of normal mice seem to produce some factor(s) which compensate for the deficiency of the adrenal cortex of the nude mice. The reticular zone of their glands, which is enlarged probably in the sense of a compensatory hypertrophy or hyperplasia, regresses to a normal width. As reported in a previous paper[9], implantation of the neonatal thymus into newborn nude mice prevents such alterations of the adrenal cortex. Thus the neonatal thymus in mice seems to control the differentiation of a 'thymus-dependent zone of the adrenal cortex', but once the alteration of the cortex is established, implantation of the adult thymus into adult animals cannot induce a reversal of the alteration. In this case, only the same factors which are present in the normal adrenals seem to be able to reverse the enlargement of the reticular zone of the immature adrenals of the nude mouse. As discussed above, the adrenal cortex of the mouse in the postnatal time can be compared to that of the human foetus where the reticular zone is extremely developed and disappears suddenly at birth. In our case, it appears that the thymus in the mouse produces factors in ontogeny which promote maturation of the adrenal glands in coincidence with maturation of the immune capacity. It is therefore probable that some adrenal factors, produced in that section of the adult adrenal cortex which was thymus-dependent for its maturation, are promoting the immunological maturation of incompetent thymus-derived or bone marrow-derived lymphocytes.

Endocrinological and immunological implications. The thymus is considered as the organ where formation of the lymphocytes which are responsible for cell-mediated immunity occurs. This type of immune response is particularly deficient in the congenitally athymic mice. As cell-mediated immunity in mice at birth is much less developed than in humans and the absence of the thymus in athymic nude mice seems to be responsible for the extreme enlargement of the reticular zone of the adrenal cortex, a correlation between the thymus, the foetal or immature adrenal cortex and the development of the immune capacity in ontogeny is likely to exist. In agreement with this, we have found that the adrenal alterations in the athymic mice can be prevented by neonatal implantation of the thymus[9]. This suggests that the thymus in ontogeny is regulating the development of some zones of the adrenal gland. These zones might produce factors, some of which first may prevent and later on promote, the differentiation of lymphocytes to immunocompetent cells. These factors are probably produced by the reticular or foetal zone of the immature adrenal glands and by the mature adrenal glands of adult animals (Figure).

The function of the thymus in ontogeny might be that of promoting the differentiation of the adrenal gland from the foetal to the adult structure and function. This change of the thymus-dependent adrenal function in neonatal and postnatal life does probably depend on a change of factors released by the adrenal glands. The fact that young nude mice are unable to reject skin grafts in spite that the number of their peripheral lymphocytes can still be up to 50–60%, seems to indicate that, not the number, but the function and the capacity of the lymphocytes is important for the animal to mount a cell-mediated immune reaction. The absence of the thymus in the nude mice prevents the maturational changes in the adrenal cortex to occur and their cell-mediated immune responsiveness remains as deficient as in newborn mice. Therefore, absence of the thymus may prolong the foetal state of immaturity of cellular immunity because the differentiative action of the neonatal thymus on the adrenal gland cannot be exerted and the adrenal cortex cannot produce the factor(s) which are necessary to promote the transformation of the lymphocyte from an unreactive to a fully immunoreactive cell. The above data to suggest that, not only the absence of the thymus in nude mice, but possibly the lack of differentiation of the thymus-dependent zone of the adrenal cortex is responsible for their deficiency in cellular immunity.

Another argument is that the reticular zone of the adrenal cortex which is so developed in the human foetus and in the nude mouse is possibly secreting a factor which inhibits immune functions of lymphocytes and prevents, e.g. during foetal development in humans, an immune reaction of the foetus against the mother which would seem possible on the basis of the development of the thymo-lymphatic tissue in the human embryo. At birth, the dramatic disappearance of the foetal zone or reticular zone would eliminate this inhibiting factor being produced and the human newborn would become fully immunocompetent. From this point of view, the rat foetus seems to be more similar to the human than to the mouse foetus[13,14]. Some experimental work supports this suggestion[15].

The hormones of the adult adrenal gland that promote differentiation of the immune lymphocytes are probably some of the known hormones of the adrenal cortex. On the contrary, the identification of the factors which might be present in the foetal zone of the adrenal cortex in humans is necessary. Also more study of the factors in the thymus promoting the differentiation of the adrenal gland from the foetal to the adult structure and function is indicated. Thus the possibility exists to identify presumably hormonal factors which are both crucial for stimulating or preventing immune responsiveness.

Zusammenfassung. Es wird vorgeschlagen, dass der neonatale Thymus in Mäusen die Differenzierung einer foetalen Thymus-abhängigen Zone der Nebennierenrinde zur erwachsenen Struktur und Funktion kontrolliert. Die mögliche Bedeutung dieser Befunde für die hormonelle Kontrolle des Immunsystems in der Ontogenese wird diskutiert.

W. PIERPAOLI and E. SORKIN

Schweizerisches Forschungsinstitut,
Medizinische Abteilung,
CH–7270 Davos-Platz (Switzerland),
24 February 1972.

[15] H. O. BESEDOVSKY, Experientia 27, 697 (1971).

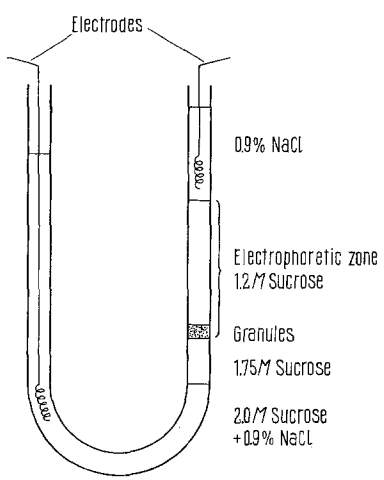
Free Flow Electrophoresis of Isolated Secretory Granules from Bovine Neurohypophyses

Recently some evidence has been presented that the secretion of the hormones vasopressin and oxytocin from the mammalian neural lobe of the hypophysis occurs by exocytosis, that is a fusion of the membrane surrounding the neurosecterory granules with the plasma membrane of the nerve terminals, followed by a perforation of the membranes, allowing the contents of the neurosecretory granule to leave the cell (for a review see DOUGLAS et al.[1]).

In the resting state of the cell the plasma membrane is electrically polarized, being negative on the inside. The secretion process is initiated by a 'depolarisation' of the plasma membrane of the nerve terminals. Also some calcium dependent steps seem to be involved in the secretion mechanism[2,3]. Since exocytosis would hardly be expected to occur, if the electrostatic forces between the membranes involved were repulsive, it was found of interest to study whether isolated neurosecretory granules carried any electrical charge and if so, whether it was positive or negative.

Methods. Purified neurosecretory granules were isolated by density gradient ultracentrifugation according to the method of DEAN and HOPE[4]. The isolated granules were recovered in 1.4 *M* sucrose. After equilibration to a temperature of 20°C this suspension was layered in a U-shaped glass tube on top of a solution of 1.75 *M* sucrose (see Figure). On top of the granular suspension was layered a solution of 1.2 *M* sucrose (the electrophoretic zone). Electrodes were placed in solutions of 0.9% NaCl in one end of the tube (upper) and in 2.0 *M* sucrose with 0.9% NaCl in the other end (lower). The granular suspension and its mobility in the electrophoretic zone could be visualized through a stereomicroscope. The U-shaped tube was submerged in a water bath (20°C). Electric fields from 40 to 100 V/cm were applied.

Results and discussion. On applying a voltage across the two electrodes the granular suspension would start to migrate towards the positive electrode. If the electrical polarity of the electrodes was changed the direction of the movement would also change. If the electrode in the upper end of the tube was positive the suspension would pass through the electrophoretic zone and gradually be concentrated at the upper end of that zone (at the border of the 0.9% NaCl solution). If the other (lower) electrode subsequently was made positive the granules would start to move downwards again. Introducing 2 m*M* $CaCl_2$ or 4 m*M* NaCl in 1.2 *M* sucrose in the electrophoretic zone reduced the mobility of the granular suspension (measured as the time required for the granules to reach the upper end of the electrophoretic zone). This effect might be due to a decrease in the electric field, caused by the increased conductivity of the zone. In 3 out of 6 experiments $CaCl_2$ was more potent than NaCl in reducing the rate of migration. Since there was no difference in conductivity of the two solutions, the additional effect of $CaCl_2$ might be due to neutralization by that ion of fixed negative charges on the secretory granules. Similar results have been obtained with chromaffin granules of the adrenal medulla[5].



Apparatus for free flow electrophoresis of isolated secretory granules from bovine neurohypophyses.

If the granules carry a net negative charge in the cytosol of the nerve terminals they would be repelled from the negative inside of the plasma membrane during non-secretory states of the cell. On secretion, however, the potential of the plasma membrane is abolished or even reversed, which might make it possible for the granules to approach and fuse with the plasma membrane. Calcium entering the cell on depolarization of the plasma membrane[6] may play a role in modifiyng the surface charge of the secretory granules.

Zusammenfassung. Sekretorische Granula aus der Neurohypophyse von Rindern wurden durch Ultrazentrifugierung isoliert. Frei-Fluss Elektrophorese dieser Partikel zeigte, dass sie eine negative Ladung besitzen. Ca^{++} und Na^{+} verminderten die Mobilität der Granula im elektrischen Feld.

H. VILHARDT[7] and T. JØRGENSEN

Institute of Medical Physiology C,
University of Copenhagen,
2200 Copenhagen N (Denmark), 11 January 1972.

[1] W. W. DOUGLAS, J. NAGASAWA and R. SCHULZ, Mem. Soc. Endocr. *19*, 353 (1971).
[2] W. W. DOUGLAS and A. M. POISNER, J. Physiol., Lond. *172*, 1 (1964).
[3] N. A. THORN, Acta endocr., Copenh. *50*, 357 (1965).
[4] C. R. DEAN and D. B. HOPE, Biochem. J. *104*, 1082 (1967).
[5] P. BANKS, Biochem. J. *101*, 18c (1966).
[6] W. W. DOUGLAS and A. M. POISNER, J. Physiol., Lond. *172*, 19 (1964).
[7] Present address: University Department of Pharmacology, Oxford OX1 3QT (England).

Spontaneous Mutants of *Staphylococcus aureus* PS 80

Staphylococcus aureus strain PS 80 serves as the propagation organism for the staphylococcal bacteriophage 80. It was obtained from the National Reference Laboratory for Phage Typing, Prague, Czechoslovakia.

Pigment mutants in stored culture. The strain was stored stabbed in nutrient agar slants overlaid with paraffin oil at room temperature in the dark for 1 year.

A suspension was prepared directly from the agar scrapings, diluted and plated on cream agar plates (WILLIS, O'CONNOR and SMITH[1]). After incubation, colonies of different size and colour developed. The majority consisted of the original wild type – i.e. orange coloured colonies, about 2 mm in diameter. The rest of the colonies occurring in a frequency 10^{-1}–10^{-2} were chromogenic mutants of different colony size. They were selected, purified and tested along with normally appearing colonies for different markers (Table I). The tests were performed as described elsewhere (SCHINDLER, MAREŠOVÁ