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

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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].

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14-day-old chick embryo telencephalon was exposed and cooled in a stream of 10–20 °C nutrient fluid and 1 mm³ of the postero-lateral angle of the right lobe excised rapidly and placed on the tip of the recording electrode at the junction of the frit and coverglass. The microchamber was then maintained at 35 °C and 100% humidity in a microscope stage incubator[10]. Spontaneous electrical activity is detectable before 24 h after explantation.

Glass micropipettes (tip diameter under 1 μ, filled with 3 M KCl and with measured resistance between 15 and 40 $M\Omega$) were positioned visually, using phase contrast microscopy and methylene blue vital staining. A Medistor A-35B negative electrometer amplifier provided first-stage amplification ($\times$ 10) and a Dana amplifier (for DC) or the vertical amplifier of a Tektronix 502A (for AC), second-stage amplification. Data were recorded by a modified Ampex 305 tape recorder ($1-{}^{7}/_{8}$ ips for slow events and $7-{}^{1}/_{2}$ ips for fast events).

The term 'spontaneous' means that the tissue was not knowingly 'stimulated' after explantation. Except during penetration of cells, data recorded for 15–30 min following electrode movement were not used (to allow stabilization of the electrode tip).

Three classes of signals have been observed using microelectrodes: (1) long duration, lasting from ${}^{1}/_{3}$–1 min; (2) intermediate duration (10–500 msec), similar to those obtained with gross electrodes (50–500 msec); (3) short duration (1–2 msec), similar to those usually encountered using microelectrodes in vivo.

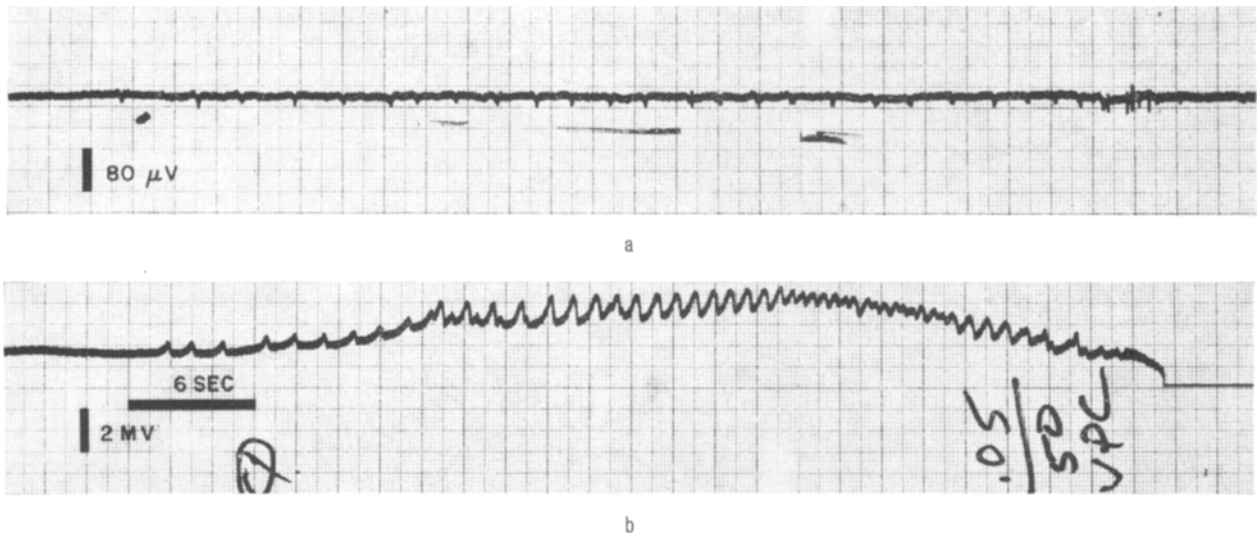

Fig. 1. Simultaneous recordings from an explant of 14-day-old chick embryo telencephalon after 36 h in vitro. (a) Using 40-gauge platinum electrode lying under the tissue (AC amplification); (b) using microelectrode within the tissue (DC amplification).

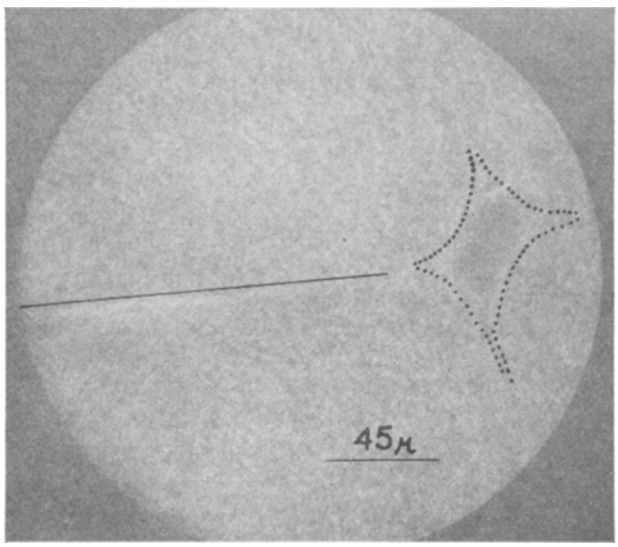

Fig. 2. Microelectrode approaching neuron whose granules are stained with methylene blue ($\times$ 640), visualized with phase contrast. A thick section was used so that accurate delineation of the cell was not possible photographically. A dotted line has been drawn outside the boundaries of the cell.

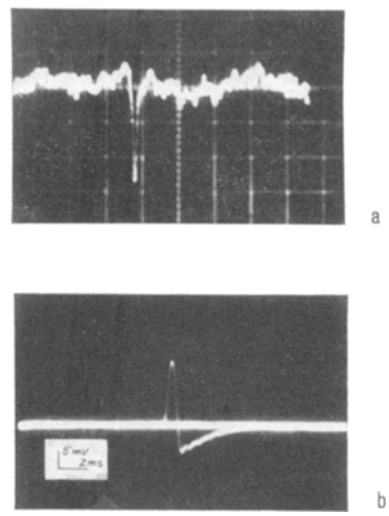

Fig. 3. (a) Negative spike potential from a microelectrode placed extraneuronally in an explant of 14-day-old chick embryo telencephalon. Vertical scale 1.25 mV/cm; horizontal scale 5 msec/cm; (b) Intracellular spike recorded from neurons in an explant of 14-day-old chick embryo telencephalon during second day in vitro.

Long-duration signals. Figure 1b shows a positive, long-duration signal recorded in an explant using a microelectrode. Intermediate-duration positive signals (50–500 msec) are superimposed on it.

Intermediate range (10–500 msec) signals. These usually occur in repetitive patterned sequences[4]. Figure 1 shows a sequence of pulses recorded simultaneously from a gross electrode (Figure 1a) underlying the explant and a microelectrode lying outside neurons within the explant (Figure 1b). A series of such simultaneous pairs of grouped pulses were recorded in this way. Each of the pair of sequences had approximately the same duration and started at the same time after the same silent interval. There was a pulse in the microelectrode recording for nearly all pulses in the gross electrode recording. Thus the gross and microelectrode activity are sufficiently similar to have been initiated at a common source. Equally similar sequences can be recorded with microelectrodes in the tissue and in the film of fluid external to the tissue. Entry of the microelectrode into the tissue was accompanied by a 7 mV negative DC shift, which varied (as much as 2 mV) between different sites in the tissue.

The sites and extents of electrically active areas within an explant can be studied (a) by placing an explant on the cut end of a microcable containing twenty 0.08 mm diameter, individually insulated platinum electrodes and observing activity from varying pairs out of the twenty, or (b) by observing the several foci of activity detectable using microelectrodes at different sites in the film of fluid at the periphery of an explant.

Signals observed with technique (a) are in the microvolt range, while those with technique (b) are in the millivolt range, but are otherwise the same. There are discrete small foci of activity within the tissue. A 200–300 μ movement of the microelectrode tip away from a focus (in technique (b)) may result in the activity's being no longer detectable, and restoration of the position of the microelectrode tip causes a return of activity. Several foci may be present in one explant, each with similar but not identical telencephalic patterns of signals. The spread of the potential from a single focus is narrowly directional.

Short duration (1-2 msec) signals. Typical negative 'extracellular' 1–2 msec potentials (Figure 3a) occur in groups whose duration and intergroup intervals are similar to those detected with gross electrodes and microelectrodes in the film of fluid around the explant. The grouped 1–2 msec signals vary in amplitude; thus they must originate in several neurons.

Figure 3b shows one of a series of fast (1–2 msec) 20 mV positive spikes (AC amplification) recorded intracellularly from a non-motile cell whose granules stain selectively with methylene blue and which had the form of a neuron (Figure 2). These spikes were preceded by a 30 mV negative DC shift when the cell membrane was penetrated. The membrane potential and the spikes are smaller than those reported from other animals in vivo, but these measurements are less in other chick embryo tissues than in the same tissues in other animals (e.g. resting potential and action potential from chick auricular muscle fiber are 29.1 and 39.2 mV, and for cat 60.4 and 65.2 mV[11]). The measurements are made during the first 72 h after explantation, so that potassium released from damaged cells may alter the potassium/sodium ratio and reduce signal amplitude.

The neuron-like cells within the active foci are electrically active, and it is likely that the sequences of signals detected by all of the above means are associated with their activity[12].

Résumé. A l'aide de micro-électrodes, des potentiels rythmiques spontanés ont été enregistrés dans du tissu télencéphalique d'embryons de poulets âgès de 14 jours, in vitro. Ces potentiels sont semblables à ceux précédemment décrits en utilisant des électrodes de 80 μ en platine. La possibilité pour ces potentiels d'être produits par les neurones de l'explant est discuté.

A. W. B. Cunningham, P. O'Lague,
R. R. Rojas-Corona, and J. A. Freeman

Center for Communication Sciences, Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge (Massachusetts 02139, USA),
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Studies on Modality Segregation and Second-Order Neurones in the Dorsal Funiculus

The dorsal funiculus of the spinal cord has been considered as composed exclusively of primary afferents ascending to the dorsal column nuclei, or terminating within the cord. This conception has been based on anatomical work, and it has been shown that the ascending fibres are arranged in a segmental way[1]. From electrophysiological studies on the gracile nucleus[2,3] it can be concluded that fibres activated from rapidly adapting hair receptors, slowly adapting receptors sensitive to touch or pressure of the skin, and vibration receptors ascend in the funiculus. There is also evidence that group I muscle afferents from the forelimb ascend in the dorsal funiculus[4,5]. This investigation has demonstrated a differential distribution of primary afferents activated from different receptors, and also the unexpected existence of a secondary tract in the dorsal funiculus.

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