here were similar to those obtained by REVERBERI with the use of sodium azide[7,8] and of sodium malonate and selenite[9], and to those observed by DE VINCENTIIS with the use of partial anaerobiosis[10] and in the presence of CO in the dark[11]. According to REVERBERI, the cytochrome oxidase, which is segregated at an early stage in the posterior vegetative blastomeres, plays a part in the morphogenesis of muscle cells by supplying the energy required for the construction of the molecules that form the first substrate of the specific substance of the muscle cells, or for the linkage of these molecules into fibrous chains, or for the elongation of the entire cell.

CROWELL[12], using certain specific inhibitors of the –SH groups (mercapto-ethanol, thiomalic acid, phenyl-mercuric chloride) at various stages of development of the eggs and embryos of *Ciona intestinalis* L., was able to show recently that the larvae which developed had distorted tails, in which the muscle cells were only little differentiated and which in addition contained hypertrophic chordal cells, while the cerebral vesicle was much reduced in size and palps remained absent.

From these various findings it may be concluded that our results could be interpreted in two different ways: (1) on the one hand, the absence of $SO_4^{=}$ ions may act on the mitochondrial systems, the accumulation of which in the posterior vegetative blastomeres appears to be associated with differentiation of muscle cells. The positivity of the Nadi reaction does not in itself prove that the mitochondria function perfectly, as[13] the Nadi reagent is oxidized at the cytochromoxidase level, and yields no information concerning the potency of the succino-dehydrogenase and NADH-dehydrogenase systems. The function of these systems is known to depend on the presence of –SH groups. (2) Further, in view of the work done by IMMERS and RUNNSTRÖM[14], who found that absence of $SO_4^{=}$ ions causes a decrease of uptake of labelled uridine, thymidine and amino acids into the embryos of the sea-urchin, it is worth considering the hypothesis that in *Phallusia*, also, the effect observed may be due to a disturbance in synthesis of the specific proteins. Cytochemical and autoradiographic studies which are still in progress will probably throw further light on this problem[15].

Riassunto. Viene studiato lo sviluppo di *Phallusia mammillata* in acqua di mare priva di ioni $SO_4^{=}$. Le anomalie riscontrate a carico della larva riguardano principalmente il sistema muscolare della coda. Viene discusso il probabile ruolo esplicato dagli ioni $SO_4^{=}$ nel corso dello sviluppo.

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[7] G. REVERBERI, Pubbl. Staz. zool. Napoli *29*, 187 (1957).
[8] M. DE VINCENTIIS, Boll. Zool. *23*, 701 (1956).
[9] G. REVERBERI, Acta Embryol. Morph. exp. *1*, 12 (1957).
[10] M. DE VINCENTIIS, Experientia *12*, 381 (1956).
[11] M. DE VINCENTIIS, Ricerca scient. *26*, 3093 (1956).
[12] J. CROWELL, Acta Embryol. Morph. exper. *5*, 285 (1962).
[13] L. LISON, in *Histochemie et Cytochimie animales* (Gauthier-Villars, Paris 1960), p. 583.
[14] J. IMMERS and J. RUNNSTRÖM, J. Embryol. exp. Morph. *14*, 289 (1965).
[15] This investigation was supported by Consiglio Nazionale delle Ricerche of Italy.
[16] We are very much obliged to the Director, Dr. PETER DOHRN and to the staff of the Stazione for effective support and help in our work.

Biotin and Fatty Acid Biogenesis in *Aspergillus flavus*

Using avian liver preparations, it has been demonstrated that biotin has a role in the synthesis of fatty acid[1]. Biotin containing enzyme acetyl carboxylase catalyses the first step in the reaction; malonyl CoA, the product of this reaction, is the intermediate in the pathway. A great deal of data is available on the mode of biogenesis of fatty acid in animals and plants. In comparison, there is little information available on the mechanism of microbial fatty acid biogenesis. Recently, two reports[2,3] have been published which indicate that biotin deficiency results in the change in fatty acid composition of *Lactobacillus plantarum* and *E. coli*. WOODBINE et al.[4] reported high lipid production in *Aspergillus flavus*. In the present experiments we have used this mould to study the relationship between biotin and lipid synthesis.

Submerged cultures of *A. flavus* were grown at 28–30 °C on 50 ml medium pH 5.7 containing glucose 40 g/l, NH_4NO_3 1 g/l, $MgSO_4$, $7H_2O$ 0.3 g/l, and KH_2PO_4 0.3 g/l in 250 ml Erlenmeyer flasks. At the end of 6 days the mycelium was harvested and the lipid of the dry felt was extracted with ether and saponified by 1 *N* alcoholic KOH. After acidification the fatty acids were extracted by ether and were determined by weighing the residue after evaporation of ether. Biotin-deficient mat of *A. flavus* was obtained by cultivating the mould on the medium containing 5 U of avidin. Reactivation of biotin-deficient mould was carried out by the addition of 0.05 μM of biotin per flask.

The results of the experiments on fat production in normal and biotin-deficient cultures are recorded in the Table. Biotin deficiency has been found to result in greatly diminished ability to synthesize fatty acid, which indicates a role of the vitamin in the biogenesis. Further, the data listed indicate that increase in the amount of avidin does not result in the total inhibition of the fatty acid synthesis, which suggests that there exist 2 pathways of fatty acid synthesis of which one is sensitive and the other is insensitive to avidin; biotin overcomes the avidin

[1] S. J. WAKIL, J. Lipid Res. *2*, 1 (1961).
[2] J. A. CROOM, J. J. MCNEILL, and S. B. TOVE, J. Bact. *88*, 389 (1964).
[3] J. J. GAVIN and W. W. UMBREIT, J. Bact. *89*, 437 (1956).
[4] M. WOODBINE, M. E. GREGORY, and T. K. WALKER, J. exp. Bot. *1*, 204 (1951).

inhibition. Normal medium with supplementation of biotin does not result in enhanced production of fatty acid.

We have made a further study of the conditions necessary to restore normal activity to biotin-deficient cultures. A number of previous reports[5,6] indicate that the role of biotin in the synthesis of some enzymes is concerned with the production of an active 4 carbon U essential for aspartate biogenesis, an essential component of the enzyme. As shown in the Table, exogenous aspartic acid cannot replace the biotin requirement in fatty acid biogenesis, which indicates that fatty acid reduction during biotin deficiency is the result of reduction in availability of biotin enzyme required for the biogenesis of fatty acid.

Similar results have been obtained in *Aspergillus nidulans* and *Phycomyces blakesleeanus*.

Fatty acid (%) in normal and biotin deficient mats of *A. flavus*

Normal	Deficient	Deficient + biotin	Deficient + aspartate (100 mg/flask)
6.1	3.8	6.4	3.8
6.0	3.2	5.75	3.9
6.2 (+ biotin)	3.6 (avidin 10 U)		

Zusammenfassung. Avidin vermindert die Biosynthese von Fettsäuren in *Aspergillus flavus*, die nur von Biotin überwunden wird. Ähnliche Phänomene werden in anderen Pilzen gefunden.

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Department of Microbiology, M.S. University of Baroda (India), December 27, 1965.

[5] J. M. Raval, B. F. Mollenhauer, and W. Shive, J. biol. Chem. *236*, 2268 (1961).
[6] P. J. Ables, J. M. Ravel, and W. Shive, J. biol. Chem. *236*, 3263 (1961).

The Estrogenic and Deciduogenic Properties of Some Estra-1,3,5(10)triene-3,17β-diol Derivatives

Following the introduction of Enovid® and Norlutin® into clinical practice in the 1950's, a large number of compounds have been prepared that have been highly effective for treating menstrual disorders and controlling fertility in women. Most of the steroids evaluated have been related chemically to 19-nortestosterone or progesterone. In the main, the primary objective with the 19-nortestosterone steroids was to increase the progesterone-like activity while decreasing its androgenic side effects. Some of these progestins, such as norethynodrel and ethynodiol diacetate are of interest because, in addition to their progestational property, estrogenic activity has been imparted to the molecule[1-4]. For reviews of the structure-function relationships of the synthetic progestins see Drill and Riegel[5] and Drill[6].

The present report describes another series of compounds, all derivatives of estradiol, that are unique in that some effects attributable to progesterone have been imparted to the basic estrogenic molecule.

Materials and methods. Estrogenic activity was determined in rats and mice using cornification of the vaginal epithelium and increases in the weights of the uteri as end-points. Methods for these tests have been described previously[7,8]. *Progestational activity* was determined in rabbits using arborization of the uterine epithelium as the index of activity[9]. In addition, the ability of these materials to produce decidual cell responses in the endometrium of rabbits served as an indication of intrinsic progestational activity. The method for assessing activity has been described[10]. *Steroids used* were 17-substituted estra-1, 3, 5(10)-triene-3, 17β-diols and their 3-acetate esters (Tables I and II). These steroids were administered to experimental animals as solutions or suspensions in corn oil according to the experimental protocols using both subcutaneous and intragastric routes of administration.

Results. Estrogenic activity, determined in mouse uterine growth assays and vaginal cornification tests in ovariectomized rats, was found to be closely related to the length of the alkyl side chain (Table I). Depending on the test method, the potency of the methyl derivative was found to be 285–1000% of estrone when administered subcutaneously and 15–40% when given intragastrically. This potency was sharply decreased when the 17α-side-chain length was increased by 1, 2, or 3 methylene groups. Unsaturation of the ethyl side chain to form the vinyl compound resulted in marked increases in estrogenic potency in both estrogen assays by both the oral or parenteral routes of administration. Further unsaturation, forming the ethynyl compound, imparted even greater estrogenic potency. When a double bond was introduced at the C-2 position of the side chain as with the propenyl, isopropenyl, and butenyl compounds, estrogenic

[1] F. J. Saunders and V. A. Drill, N.Y. Acad. Sci. *71*, 516 (1958).
[2] V. A. Drill and F. J. Saunders, *Proc. Symposium on 19-Nor Progestational Steroids* (Searle Research Laboratories, 1957), p. 543.
[3] R. L. Elton and E. F. Nutting, Proc. Soc. exp. Biol. Med. *107*, 991 (1961).
[4] F. J. Saunders, F. B. Colton, and V. A. Drill, Proc. Soc. exp. Biol. Med. *94*, 717 (1957).
[5] V. A. Drill and B. Riegel, Recent Prog. Horm. Res. *14*, 29 (1958).
[6] V. A. Drill, Fedn Proc. Am. Socs exp. Biol. *19*, 1040 (1959).
[7] R. A. Edgren and D. W. Calhoun, Am. J. Physiol. *189*, 355 (1957).
[8] R. A. Edgren, D. W. Calhoun, R. L. Elton, and F. B. Colton, Endocrinology *65*, 265 (1959).
[9] R. L. Elton and R. A. Edgren, Endocrinology *63*, 464 (1958).
[10] R. L. Elton, P. D. Klimstra, and F. B. Colton, in press.