An Attempt at the Achievement of Acquired Tolerance of Skin Homografts in Post-Natal Life

It is possible to achieve the permanent development of skin homografts in grown-up individuals, not related to each other, if a host gets either the living cells of a donor[1] or desoxyribonucleoproteins from the donor's split cells[2]. A similar effect can be obtained in some rodents when given intraperitoneally the living cells of a donor in the first hours after birth[3]. Fetal period, or an early period after birth, during which the host's organism becomes – so as to speak – acquainted with protein (with which it also gets in touch in its later life), without producing any immunological reaction to it, which is called the plastic period of an organism.

Because of a certain association of ideas, I have put forward a hypothesis that it would be possible to recreate the plastic period of an organism if grown-up individuals were administered thymic extracts from young fetus.

The first proof of the hypothesis was a successful attempt to achieve agammaglobulinemia, lasting in grown-up rats[4], and in one guinea pig[5], for several weeks. The result was obtained by the hypodermic or intraperitoneal administration of a great quantity of thymus of young fetal pigs. It is a well-known fact that γ-globulins are produced only between the 4th and 6th week after birth[6–9].

When dissolved protein (e.g. human or bovine serum) is administered to the fetus of a rabbit, then there is no immunological reaction to many injections of the same antigen after the fetus has become a grown-up individual[10]. The experiment was repeated with guinea pigs and a rabbit, the assumption being that the final period of the administration of thymic extracts from young fetal pigs corresponds probably to the plastic period. Having received (as it has already been mentioned) dissolved protein, the experimental animals did not react to it in their later life. They displayed a marked immunological reaction to some other antigens, with which they had not come into contact when they had remained under the influence of thymic extracts from young fetal pigs[5].

These results have been followed by research concerning the prolonged survival of skin homografts in grown-up mice subject to the influence of thymic extracts from young fetal pigs. Although the investigations are in their initial stage, I present them in the form of a temporary report because of their positive results.

Materials and Method. Experimental animals (mice) were given hypodermically thymic extracts from young fetal pigs of the length of Si from 1 cm to 8 cm. The extracts were prepared in the following way: they were homogenized in physiological salt (NaCl 0.9%), centrifuged, and the liquid was separated from the sediment. On an average, about 1.5 ml of supernatant, containing from 90 to 140 mg% of protein, was obtained from a hundred homogenized thymi. Injections of this extract, either newly prepared or kept in the frozen state, were administered hypodermically before operation thrice a week in 21 days' time. No thymic extracts were given to control mice. Ether anesthetics were used in all the operations. Thick-split grafts, the surface of each graft being equal to 1 cm², were transplanted after previous depilation of the operation area. The grafts were stitched with single sutures and covered with thin colloidal membranes.

Two mice, each of them a four-month-old male, were used in the first experiment. The donor was a white mouse of the R_3 strain, and the host was a dark-brown mouse of the F_1 strain (A × Swiss). The F_1 strain mouse was given before operation a thymic extract from 250 young fetal pigs; besides which, in the period from the 4th to 14th day after operation, the mouse was administered an extract (in 4 injections) from 150 young fetal pigs.

Two mice were used in the second experiment, one of them a male of the A strain, and the other mouse a five-month-old male of the C_{57} strain. Both mice were given thymic extracts only before operation, the total amount being equal to 300 thymi per mouse.

The Description of the Experiments. In the first experiment the skin graft from a brown mouse of the F_1 strain to a white mouse of the R_3 strain (the latter had not received any thymic extract) began to fade on the 7th day after operation. The skin graft from the white mouse of the R_3 strain to the brown mouse of the F_1 strain (the latter was getting thymic extracts) continued to survive. On the 14th day after operation (before taking a documentary photograph to get a more exact picture of the case) the colloidal membrane was removed from most of

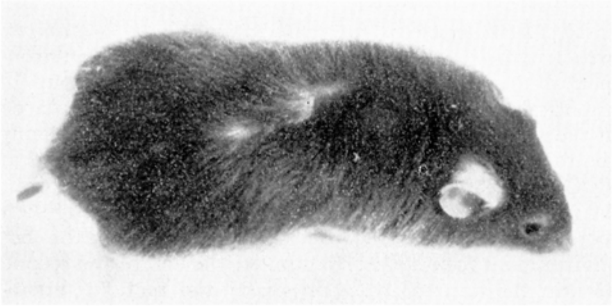

Fig. 1. $R_3 \rightarrow F_1$ (A × swiss)

Fig. 2. A $\rightarrow C_{57}$

[1] R. E. BILLINGHAM, L. BRENT, and P. B. MEDAWAR, Nature *172*, 603 (1953).
[2] R. E. BILLINGHAM, L. BRENT, and P. B. MEDAWAR, Nature *178*, 514 (1956).
[3] M. F. A. WOODRUFF and L. SIMPSON, Brit. J. exp. Pathol. *36*, 494 (1955).
[4] J. CZAPLICKI, Surgery *50*, 817 (1961).
[5] J. CZAPLICKI, Surgery *51*, 645 (1962).
[6] R. A. BRIDGES, R. M. CONDIC, S. J. ZAK, and R. A. GORD, Fed. Proc. *16*, 352 (1957).
[7] E. L. KNAPP and J. I. ROUTH, Pediatric *4*, 508 (1949).
[8] M. B. S. C. MILLER, The Lancet *30*, 748 (1961).
[9] D. H. MOORE, R. M. DU PAN, and C. L. BUSTON, Amer. J. Abst. Gynecol. *57*, 312 (1949).
[10] R. T. SMITH and R. A. BRIDGES, Transplantation Bull. *3*, 145 (1956).

the graft's surface. The exposed graft became dark and stiff in a few minutes. This symptom was recognized as an initial fading of the whole graft. It turned out, however, that a slip of the grafted skin left under the intact colloidal membrane developed and became covered with white hair; it has survived up to now, i.e. for 220 days, and does not show any symptoms of fading (Table).

In the case of 6 control grafts, transplanted crosswise to mice of the same breed, all the grafts faded between the 6th and 11th day after operation.

In the second experiment, skin grafts were made edgewise on the 3rd day following the last injection of thymic extracts. The skin graft from a black mouse of the C_{57} strain to a white mouse of the A strain had faded by the 10th day after operation. The skin graft from the white mouse of the A strain to the black mouse of the C_{57} strain developed edgewise and became covered with white hair. It has already survived for 280 days and does not show any symptoms of fading.

As regards 10 control grafts made crosswise in male mice of the same breed as the experimental mice, all the skin grafts faded between the 6th and 10th day after operation.

Zusammenfassung. Während 3 Wochen wurden zwei geschlechtsreifen Mäusen Thymusextrakte aus Schweinefrühfrucht subkutan bzw. intraperitoneal injiziert. Implantierte Hautläppchen aus gattungsfremder Maus ergab Einwachsen vom Rande her und dauerhafte Implantate ohne Schwundtendenz.

J. Czaplicki

Department of Histology and Embryology, Silesian Academy of Medicine, Zabrze (Poland), June 22, 1962.

Nystagmus and Related Phenomena in *Sepia officinalis*

The statocyst of cephalopods contains not only one or more 'maculae', i.e. statolith-bearing groups of sensory hairs (one in *Octopus*, three in *Sepia*), but also three 'cristae', rows of delicate, unloaded sensory hairs, arranged more or less perpendicular to each other. Whereas the maculae may serve as gravity receptors, the structure of the cristae suggests another function: namely the detection of endolymph movements within the statocyst as caused by active or passive rotation of the animal[1]. In *Octopus*, this view has been confirmed experimentally. On a turn-table, blinded octopuses show clear post-rotatory eye-nystagmus and corresponding after-movements of the head. These post-rotatory reflex movements persist after removal of one statocyst, but they disappear completely after removal of the remaining one[2].

Turn-table experiments of this kind were now performed with cuttle-fish (*Sepia officinalis*). First, an intact animal was put in a rather tightly fitting rectangular glass vessel and fixed in its 'normal' position within the vessel. Care was taken to provide the animal with sufficient fresh sea water for undisturbed respiration. The vessel was placed on a horizontal turn-table. On rotation of the table (by hand), the animal was turned around its vertical axis. The compensatory eye and head movements which occurred during rotation might have been due partly or even entirely to optical stimulation, although the turn-table was surrounded by a grey card-board in order to reduce visual landmarks as far as possible. However, when the rotating turn-table was suddenly arrested and stopped after a couple of revolutions, clear 'after-nystagmus movements' of the head were observed. After clockwise rotation, for example, the animal swayed its head clockwise and this movement was interrupted by two or three relatively slow anti-clockwise 'nystagmus' movements. Weak similar post-rotatory eye movements were also observed. After-reactions of this kind can of course not be due to optical stimulation; visual perception of the resting environment will rather tend to inhibit such post-rotatory reactions, as appeared already in earlier similar experiments with *Octopus*[2].

In addition to experiments with rotation around the animal's vertical axis, the reactions of *Sepia* on rotation around its horizontal axes were investigated. With the animal in its 'normal' position, any rotation around its horizontal axes would involve a change of position with respect to the direction of gravity, and thus additional stimulation of the gravity receptors would occur. In order to avoid this, the glass vessel with the fixed cuttle-fish was tilted by 90° and placed again on the turn-table. In this way the animal was laid on its side, one eye looking upward, the other eye looking downward. Turn-table rotation now made the animal rotate around its transverse axis, whereas no change of position with respect to gravity occurred. Notwithstanding the fact that the animals were kept in a forced, 'abnormal' position during the experiments, they showed typical after-nystagmus movements of the eyes with the well-known slow and quick phases in opposite directions, and similar movements of the whole head. Likewise, on rotation of an animal around its long axis (head pointing upward, 'tail' downward), clear post-rotatory head movements were observed.

Experiments of this kind were performed with three intact specimens of *Sepia officinalis*, measuring 12 to 15 cm in length. It was intended originally to blind the animals previously by cutting their optic tracts in order to eliminate visual orientation. However, after section of one optic tract in a cuttle-fish (anaesthetized in 1.5% urethane in sea water) the animal recovered only temporarily; it swam backwards, circling towards the operated side (asymmetrical locomotion after section of one optic tract is also known from *Octopus*[2]), and died soon after the operation. Since even intact cuttle-fish used to die within a week after arrival at the station, the intention to work with blinded or otherwise operated animals was abandoned. However, the occurrence of post-rotatory reflexes even in cuttle-fish with normal eye-sight stresses all the more the strength of these rotatory stimuli, occurring on turning around all three main body axes. On the basis of what has been found in *Octopus*, there seems little doubt that the stimuli involved arise within the statocysts with their highly developed cristae[3].

[1] J. Z. Young, Proc. R. Soc. B *152*, 3 (1960).
[2] S. Dijkgraaf, Pubbl. Staz. Zool. Napoli *32*, 64 (1961).
[3] The work was supported by grants from the Centre National de la Recherche Scientifique (Paris) and the Netherlands Organisation for the Advancement of Pure Research (Z.W.O.). I am indebted to Dr. P. Bougis and his staff of the Station zoologique at Villefranche for putting animals and apparatus at my disposal.