Maintenance of Normal Morphology in Adenohypophysial Transplants By Topical Administration of Hypothalamic Extracts with a Simple Method for Transplantation in a Pneumoderma Pouch.* (31373)

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Transplantation of the pituitary in a site remote from the sella turcica is followed by a progressive loss of the normal staining characteristics of the pituitary(1-13). Normal adenohypophysial morphology can be restored upon replacing the pituitary transplant in contact with the primary plexus of the hypothalamo-hypophysial portal vessels (8,11,14) or, according to Nikitovitch-Winer (15,16), upon chronic perfusion of hypothalamic extracts by an ingenious technique involving permanent aortic catheterization in rats bearing a pituitary transplant under the kidney capsule. This note describes an unusually simple technique by which one or several pituitary glands can be transplanted in a "pneumoderma pouch"(17) under the dorsal skin of the adult rat. The chronic administration of hypothalamic extracts directly into the pneumoderma pouch maintains the staining characteristics of the PAS positive cells in these transplanted adenohypophyses; no efforts were made in this series of experiments to study the functions of the pituitary transplants. Data obtained in earlier studies not reported here showed that systemic injections of the hypothalamic material were not effective in contradistinction to results obtained by topical administration in the pouch, at the doses tested. This does not preclude that systemic injections of still larger doses may not eventually affect the peripheral transplant; the possibility of topical administration as in the technique described here is certainly one of its virtues as problems of toxicity are encountered when very large quantities of crude tissue extracts are injected systematically. Activity of the hypothalamic substances in topical application may be related also to their reported lability in the peripheral circulation.

Material and methods. 1. Rats. 33 male or female rats (Cheek-Jones Farms, Houston, Texas) 150 to 220 g B.W. were used. They were fed a normal diet of Purina lab chow and tap water. *2. Pneumoderma pouch technique.* This is similar to Selye's granuloma pouch used for assessment of anti-inflammatory compounds(17). In contradistinction to this technique, it was proposed, however, that an irritating agent be chosen for production of the thin layer of granuloma tissue which is to line the inside of the pneumoderma pouch, that would disappear from the pouch after creating the initial granulomatous reaction so as not to interfere with the survival of the transplant. Hypertonic saline (5%) and dilute dextran (1:35) solutions were chosen. The technique used is as follows: under ether anesthesia, the dorsal skin is shaved. A 25-gauge hypodermic needle is inserted under the skin in the loose connective tissue and a mixture of oxygen (95%) and CO_2 (5%) is injected in less than one second with the high pressure from a tank of compressed gas in order to form a localized pouch of approximately 3 $\times$ 4 cm in diameter. Three to 4 ml of the mild irritant (saline or dextran) are injected into the pouch through a 27-gauge needle and the anesthetized animal is gently rotated so that the whole inside of the pouch comes in contact with the irritant. During the next 3 to 7 days the air-pouch is reinjected once or twice through a syringe with air or 95% O_2-5% CO_2 and 3 to 4 ml of physiologic saline. *3. Pituitary transplantation.* Whole or halved adenohypophyses from donor rats, collected rapidly in Krebs solution or in Difco medium TC 199, are aspirated into the bore of a #17 spinal puncture needle and introduced rapidly into the pouch, together with 3 to 4 ml of Krebs solution or Difco medium TC 199. The transplantation is done usually 7 to 10 days after creation of the pouch. Every 2 to 3 days, 3 to 4 ml of saline or Difco medium TC 199 followed by 2 to 3 ml of

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air or 95% O_2-5% CO_2 are injected into the pouch containing the pituitaries using a 10 ml syringe through a 27-gauge needle. In these experiments one to 4 adenohypophyses have been transplanted. *4. Hypophysectomy* was performed in 12 animals by the classical parapharyngeal approach one week prior to making the pneumoderma pouch, one week after, or at the same time. *5. Histological studies.* 7, 13, and 21 days after transplantation the histological appearance of the adenohypophysial grafts was studied after H-E and PAS-Orange G staining of serial sections cut at 4 μ. *6. Preparation of the hypothalamic extract.* 1600 frozen hypothalamic fragments of sheep origin were extracted with 4 N acetic acid (500 ml/200 g of fresh tissue). The lyophilized material was dissolved in saline, spun at 12,000 rpm to remove insoluble material and finally the pH of the supernatant was adjusted to 7.2. One ml of the final solution contained approximately the material extracted from 4 hypothalamus. *7. Experimental protocol to assess the effect of ovine hypothalamic extracts directly on grafted adenohypophyses.* Two groups of 4 rats were prepared with a pneumoderma pouch. Three adenohypophyses taken from male rats (120 g B.W.) were implanted as above in each pneumoderma pouch. Two days later, and for the next 11 days, 5 ml of saline (control group) and 5 ml of hypothalamic extract (experimental group) were injected twice daily directly into the pouch.

Results. 1. Macroscopic appearance of transplanted pituitary, effects of hypophysectomy. The grafts were easily located after 7, 13 and 21 days in all the 33 animals studied. Under the stereoscopic microscope the grafts were seen surrounded by a highly vascularized area with many small vessels penetrating the transplant (Fig. 1). In several instances, the transplants were observed to beat, *i.e.*, to rhythmically increase and decrease volume synchronously with the arterial pulse seen in the neighboring vessels. Hypophysectomy performed before, jointly with or after the implantation of pituitary tissue into the pneumoderma pouch did not appear to interfere with its survival or macroscopic appearance, particularly regarding vascularization. A transient loss of body weight averaging 10 to 20 g was consistently observed as a result of the pneumoderma-pouch in the intact and hypophysectomized rats.

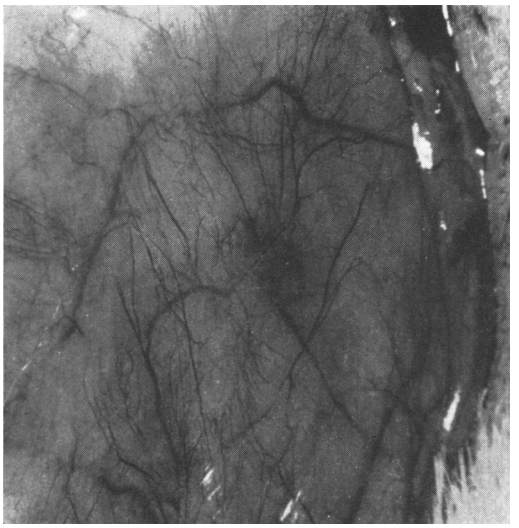

FIG. 1. Macroscopic appearance of one adenohypophysis 21 days after transplantation in a pneumoderma pouch.

2. Microscopic examination of the transplanted pituitary. a) 7 days after implantation the graft is usually found to be encircled by a thin layer of connective tissue. The outer layer of the pituitary tissue is composed of eosinophils, chromophobes and a few reduced size PAS positive cells, whereas the central region of the graft exhibits an infarcted zone (see also Fig. 2). The sinusoids are filled with red blood cells; b) 13 days after implantation some PAS positive cells reduced in volume are seen occasionally; their shape is neither that of classical thyrotrophs or gonadotrophs; c) 21 days after implantation the graft is encapsulated by a thick layer of connective tissue which tends to invade the pituitary tissue. The cells of the graft are mostly chromophobic and agranular with the exception of the eosinophils staining with Orange G which are observed in the peripheral areas. In some sections are seen some rare cells of small size with their cytoplasm staining a pink hue with the PAS method, with no visible granulations. Hypophysectomy performed before, jointly, or after the implantation of the pituitary does not change

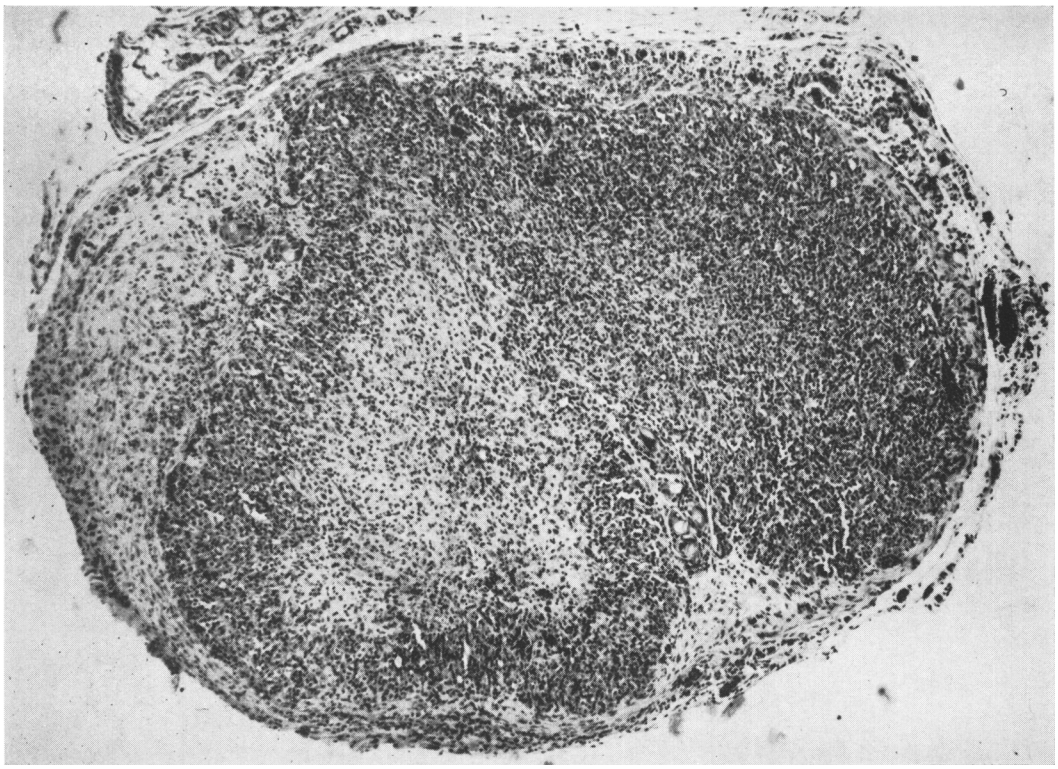

FIG. 2. Typical aspect of the graft after 13 days (×45). Note central necrosis and outer layer of pituitary cells.

the histologic picture of the graft, although the eosinophils appear to be more numerous.

3. Effect of local injections of ovine hypothalamic extracts into the pneumoderma pouch. a) The grafts in the control group (13 days) present the same histological picture as described above (see 2b, 2c, Fig. 3); b) local administration of hypothalamic extracts results in maintenance of numerous PAS positive cells located in the outer layer of the graft. The two classical types of basophils are observed: large round cells with vacuoles and coarse granules well stained with PAS and irregular-shaped cells, variable in size, with fine PAS positive granules and sometimes a few dark-red stained particles. Orangeophil cells darkly stained are also observed throughout the transplant.

Discussion. Many methods are available for transplantation of the pituitary in the rat. The adenohypophysis has been grafted under the kidney capsule(1,5,6,10,11,15,16,18), in the anterior chamber of the eye(2,4,9,13,19, 20,21), in the abdominal cavity(7), in the brain(8), near the thyroid(3) and in muscles (7,12). The pneumoderma pouch technique, unusually simple to perform, allows the transplant of one or many pituitaries into one pouch. This technique should be applicable to the transplantation of other organs in view of the ease with which the vascularized granuloma layer seems to favor implantation.

Maintenance of the normal histology in the adenohypophysial transplants following local administration of the hypothalamic extracts is well in keeping with the conclusions of others on this subject using more involved techniques(8,15,16). These results are part of the now well established evidence that adenohypophysial functions are controlled by substances of hypothalamic origin, the so-called hypothalamic releasing factors or hypophysiotropic hormones. When large quantities of pure specific hypothalamic-releasing factors become available, such as CRF, TRF, LRF, a simple technique as the one described

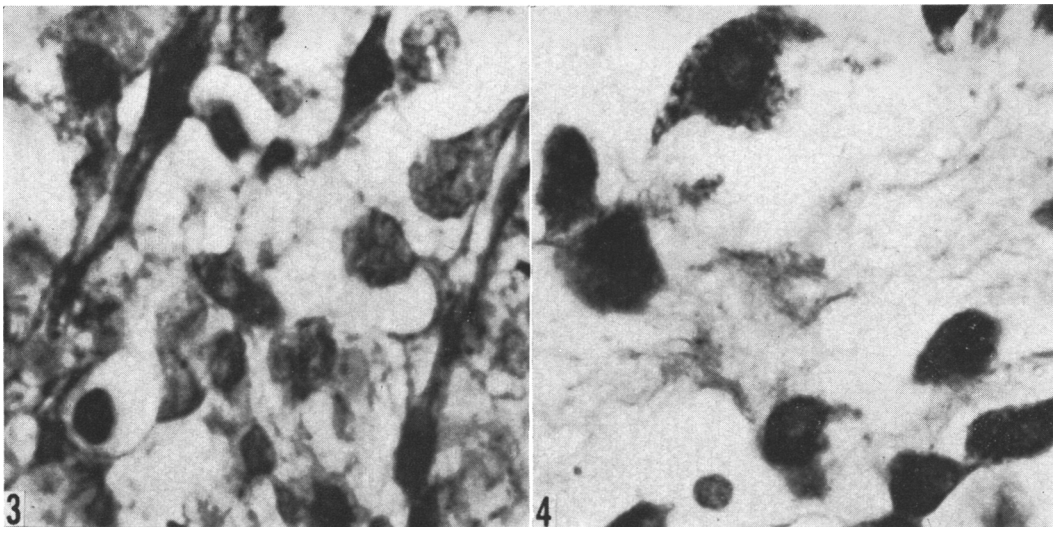

FIG. 3. 13 days after transplantation; eosinophils, chromophobes and some picnotic nuclei (×875). PAS-Hematoxylin stain.

FIG. 4. 13 days after transplantation, 11 days of topical administration of hypothalamic extracts. Note coarse granules in the large PAS positive cells. A noticeable degree of intercellular edema was noted in all transplants receiving hypothalamic extracts (×875). PAS-Hematoxylin stain.

here could be used to investigate the role of specific hypophysiotropic hormones on the maintenance of the differentiation and morphological characteristics of the pituitary cells transplanted peripherally, or on the reestablishment of such characteristics in already dedifferentiated cells. Local administration of a total extract of hypothalamic tissue, as here, maintains a normal morphology with persistence of all cell types. It would be of considerable interest to see whether the local administration of, for instance TRF, would produce a tissue entirely composed of thyrotrophs to the exclusion of all other cell types, or whether some inherent genotypic characteristics of only the original thyrotrophs would allow their maintenance by TRF, other cell types dedifferentiating as in the control transplants.

Summary. A simple technique is described for transplanting one or several pituitaries in a pneumoderma pouch insufflated under the dorsal skin of a rat. Topical administration of hypothalamic extracts into this pneumoderma pouch maintained the normal staining characteristics of all adenohypophysial cell types, whereas the saline injected control tissues exhibited the classical changes of dedifferentiation. The pneumoderma pouch technique could be used for transplantation of tissues other than pituitary; it allows application of substances directly in contact with the transplant by injecting into the pouch.

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1. Ahren, K., Acta Endocr., (Kbh), 1961, v38, 449.
2. Buxton, C. L., Anat. Rec., 1936, v64, 277.
3. Courrier, R., Colonge, A., C. R. Acad. Sci., 1957, v245, 388.
4. Cutuly, E., Anat. Rec., 1941, v80, 83.
5. Desclin, L., Grégoire, C., C. R. Soc. Biol., 1936, v121, 1366.
6. ———, ibid., 1957, v151, 1774.
7. Emery, F. E., Anat. Rec., 1936, v66, 253.
8. Harris, G. W., Jacobsohn, D., Proc. Roy. Soc. London B, 1952, v139, 263.
9. May, R., C. R. Soc. Biol., 1935, v120, 867.
10. Nikitovitch-Winer, M., Everett, J., Endocrinology, 1958, v63, 916.
11. ———, ibid., 1959, v65, 357.
12. Phelps, D., Ellison, E. T., Burch, J. C., ibid., 1939, v25, 227.
13. Richter, C. P., Eckert, J. F., ibid., 1937, v21, 481.
14. Greer, M. A., Matsuda, K., Stott, A. K., ibid., 1966, v78, 389.
15. Nikitovitch-Winer, M., Evans, J. S., Proc. II Int. Cong. Endocr., London, 1964, 1278.

16. Evans J. S., Nikitovitch-Winer, M., Fed. Proc., 1965, v24, 190.
17. Selye, H., Proc. Soc. Exp. Biol. and Med., 1953, v82, 328.
18. Critchlow, V., Lipscomb, H., Guillemin, R., J. Endocrinol., 1963, v25, 465.
19. Fortier, C., Selye, H., Am. J. Physiol., 1949, v159, 433.
20. Cheng, C. P., Sayers, G., Goodman, L. S., Swingard, C. H., ibid., 1949, v159, 426.
21. Martini, L., De Poli, A., Pecile, A., Saito, S., Tani, F., J. Endocrinol., 1959, v19, 164.

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Demonstration of Tissue Receptors for Viruses. (31374)

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Myxovirus particles attach to mammalian or avian cells which possess receptors suitable for their attachment. The suitability of receptors for this attachment is dependent on the species origin of the cells and on the virus strain examined. Many previous studies of virus-cell attachment have used red blood cells (RBC) since their agglutinability by virus suspensions may conveniently serve as evidence for the presence of receptor sites.

For certain purposes, however, it would be helpful if a simple and convenient method were available for demonstration of viral receptors on cells of parenchymatous organs. Since growth of cells in *in vitro* cultures may be associated with the structural alteration of their surfaces, such a method should be applicable to native tissues.

The present paper describes a method for testing cryostat sections of animal tissues for myxovirus receptors, based on a principle analogous to that of mixed agglutination(1). The latter principle has previously been applied to the demonstration of antigens on tissue sections, using immune sera and suitable RBC(2). Under these circumstances, RBC adhere to tissue with similar antigenic sites due to bridging by specific antibody. In a similar manner, the present test employs the adherence of RBC to tissues in the presence of virus suspensions as an indication of the presence of receptors common to both the tissues and RBC used.

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Materials and methods. Virus suspensions of the Victoria strain of Newcastle disease virus (NDV) and the PR-8 strain of influenza virus consisted of allantoic fluids harvested from infected chicken embryos. These materials were heated at 56°C for 30 minutes and centrifuged at low speed to remove debris. Measles virus was purchased from Microbiological Associates, Inc. as "Measles Hemagglutinating Antigen" and parainfluenza Type 1 was obtained from Flow Laboratories, Inc. as "Parainfluenza 1, CF Antigen."

Tissue sections were prepared from livers of apparently healthy individuals of various species. Following sectioning in a cryostat, the sections, approximately 10 μ thick, were fixed to coverslips by air drying and stored at −5°C until used.

RBC were obtained from blood drawn into ACD solution from a group A human donor and from healthy rhesus monkeys. Prior to use, the RBC were washed with physiologic saline and finally suspended in phosphate buffered saline, pH 7.4.

Hemadsorption tests were performed using a modification of a slide chamber method previously described(3). The wells of microculture slides were filled with the RBC suspension. After applying the coverslip with its attached tissue, the slides were placed in the inverted position at room temperature for 20 minutes. They were then reverted, coverslip up, and observed microscopically after 10 minutes (Fig. 1). Since slight hemadsorption of certain RBC to some tissues (*e.g.*,