, in the large mass of accumulating yolk. The remnants of the cytoplasm form a network around the germinal vesicle. The follicle cells secrete materials into the follicular space during the slow period of yolk deposition; during the period of major yolk deposition the follicle cells permit blood plasma to flow between them towards the center of the follicle. At the same time, they transport sodium, water, and many solutes from the plasma back to the blood. Some major plasma proteins, potassium, lipids, and other substances are left behind in the enlarging follicle, and form the egg yolk.

The above proposal is based on the view that the major functional unit of yolk deposition is the ovarian follicle, not the oocyte. Certain facts favor consideration of such a proposal. The transport of intact blood proteins[2] and subcellular constituents[3] into the avian egg has been well documented. Although not yet observed in the avian ovary, it has recently been shown that in the moth, protein materials pass between and not through the follicle cells[4]. BELLAIRS et al.[5] have shown that the vitelline membrane of mature avian eggs is a non-cellular, open network of fibers which would offer little resistance to the flow of large molecules.

Current theories of yolk formation require that the germ cells actively transport large amounts of material across their cell membranes. Active transport processes demand readily available sources of energy. In the mature ovum the region of the egg closest to the follicle cell is seemingly devoid of mitochondria[6] but there are many of these particles in the follicle cells. Moreover, there is no definitive evidence to indicate that the living margin of avian oocytes is apposed to the follicle cells during later stages of oogenesis.

Our observations of living oocytes, conducted with phase-contrast microscopy, reveal little gross structure inside the developing follicle. However, what is seen differs from that found in fixed and stained sections, and indicates that a clear region, interpretable as a follicular cavity, is present in oocytes of 0.1 to 1.0 mm diameter. This cavity lies between the follicle cells and numerous highly refractile globules (the primordial yolk[7]) which surround the transparent, eccentrically located germinal vesicle.

SOTELO and PORTER[8] have reported that mammalian follicle cells are separated from the surface of the developing germ cells by the zona pellucida, a non-cellular, jelly-like region. They noted that long microvilli extended from the follicle cells through the zona pellucida to the egg cell surface and that short, slender projections extended from the egg cell into the same region. If the zona pellucida of mammalian follicles were much enlarged and yolk were deposited within it, the situation would be similar to that which we envisage for birds.

We propose first that follicle cells extract sodium, water and other substances from the plasma as a major part of their role in yolk formation. Second, we hold that bird yolk is primarily extracellular and that transport of yolk material by avian oocytes is not required for yolk formation.

Zusammenfassung. Es wird die Auffassung vertreten, dass bei Vögeln die grosse Masse des Eidotters extrazellulär ist. Dieser wird durch selektive Absorption von Blutplasma abgelagert, welches zwischen den Follikelzellen in die Follikelhöhlung eindringt. Wasser, Natrium and andere gelöste Stoffe werden wieder ins Blut abgegeben, und der kaliumreiche Eidotter bleibt zurück. Das Cytoplasma der Eizelle geht funktionell im Eidotter auf und spielt bei der Ablagerung des Dotters nur eine passive Rolle.

C. R. GRAU and B. W. WILSON

Department of Poultry Husbandry, University of California, Davis (U.S.A.), September 24, 1963.

[1] J. BRACHET, *The Biochemistry of Development* (Pergamon Press, New York 1960), p. 3.
[2] P. F. KNIGHT and A. M. SCHECHTMAN, J. exp. Zool. *127*, 271 (1954).
[3] O. A. SCHJEIDE, R. G. MCCANDLESS, and R. J. MUNN, Growth *27*, 111 (1963).
[4] W. H. TELFER, J. Biophys. Biochem. Cytol. *9*, 747 (1961).
[5] R. BELLAIRS, M. HARKNESS, and R. D. HARKNESS, J. Ultrastruct. Res. *8*, 339 (1963).
[6] R. BELLAIRS, J. Biophys. Biochem. Cytol. *11*, 207 (1961).
[7] V. D. MARZA, Quart. J. Microscop. Sci. *78*, 191 (1935).
[8] J. R. SOTELO and K. R. PORTER, J. Biophys. Biochem. Cytol. *5*, 327 (1959).