Electric Polarity in the Chorioallantoic Membrane of the Chick Embryo.* (28360)

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The chorioallantoic membrane (CAM) of the chick embryo has been used in many investigations as a site for tissue grafts of various kinds(2,3,4). This site has the advantage of being readily accessible, and it is well vascularized, ensuring adequate blood supply.

Weiss and Taylor(6) recently used it to secure more advanced development of dissociated and reaggregated cells from the kidney, liver or skin of the chick embryo than had been attainable by culturing them *in vitro*. Clusters of such cells incorporated on the CAM developed not only the specific histiotypic characters of the respective organs of origin, but formed well organized miniature organs of typical structure. These organotypic formations proved to be definitely polarized relative to the CAM. Thus, kidney tubules, for instance, coursed in a general transverse direction, became confluent and opened through the outer (chorionic)‡ wall.

The purpose of the present investigation was to examine whether this characteristic polarization of the morphogenetic reconstitution process might have a counterpart in an electric polarity from the outer to the inner side of the membrane. Although a few studies of electric potentials in chick embryos have been reported, these have dealt with the whole blastoderm(5), whereas the electric properties of embryonic membranes do not appear to have been investigated.

The CAM is more complex than most biological membranes that have been studied electrically. It is formed between the fourth and ninth days of incubation by the fusion of the chorion on the outside with the underlying wall of the allantois. The composite CAM then consists of 3 layers, an outer and an inner epithelial one, separated by a mesodermal one, in which a network of blood vessels and capillaries is embedded. The basoapical axes of the cells of the outer and inner epithelial layers point in opposite directions.

Experimental technique. Eggs incubated for periods within the range of 4-10 days were used in these experiments. A circular piece of shell about 8 mm in diameter was first removed from the upper surface of the egg using a small motor-driven grinding wheel. Care was taken to avoid damage to the underlying tissue. The inner shell membrane was then peeled off exposing the intact chorioallantoic membrane, which would sag down, leaving a shallow depression above it.

For the electric measurements, glass micropipettes were used. These were from 1-4 μ in external diameter at the tip, with a lumen of approximately two-thirds of the external diameter, filled for most experiments with 1 M NaCl. In a few experiments, 3 M KCl was used. One of these micropipettes was mounted on a de Fonbrune micromanipulator so that it could be thrust axially through the membrane; it was connected by means of a salt bridge to a mercury-calomel half-cell. The reference electrode consisted of another half-cell, which was connected through a salt bridge to a cotton wick that dipped into the medium bathing the outer surface of the membrane. The potential was measured with a Keithley electrometer (model 603).

In one set of experiments, a circular segment of the membrane about 2 cm in diameter was excised. This was done by making a deep cut around a circular piece of shell so that the inner shell membrane and the CAM remained attached. The shell was then separated and the CAM segment could be floated off onto a flat plastic mount. A small hole in the mount allowed electric contact to

* These investigations form part of a program of study under the direction of Dr. Paul Weiss as principal investigator, aided in part by grants from Am. Cancer Soc., Nat. Cancer Inst. (Nat. Inst. Health, USPHS), and National Science Foundation.

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‡ Not on the allantoic side, as was erroneously stated in the above publication—(Weiss, personal communication).

be made with the under surface of the membrane. The experiments were performed in a room maintained at about 25°C.

Results. When the pipette was pushed against the intact membrane it did not appear to penetrate it gradually. Instead the membrane was observed to become deformed locally around the tip, gradually enveloping it, until suddenly the tip passed through into the allantoic cavity. During the passage the potential at the tip became negative but in an erratic and non-reproducible manner. It finally became steady only after the tip had pierced into the allantoic cavity. Because of this variability no attempt was made to determine potentials within the membrane, and only the total potential difference between the inner and outer surfaces was recorded.

In every case examined in an intact CAM, there was an inwardly directed electric field, the fluid in the allantoic cavity being negative in relation to the external medium.

In 2 experiments the external medium consisted of the small amount of vitelline fluid normally present between the inner shell membrane and the CAM. In these cases the potential differences were −16.0 and −17.5 mV, respectively, each representing the average of 5 readings. However, these electric measurements were found to be variable, due presumably to changes in salt concentration in the small volume of external fluid which was apt to evaporate easily. In most experiments, therefore, an artificial bathing solution (either Ringer's solution or Earle's solution) was used. The potential values were approximately the same for these 2 media.

Some dependence of the potential on the age of the embryo was observed. For 6-day embryos, with Earle's solution as external medium, the potential was −8.8 mV (mean value for 4 embryos with 5 readings taken for each). For 8- and 10-day embryos, the mean potentials were −13.8 mV and −11.1 mV respectively (4 embryos each). This appears to indicate that the magnitude of the potential rises to a maximum at about 8 days, although the subsequent fall may not be significant.

In the above experiment the micropipettes were filled with 1 M NaCl. Similar results were obtained if 3 M KCl was used. With this the mean potential for 3 eight-day embryos was −12.8 mV.

It was not possible to decide from these observations whether the observed potential difference was due to an intrinsic electrical asymmetry in the membrane or whether it was caused by differences in the ionic composition of the media bathing its inner and outer surfaces. To distinguish between these 2 possibilities, one experiment was performed in which fluid from the allantoic cavity of one embryo was used as external medium for another embryo of the same age, so as to have identical media on both sides. A potential reading of −13.5 mV was obtained for a 10-day embryo. The potential is therefore seen to arise not merely as a passive response of the membrane to diffusion of ions through it. It is a product of the activity within the membrane, evidently involving the expenditure of metabolic energy.

The effects of narcosis and chilling on the membrane potential support this conclusion. In 2 eight-day embryos, a small cotton pad soaked in ether was held over the opening in the shell. The magnitude of the potential then fell to a low value (about −2 mV) in 15 minutes. Following the removal of ether vapor and readmission of air there was a partial recovery to −6 mV in 40 minutes. During this treatment there was a reduction, but no cessation, of blood circulation.

In 2 further experiments the embryo was chilled by placing it in the freezing compartment of the refrigerator for a few minutes. Blood flow was found to have stopped and the potential had fallen to −3 mV with no subsequent recovery.

Attempts were made to determine whether there was a potential difference across a segment of membrane isolated and mounted as described earlier. A small area in the center of the upper and lower surfaces was kept moist with Earle's solution and the surrounding areas were made as dry as possible with filter paper to minimize electrical leakage over the surface of the segments. No potential difference could be detected between the upper and lower moistened areas, the limit of resolution being 0.2 mV. This result might be interpreted as indicating that removal from blood supply inhibits the metabolic

processes which underlie the generation of potential differences. However the possibility of electrical shunting by an external pathway across surfaces that were not sufficiently dry cannot be ruled out.

Measurements were made on 4- and 5-day embryos of the potential across the chorion alone before it became fused to the allantois. In 2 experiments the inner (basal) surface was found to be at a potential of +4 mV relative to the external bathing medium (Earle's solution). If the chorion, once it has fused to the allantois, retains this reversed sign for the potential across it, as compared to that across the combined CAM, it is evident that the interior of the CAM is more positive than either its inner or outer surfaces. In view of the opposite orientation of the cells in the chorionic and allantoic layers of the membrane it can be concluded that both epithelial layers are negative on their free apical surfaces, but that the potential difference across the allantoic layer exceeds that of the chorionic layer, hence imparts its sign to the net difference across the whole membrane.

Conclusions. The experiments discussed here indicate that a potential difference, requiring the expenditure of metabolic energy, is maintained across the chorioallantoic membrane, the inner surface being negative. Its value is somewhat smaller than the potential observed across most other biologically active membranes(1), and it is unlikely that these small electric forces would act directly as organizing agents in a tissue graft on the membrane. Nevertheless the potential difference may be an expression of an underlying metabolic asymmetry, maintained for example by a gradient of CO_2 or O_2 across the membrane, which could be related more relevantly to the observed polar reconstitution of the organs in the experiments of Weiss and Taylor.

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Received April 5, 1963. P.S.E.B.M., 1963, v113.

Contamination of Adenovirus Stocks with SV40 (Papovavirus Group)*† (28361)

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Since Sweet and Hilleman(1) demonstrated the presence of vacuolating virus (SV40)—a member of the papovavirus group(2)—in normal rhesus and cynomolgus monkey kidney cell cultures, the biology of the virus has been studied intensively. Although only low grade subclinical infection has been observed in human beings infected with SV40(3,4), the agent is able to produce fibrosarcomas (5,6) and ependymomas(7) in newborn hamsters, and to transform human cells(8,9) and hamster cells *in vitro*(10-12). With SV40 occurring as an adventitious agent of monkey kidney cultures, it is not surprising that it has been discovered in polio- and adenovirus vaccines(13,14).

The above findings led us to search for SV40 contamination in experimental adenovirus vaccines being prepared by means of $MgCl_2$-inactivation of the virus(15). Such contamination of our adenovirus stock and vaccine was found. In this paper we de-

* Aided by grants from Nat. Cancer Inst., and from Nat. Inst. of Allergy and Infect. Dis., Nat. Inst. Health, U.S.P.H.S.

† Papovavirus was officially accepted as the name for the papilloma-polyoma-vacuolating group of viruses by the International Subcommittee on Viral Nomenclature, International Conference on Microbiology, meeting on Aug. 20, 1962, in Montreal.