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.

activity was reduced by 10–400 fold when compared to the potency of the ethylene compound. In fact, these materials had potencies of the same order of magnitude as their saturated analogues.

Similarly, the introduction of a methylene group between the steroid molecule and the ethynyl group (2-propynyl) reduced estrogenic potencies in the uterine growth and vaginal smear assays. Addition of a methyl group on the terminal carbon of the propynyl side chain greatly enhanced its activity. This substance [17-(2-butynyl)estra-1,3,5(10)-triene-3,17β-diol 3-acetate] was the most potent estrogen tested in this group of steroids by either route of administration.

Progesterone-like activity, as measured by the Clauberg-type assay, was absent for all of the materials tested except the 2-methyl-2-propenyl (2-methallyl) analog. It had approximately 5% of the activity of progesterone (Table I).

Decidual cell responses were observed in the uteri with 8 of the 12 compounds tested (Table II). To obtain a decidual response in a test system such as this one, one must employ both a progestin and an estrogen[11] or

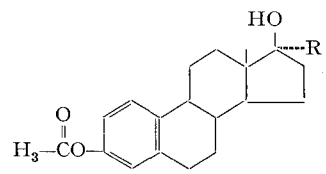

Table I. Estrogenic and progesterone-like activities of some 17-(substituted)estra-1,3,5(10)-trien-3,17β-diol 3-acetates

R	Estrogenic activity[a,b]								Progestational activity Clauberg-rabbit SC[a,c]	
	Rat vaginal smear				Mouse uterine growth					
	IG		SC		IG		SC			
	N	RP (%)	*N*	RP (%)	*N*	RP (%)	*N*	RP (%)	*N*	RP (%)
CH_3	3 (45)	40	7 (179)	1000	4 (40)	15.0	12 (160)	285.0	1 (4)	Inactive
CH_2–CH_3	1 (13)	< 0.25	6 (150)	2	4 (50)	0.02	6 (70)	2.4	1 (4)	Inactive
CH_2–CH_2–CH_3	3 (44)	0.17	3 (58)	1	5 (60)	0.06	3 (40)	0.3	1 (4)	Inactive
CH_2–CH(CH_3)–CH_3	3 (45)	0.25	4 (60)	3	3 (40)	0.38	3 (40)	0.5	1 (3)	Inactive
CH_2–CH_2–CH_2–CH_3	3 (45)	< 0.25	3 (45)	0.25	4 (40)	0.04	3 (30)	0.06	1 (4)	Inactive
CH=CH_2	5 (75)	5	4 (60)	250	4 (40)	5.0	10 (120)	120.0	1 (4)	Inactive
CH_2–CH=CH_2	6 (88)	0.3	8 (172)	2.5	5 (130)	0.03	7 (80)	1.1	2 (8)	Inactive
CH_2–C(CH_3)=CH_2	3 (43)	0.7	6 (120)	2.5	5 (60)	0.08	7 (210)	0.3	4 (29)	5
CH_2–CH=CH–CH_3	4 (59)	0.7	4 (59)	3.3	3 (30)	0.1	6 (60)	0.4	1 (4)	Inactive
C≡CH	4 (59)	25	4 (60)	1000	6 (100)	67.0	12 (150)	1000	1 (4)	Inactive
CH_2–C≡CH	3 (43)	5	4 (55)	80	4 (40)	22.0	3 (30)	25.0	1 (3)	Inactive
CH_2–C≡C–CH_3	6 (88)	170	5 (75)	1250	4 (80)	8.3	9 (100)	100.0	2 (8)	Inactive

[a] IG and SC indicate intragastric and subcutaneous routes of administration, respectively; *N* is the number of doses used to estimate the potency and the number in parenthesis is the total number of animals used; RP is the relative potency. [b] Relative potency of subcutaneously administered estrone is 100%. [c] Relative potency of SC progesterone is 100%.

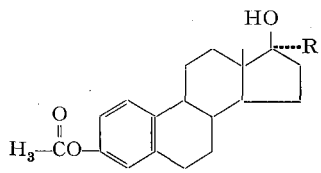

Table II. Deciduogenic effects of some estradiol derivatives

R	Daily dose (mg/rabbit)[a]				Activity
	1	2	4	10	
CH_3	–	–	0/8	–	Inactive
CH_2–CH_3	–	–	1/4	–	Weakly active
CH_2–CH_2–CH_3	–	–	0/7	–	Inactive
CH_2–CH(CH_3)–CH_3	–	–	1/6	–	Weakly active
CH_2–CH_2–CH_2–CH_3	–	–	0/3	0/4	Inactive
CH=CH_2	–	–	1/8	–	Weakly active
CH_2–CH=CH_2	0/3	5/8	3/4	–	Active
CH_2–C(CH_3)=CH_2	0/3	1/3	15/17	5/11	Active
CH_2–CH=CH–CH_3	–	–	1/4	3/4	Active
CH≡CH	–	1/4	3/4	–	Active
CH_2–C≡CH	–	–	1/12	–	Weakly active
CH_2–C≡C–CH_3	2/6	1/7	7/7	–	Active
Ethynodiol diacetate[b]	2/8	3/4	3/3	3/4	Active

[a] Number of animals with decidual responses in their uteri over the number of animals treated. [b] This substance is included for comparative purposes.

materials possessing a combination of these effects, such as ethynodiol diacetate (Table II)[10]. The presence of decidual responses can be taken as evidence for both estrogenic (which was measurable) and progestational (measurable with only 1 compound) properties.

Discussion. The introduction of various side chains into the 17α-position of the estradiol 3-acetate molecule had a marked effect upon estrogenic potency. When comparing the saturated and unsaturated side chains of comparable homology, lengthening the alkyl side chain decreased potency while unsaturation increased activity. Reports in the literature indicated that these same alkyl side chains when introduced into the 19-nortestosterone molecule increased progestational potency[4-6]. Thus, while progestational activity per se was observed only in animals following treatment with the 17-(2-methallyl) derivative of estradiol it is interesting that when this group is introduced into the 19-nortestosterone molecule it is one of the most potent progestins of that series[6,9]. It appears then that some of the decreases in estrogenic activity observed following introduction of the 17-alkyl, alkenyl or alkynyl groups into the estrogenic molecule may be due in part to incorporation of some of the properties of progesterone. However, because of the estrogenic activity of these substances the progestational properties, in the classical sense, were observed only in the deciduogenic test and not in the Clauberg assay.

Compounds such as ethynodiol diacetate, norethynodrel and norethindrone acetate which possess both progestational and estrogenic activities have been shown to produce decidual effects in rabbits[10,12]. Other studies have shown that the response is dependent upon the combined effects of both progesterone and estrone[11]. It has been shown also that estrogens or progestins are not capable of producing this response if administered separately. Thus, any decidual responses must reflect progesterone-like as well as estrogenic activity. This is the first time, to our knowledge, that progesterone-like activity has been imparted into a steroid with an aromatic A-ring structure. It is curious that the steroid with the highest degree of decidual activity, 17-(2-butynyl)estra-1,3,5(10)-triene-3,17β-diol 3-acetate is also the most potent material in the estrogenic studies. On the other hand, the 17-allyl derivative, which is comparable to the above material as a deciduogen is very weak as an estrogen. At present there is no way in which the intrinsic estrogenic and progestational properties of these steroids can be separated to provide an answer to this disparity.

Zusammenfassung. Die östrogene Aktivität von einigen substituierten Östrodiolderivaten wurde in Rattenvaginalabstrichen und Mäuseuterus-Wachstumversuchen nachgewiesen. Die Einführung von 17α-Alkyl-, Alkenyl- oder Alkinylgruppen in die Östradiolgruppierung hatte einen signifikanten Effekt auf die östrogene Aktivität. Das 17-(2-Butinyl)-Derivat von Östradiol war eine der wirksamsten Substanzen in den Östrogen- und Deciduomaversuchen.

R. L. Elton, R. E. Counsell,
P. D. Klimstra, and E. F. Nutting

Divisions of Biological and Chemical Research, G. D. Searle & Co., Chicago (Illinois, USA), February 24, 1966.

[11] R. L. Elton, D. W. Calhoun, and E. F. Nutting, in press.
[12] R. L. Elton, P. D. Klimstra, F. B. Colton, and V. A. Drill, in press.

Microelectrode Studies of Spontaneous Potentials from Chick Embryo Telencephalon in vitro

Spontaneous potentials have been reported in amphibian[1], fish[2], insect[3], chick embryo[4,5] and human adult brain tissue[6] in vitro, using 0.12–0.2 mm diameter metallic electrodes. Their responses to environmental changes and administration of drugs[4] are also recorded. Using microelectrodes, resting potentials and potentials in response to direct mechanical[7] and electrical[7,8] stimulation of brain cells in vitro have been described. Similar work has been done in isolated brain slices in cat[9].

This paper describes microelectrode studies of spontaneous potentials in chick embryo telencephalon in vitro and discusses the relationship between potentials recorded with microelectrodes and 0.08 mm diameter (gross) platinum electrodes.

Our culture chamber was an 18 mm diameter, 4.3 mm high, 1.5 mm thick glass tube with a 4 mm arc-shaped gap in its wall. 18 mm coverglasses formed the top and bottom of the chamber, which was filled by a coarse-porosity glass frit except for a sector opposite the gap in the wall. A bare gross platinum reference electrode lay between the frit and lower coverglass; a similar platinum electrode, coated with Teflon to within 1 mm of its tip, lay between the upper coverglass and frit, with its tip at the angle of the missing sector of the frit. The frit was saturated with nutrient fluid (different from previous studies[4] in the use of chick serum and addition of 1% each of concentrated methylene blue solution in balanced salt solution, multivitamin (Eagle) and amino acid solutions (Eagle). Methylene blue increases persistence and amplitude of signal sequences (possibly due to its known depolarizing effect) and vitally stains neuronal granules, which facilitates their visualization[10].

[1] R. W. Gerard and J. Z. Young, Proc. R. Soc. London *122B*, 343 (1937).
[2] E. D. Adrian and F. J. J. Burtendijk, J. Physiol. *71*, 121 (1931).
[3] E. D. Adrian, J. Physiol. *72*, 132 (1931).
[4] A. W. B. Cunningham, J. gen. Physiol. *45*, 1074 (1962).
[5] A. W. B. Cunningham and B. J. Rylander, J. Neurophysiol. *24*, 141 (1961).
[6] A. W. B. Cunningham, Nature *190*, 918 (1961).
[7] W. Hild and I. Tasaki, J. Neurophysiol. *25*, 227 (1962).
[8] S. Crain, in Symposium: *Neurological and Electroencephalographic Correlative Studies in Infancy* (Eds. P. Kellaway and I. Petersen; Grune & Stratton, New York 1964), p. 12.
[9] B. D. Burns, *The Mammalian Cerebral Cortex*, Monographs of the Physiological Society, No. 5 (Edward B. Arnold Ltd., London 1958).
[10] A. W. B. Cunningham, R. R. Rojas-Corona, J. A. Freeman, and P. H. Levine, Q. Prog. Rep. No. 79, Research Lab. Electron. M.I.T., Cambridge, Mass. (Oct. 15, 1965), p. 257.