Absence of Electrical Activity of the Pigeon's Pineal Organ in Response to Light

Recently, environmental illumination has been shown to affect the enzymatic activity required for the synthesis of melatonin in the pineal organ of rat and hen[1,2]. Moreover, illumination of young ducks resulted in acceleration of the development of sexual organs. Since this has been found both before and after removal of the lateral eyes, it has been concluded that part of the brain itself may be sensitive to light[3]. In order to obtain direct evidence of light sensitivity of the avian pineal organ comparable to that of lower vertebrates[4-6], the *epiphysis cerebri* of 8 adult pigeons, *Columba livia*, was surgically exposed under urethane anaesthesia and systematically explored by microelectrodes[7] inserted into the organ. With all animals investigated the evidence was entirely negative, i.e. direct illumination of the pineal organ or of the lateral eyes did not produce any electrical activity related to the onset or the cessation of light. This result cannot be due to technical reasons since, when the tip of the electrode was pushed deeper into the pineal organ, a sustained train of impulses of small size was seen which did not respond to illumination[8]. With a somewhat more backward and deeper position of the electrode, large spike potentials of the cerebellum were observed. The evident lack of electrical activity of the pigeon's pineal organ in response to light corresponds to recent electron microscopic findings, according to which synapses and typical photoreceptor cells with regular outer segments in the pigeon and in the duck are absent[9].

Zusammenfassung. Im Gegensatz zur Epiphyse von Fischen, Amphibien und Reptilien erweist sich das Pinealorgan der Taube bei Untersuchung mit Einstichelektroden als nicht lichtempfindlich.

Y. MORITA

William G. Kerckhoff-Herzforschungsinstitut der Max-Planck-Gesellschaft, Bad Nauheim (Germany), March 28, 1966.

1 R. J. WURTMAN, J. AXELROD, and L. S. PHILLIPS, Science *142*, 1071 (1963).
2 J. AXELROD, R. J. WURTMAN, and CH. M. WINGET, Nature *201*, 1134 (1964).
3 J. BENOIT and I. ASSENMACHER, Recent Prog. Horm. Res. *15*, 143 (1959).
4 E. DODT and E. HEERD, J. Neurophysiol. *25*, 405 (1962).
5 W. H. MILLER and M. L. WOLBARSHT, Science *135*, 316 (1962).
6 E. DODT, Experientia *19*, 642 (1963).
7 Y. MORITA, Pflügers Arch. ges. Physiol. *286*, 97 (1965).
8 The position of the tip of the recording electrode was identified to be close to a bundle of unmedullated fibres in the basal part of the pineal organ. A full account of this work will appear elsewhere.
9 A. OKSCHE und M. VAUPEL-VON HARNACK, Z. Zellforsch. *69*, 41 (1966).

Differentiation of the Epithelium in Early Grafts of the Mouse Müllerian Vaginal Region

Recent investigations[2-4,8] have disclosed a dual origin of the mouse vaginal epithelial lining: the anterior part from the müllerian epithelium, the posterior part from the sinus epithelium.

In an earlier paper, FORSBERG[3] reported studies on the differentiation of the epithelium in homografts of the vaginal müllerian epithelium from new-born female mice that were allowed to grow intramuscularly in female litter-mates. The results were interpreted as excluding any contribution of the sinus epithelium at the differentiation of the epithelium in the anterior part of the vagina, a thing that was formerly widely believed to occur. However, apart from a cellular contribution, an inductive stimulus from the sinus epithelium on the müllerian epithelium could not be excluded. Another possibility is that the mesenchyme plays some role in this connection (GROBSTEIN[5]). In this investigation, we wish, among other things, to throw light on this problem by using early fetal grafts of the mouse müllerian vaginal epithelium.

Material and methods. Albino mice from a closed stock were used in this investigation. At a gestational age of 14.5 days, calculated from the time of an observed spermal plug, the pregnant mother animal was killed and the fetuses removed. Under a dissecting microscope, the anterior part of the müllerian vaginal region was removed for transplantation (see Figure 1). The rest of the fetuses were fixed in Bouin's fluid, paraffin embedded, and sectioned transversely in 5 μ sections. These were stained in hematoxylin and eosin. The donor fetuses were studied in order to exclude any contamination of sinus epithelium in the grafts. In some cases, a piece of one of the fetal uterine horns was also removed for transplantation.

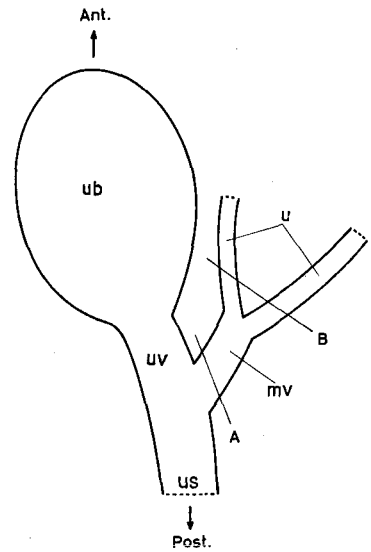

Fig. 1. Schematic presentation of the müllerian vaginal region as seen in the dissecting microscope in a 14.5 day fetus. mv, müllerian vagina; u, uterine horn; ub, urinary bladder; ur, urethra; us, urogenital sinus. The müllerian vagina was cut at A and the uterine horns at B. The region between A and B was transplanted into newborn female mice.

The grafts were transplanted into the thigh musculature of new-born females according to the method described earlier[2]. After 6 or 12 days, the hosts were killed and the thigh musculature containing the graft was fixed in Bouin's fluid. The fixed preparations were paraffin embedded and sectioned transversely in 5 μ sections. These were stained in hematoxylin and eosin.

Results. In all, 41 transplantations of the anterior part of the vaginal region were made. An examination of the section series of the 14.5 day fetuses from which the grafts were taken revealed that the posterior part of the müllerian ducts was attached to the dorsal wall of the urogenital sinus in 30 cases. The grafts from these fetuses could thus be considered free from sinus epithelium.

Of the 30 grafts in which the grafted müllerian vaginal region was definitely free from sinus epithelium, 16 were allowed to grow for 12 days; 11 of these grew well for 12 days. The epithelium in all of these showed the typical vaginal epithelial differentiation with the appearance of a basal and a superficial epithelial zone (Figure 2). The cells in the basal zone are more palely stained and lower than those in the superficial zone, just as is seen during the normal development of the vaginal region in situ.

14 grafts, definitely free from contamination by sinus epithelium, were allowed to grow for 6 days. 12 of these showed a luxuriant and proliferative epithelium but there was no differentiation into the two zones mentioned above (Figure 3). 7 grafts of a part of one of the fetal uterine horns grew for 12 days. In all of these grafts the epithelial differentiation proceeded normally.

Discussion. The posterior tips of the müllerian ducts reach the dorsal wall of the urogenital sinus some time between 14 and 14.5 days post coitum[1]. In all the 14.5 day fetuses studied in this investigation (the donors of the grafts), the tips of the ducts contacted the sinus epithelium. The border between the sinus epithelium and the müllerian epithelium is clear-cut and even if degeneration granules appear in the müllerian tips immediately upon these contacting the urogenital sinus, this does not indicate an early infiltration of sinus cells into the müllerian epithelium[1,6]. As the same type of degeneration appears also in lower vertebrates, it may have some phylogenetical meaning[7,8].

In this investigation, birth has been considered to occur 19 days after the time of the observed spermal plug. Thus, a growth of the grafts for 12 days should correspond to a post partum age of about 7.5 days. At this time, the epithelium in the müllerian vaginal part in the organ in situ is differentiated into a basal zone and a superficial zone, which differentiation begins about 3 days after birth. This differentiation can also be seen in the grafts after growth for 12 days.

When a piece of one of the uterine horns was transplanted, the epithelial differentiation progressed as in the uterine horns in situ: the epithelium retained its pseudostratified columnar character. Thus, the muscular milieu does not interfere with the differentiation.

As both an early infiltration of sinus cells into the müllerian epithelium and the milieu factor can be excluded from having any influence on the epithelial development in the grafts, the results gained from this investigation agree with the view that the anterior part of the mouse vaginal epithelial lining (approximately $^3/_5{}^1$) is formed from the müllerian epithelium.

If estradiol is administered to neonatal mice, the basal zone undergoes a cornification and the superficial zone a mucification[4,9]. Thus the müllerian epithelium is able to give a squamous response (in the sense of ZUCKERMAN[10]) to estrogen, and this response is not limited to epithelia derived only from the sinus epithelium (for a discussion, see RAYNAUD[11]).

A growth of the grafts for 6 days corresponds to a postnatal age of about 1.5 days. At this time there is no visible 'zonal' differentiation in the müllerian epithelium in the vaginal region in situ, nor is there any in the grafts. The epithelial differentiation in the grafts thus

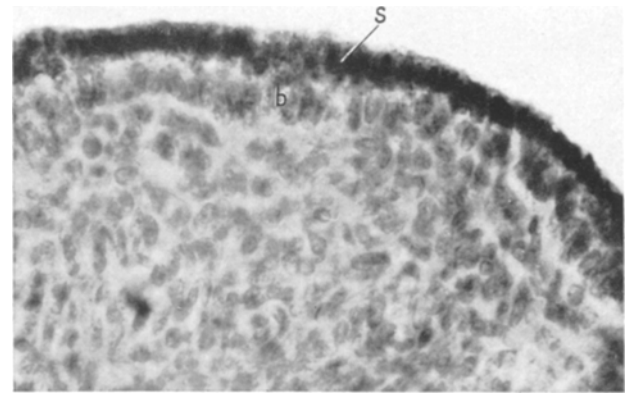

Fig. 2. Microphotograph showing the appearance of the epithelium in a graft of the müllerian vagina 12 days after the transplantation. The differentiation is the same as in the organ in situ. b, basal zone; s, superficial zone. Magnification × 560.

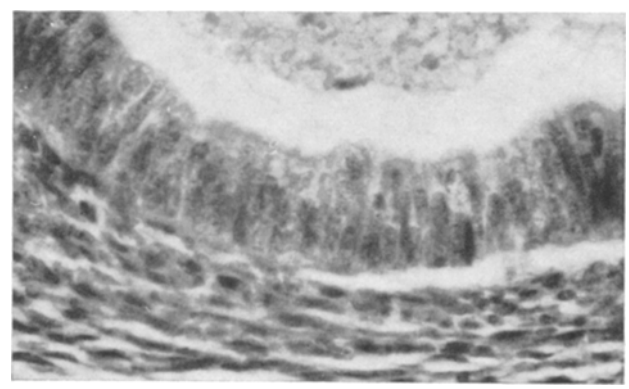

Fig. 3. 6 days after the transplantation the epithelium in the grafted müllerian vagina is high pseudostratified columnar. Magnification × 560.

[1] J.-G. FORSBERG, *Derivation and Differentiation of the Vaginal Epithelium* (Thesis, Lund 1963).
[2] J.-G. FORSBERG, Acta anat. *62*, 266 (1965).
[3] J.-G. FORSBERG, J. Embryol. exp. Morph. *14*, 213 (1965).
[4] J.-G. FORSBERG and H. OLIVECRONA, Z. Zellforsch. *66*, 867 (1965).
[5] C. GROBSTEIN, *Role of Connective Tissue in Embryologic Development;* in *Congenital Malformations*. Papers and discussions presented at the Second International Conference on Congenital Malformations (International Medical Congress Ltd., New York 1964), p. 224.
[6] J.-G. FORSBERG and H. OLIVECRONA, Z. Zellforsch. *63*, 362 (1964).
[7] J.-G. FORSBERG and H. OLIVECRONA, Z. Anat. Entw. Gesch. *124*, 83 (1963).
[8] A. RAYNAUD, C. r. Acad. Sci. Paris *260*, 5888 (1965).
[9] J.-G. FORSBERG, Acta anat., in print.
[10] S. ZUCKERMAN, Biol. Rev. *15*, 231 (1940).
[11] A. RAYNAUD, in *The Ovary* (Ed., S. ZUCKERMAN; Academic Press, London and New York 1962), vol. II, p. 179.

follows an inherent trend. It has earlier been assumed[9,12] that the differentiation of the vaginal müllerian epithelium is induced by the sinus epithelium. The results from this investigation suggest that this is unlikely. If the sinus epithelium acted as an inductor, the induction should take place immediately the tips of the müllerian ducts reach the dorsal wall of the urogenital sinus, and the effect of this induction would not be evident until 7 or 8 days later. Instead, it seems more reasonable to regard the mesenchyme-epithelial relationship as an important factor in the differentiation process of the müllerian vaginal epithelium, this agreeing with the results from several other tissues[5]. The failure so far to get a normal differentiation of the müllerian epithelium by in vitro experiments has seriously hampered further analysis of our system[13].

Zusammenfassung. Die Resultate dieser Untersuchung unterstützen die Auffassung, dass das Epithel im kranialen Teil der Vagina ein Derivat des Müllerschen Epithels ist. Seine Differenzierung erscheint durch Zusammenwirken von Mesenchym und Epithel verursacht.

J.-G. FORSBERG and KRISTINA NORELL

Department of Anatomy, University of Lund (Sweden), January 10, 1966.

[12] D. BULMER, Acta anat. *57*, 349 (1964).

[13] Acknowledgment: This investigation was supported by grants from the Swedish Medical Research Council (No. Y 479) and the Swedish Cancer Society (No. 64:164).

On the Incorporation of Uridilate into Cerebro-Cortex Ribonucleic Acid

The synthesis of ribonucleic acid (RNA) in nerve cells presents points of particular interest. It is known that unusual amounts of RNA are present in neurons where they could be involved in the storage mechanism of memory[1]. Moreover, an alteration of the base ratio composition of nuclear and cytoplasmic RNA has been registered in those neurons directly involved in establishing particular patterns of sensory and motor abilities[2,3]. The presence has also been reported, in both grey and white matter, of a DNA-directed RNA-polymerase[4]. In this note the incorporation of uridine triphosphate (UTP) into a RNA-like polymer is described. The incorporation is catalysed by a subcellular fraction prepared from the cortex of calf brain.

Preparation of the enzyme. The cortex layer was scraped from the surface of meninges-free calf brain and the tissue was homogenized with an equal volume of sucrose buffered solution (0.25 *M* sucrose, 0.15 *M* *tris*-HCl, 0.05 *M* 2-mercaptoethanol, 0.1 *M* versene, pH 7.5). After centrifugation at 20,000 *g* for 15 min the supernatant was diluted with an equal volume of *tris*-HCl 0.01 *M*, pH 7.5, containing 0.05 *M* 2-mercaptoethanol and centrifuged for 120 min at 105,000 *g*. The sediment was then suspended in 0.4 *M* *tris*-HCl, pH 7.5, containing 0.05 *M* 2-mercaptoethanol and used as the source of enzyme. This fraction contained no DNA and a RNA/protein ratio of 0.06. All the operations were carried out at 0–2 °C. DNA was determined by the BURTON procedure[5], RNA by a modification of the orcinol procedure[6], and the protein by the LOWRY method[7].

Preparation of labelled UTP. Uridine triphosphate labelled with ^{32}P in the α-phosphate was prepared as follows. *Escherichia coli* was grown on $H_3{}^{32}PO_4$, the nucleates were extracted with phenol, the DNA digested with deoxy-ribonuclease, the RNA hydrolysed with snake venom diesterase, the labelled 5′-mononucleotides were separated by Dowex I formate chromatography and phosphorylated to triphosphates with *E. coli* kinases, and finally the triphosphates were purified by column chromatography[8]. Uridine 5′-triphosphate-2-C^{14} was obtained from Schwarz BioResearch Inc., USA.

Experimental procedure. The incubation mixture contained the following components in 11 ml: 880 μmoles *tris*-HCl pH 7.5, 220 μmoles 2-mercaptoethanol, 110 μmoles potassium 3-phosphoglycerate, 55 μmoles $MgCl_2$, 5.5 μmoles UTP^{32} (specific activity $11 \cdot 10^6$ counts/min per μmole) or UTP 2-C^{14} (specific activity $4.1 \cdot 10^6$ counts/min per μmole), 5.5 mg muscle glycolytic enzymes[9], and 68 mg protein (enzyme).

All the incubations were carried out at 38 °C for 20 min. At the end of the incubation, the mixture was brought with perchloric acid (PCA) to a final concentration of 0.4 *M* and to a final volume of 35 ml. After 5 min standing in an ice bath, the mixture was centrifuged and the precipitate washed 4 times with 35 ml cold PCA 0.4 *M*. The nucleic acids were extracted from the precipitate at pH 7.4 with 4.5 ml hot 2 *M* NaCl in 2 successive 30 min extractions. The nucleates were precipitated from the extract with 2 volumes of absolute ethanol at − 15 °C, allowing 30 min before centrifugation. The precipitate was redissolved in 2 ml 2% (w/v) potassium acetate and the alcohol precipitation was repeated. After a washing with ethanol 75% the precipitate was dissolved in 1 ml potassium hydroxide (KOH) 0.3 *M* and let stand for 18 h at 37 °C. The hydrolysed mixture was then neutralized with PCA and the insoluble perchlorate was centrifuged out.

Aliquots of the supernatant were used from chromatography and counting. The ribonucleotides were separated by column chromatography on Dowex 1 formate[10]. A two-dimensional thin layer chromatography was also carried out using cellulose 300 MN as support and isobutyric

[1] H. HYDEN, *The Cell* (Eds., J. BRACHET and A. MIRSKY; Academic Press, New York 1960), vol. 4, p. 215.
[2] H. HYDEN and E. EGYHAZI, Proc. natn Acad. Sci USA *48*, 1366 (1962).
[3] H. HYDEN and P. W. LANGE, Proc. natn Acad. Sci USA *53*, 946 (1965).
[4] S. H. BARONDES, J. Neurochem. *11*, 663 (1964).
[5] K. BURTON, Biochem. J. *62*, 315 (1956).
[6] R. B. HURLBERT, H. SCHMITZ, A. F. BRUMM, and VAN R. POTTER, J. biol. Chem. *209*, 23 (1954).
[7] O. M. LOWRY, N. J. ROSEBROUGH, A. L. FARR, and R. S. RANDALL, J. biol. Chem. *193*, 265 (1951).
[8] R. B. HURLBERT, results to be published.
[9] E. RACKER, J. biol. Chem. *167*, 843 (1947).
[10] T. TAKAHASHI, R. B. SWINT, and R. B. HURLBERT, Exptl. Cell Res., Suppl. *9*, 330 (1963).