pig sera prepared by immunizing animals with monkey kidney grown virus, gave strong monkey kidney tissue reactions. However, fluorocarbon treatment removed the nonspecific tissue reaction and "purified" antigens gave specific reactions in CF tests with ECHO antisera prepared in guinea pigs and in monkeys.

The valuable assistance of Hernon Fox in preparing the antigens is gratefully acknowledged.

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Functional Adrenocortical Homotransplants in the Golden Hamster.* (23797)

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No previous reports of transplantation of adrenal cortex in the hamster have been found in the literature. In this laboratory we have had no success in autologous transplantation of the adrenal cortex in adult hamsters, although such procedures are routine in the rat(1). However, we have found that fetal hamster adrenal cortex transplanted homologously to the cheek pouch of the adult hamster will grow, differentiate and function. This paper describes the technic and presents evidence for the first known cases of successful adrenocortical transplantation in the hamster.

Materials and methods. Two female golden hamsters (Mesocricetus auratus) of 14 days pregnancy were obtained from the Boston University Mammalian Genetics and Breeding Laboratory, following their observed matings, one with a sire of albino phenotype, the other with a sire of golden phenotype. The golden hamsters used as hosts were procured from a local independent hamstery and were about 3 months old, weighing from 60 to 80 g. The pregnant female was anesthetized with Nembutal, and the gravid uterus removed *in toto* to Ringer's solution. One by one, each fetus was excised from uterus and membranes, and the adrenal glands (where recoverable) were dissected out and placed on sterile gauze moistened with Ringer's solution. While the dissections were being performed by one person, the other operator prepared the cheek pouches of the anesthetized hosts and did the transplantations according to the technic for tumors described by Lutz *et al.*(2). No more than 5 minutes elapsed between excision of a fetal adrenal gland and its transplantation into a cheek pouch. Instruments were sterilized by boiling. Host cheek pouches were swabbed with a dilute concentration of Zephiran chloride (Winthrop) in Ringer's solution prior to transplanting the fetal organs. Eight fetal adrenal glands were transplanted homologously to cheek pouches of 6 intact host hamsters (Table I). On the 22nd day after transplantation the hosts were bilaterally adrenalectomized by way of 2 small dorsolateral incisions, in a single operation under light Nembutal anesthesia. Over a period of 15 to 28 days (Table I) after adrenalectomy these animals received 6 subcutaneous doses of cortisone (Cortone acetate, Sharp &

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TABLE I. Homotransplantation of Fetal Adrenal Glands to Adult Hamster Cheek Pouches; Summary of Procedure, Survival of Hosts, and Growth of Transplants.

Host hamster No.	Sex	No. fetal adrenals transpl.	Days after transplantation: Host adrenalectomy	Cortisone, 1.5-2.5 mg per dose	Saline water	Transplant excised from living host	Death of host	Vol of transplant at excision from host, in mm³
1	♂	1	22	22-50 (6 doses)	22-50	154	159	8.0
2	♂	1	"	22-37 (6 doses)	22-39	67	73	10.0
3*	♂	2	"	"	"		53	.35
4*	♂	1	"	"	"		52	.4
5	♀	2	"	"	22-50	78	82	7.0
6	♂	1	"	"	"	78	83	6.5

* Died at 14 and 13 days after cessation of post-operative therapy.

Dohme, 25 mg/cc saline suspension) totaling about 14 mg, and saline drinking water (0.5% sodium chloride, 0.5% sodium citrate in tap water). Following cessation of all post-operative therapy, each animal was allowed to live for a further period of time equalling at least 4 times the expected survival time (7 days maximum) of adrenalectomized and untreated hamsters without transplants(3): *i.e.*, for at least 28 days (barring death of the animal before that interval elapsed). The transplant was then excised from the cheek pouch (excision of transplants from the pouch without harm to the animal is done routinely with tumors in this laboratory), and the animal was returned to its cage to determine the length of survival without the adrenal transplant. At death the hamster was examined for other possible causes of death (*e.g.* acute infection, diarrhea) and to ascertain the completeness of the adrenalectomy. During the course of the experiment observations and measurements of the transplants were made *in vivo* in anesthetized, everted pouches at magnifications up to 45X using transillumination. All hamsters were maintained in individual cages with Purina Laboratory Chow and drinking water (either saline or ordinary tap water) *ad libitum*. For histological examination all transplants and control adult normal adrenal glands were fixed in Helly's fluid, embedded in paraffin, sectioned at 6 μ and stained with Harris' hematoxylin and eosin Y. Specimens of fetal adrenal glands taken at time of transplantation were stained similarly but were fixed in Bouin's fluid and sectioned at 8 μ.

Results. Table I summarizes the procedure, growth of transplant, and survival time of each of the 6 animals. Body weights dropped 6 to 16% during the first 14 days after adrenalectomy. An apparent return to good health in 4 of the 6 animals was indicated by a slow gain in weight in Hamster 1, normal gains in Hamsters 2, 5 and 6, and normal activity and alertness of these animals. Regular observations of the transplants revealed vascularization by the host within 4 to 6 days after transplantation and a slow increase in size of the transplants over the approximate volume of 0.12 mm³ as measured at time of excision from the fetuses. (The mean volume of 4 adult male hamster adrenals was 15 mm³, and that of 10 female adrenals was 9 mm³.) Body weights of Hamsters 1, 2, 5 and 6 dropped 11 to 23% during the 4 to 6 days between excision of transplant and death. In Hamsters 3 and 4, marked losses in weight beyond levels reached 14 days after adrenalectomy started on the day when therapy was withdrawn. Volumes of these transplants, fixed at time of death, were much smaller than those of the transplants in surviving animals.

Post-mortem examination of each animal revealed in no instance any gross evidence of acute infection which might have caused death, and verified the completeness of adrenalectomy in all cases.

Additional evidence for the adrenocortical nature of the transplants was provided by histological examination of the tissues.

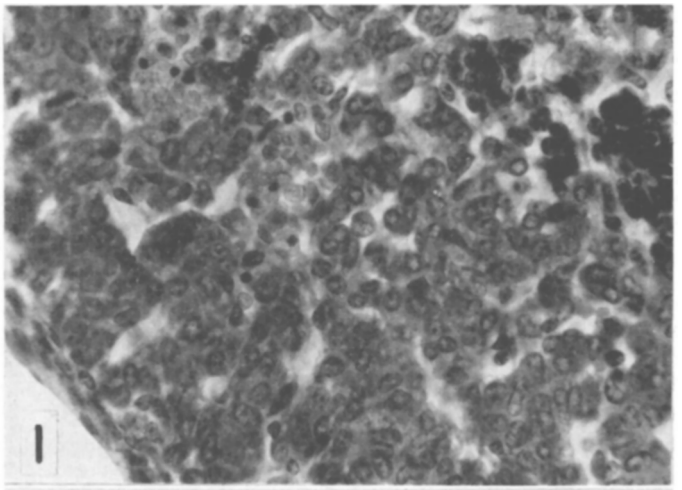

FIG. 1. 14-day fetal hamster adrenal × 430. Part of capsule at lower left. Note lack of cellular differentiation.

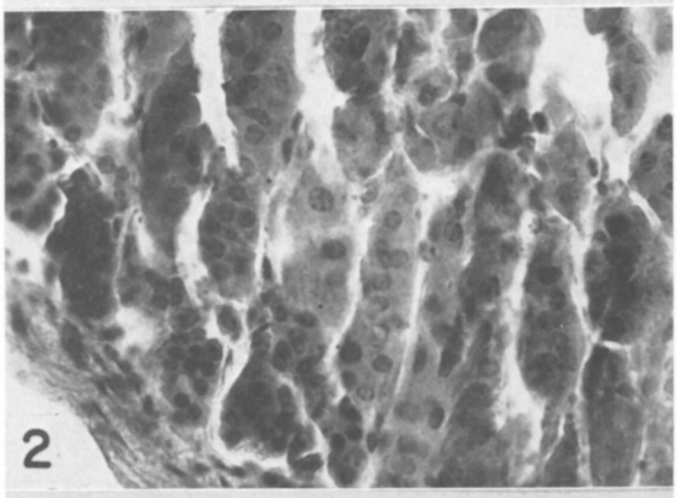

FIG. 2. Fetal adrenal homotransplant after 78 days in cheek pouch of Hamster #6 × 430. Part of capsule at lower left. Note zonation and cord-like arrangement of differentiated cortical cells.

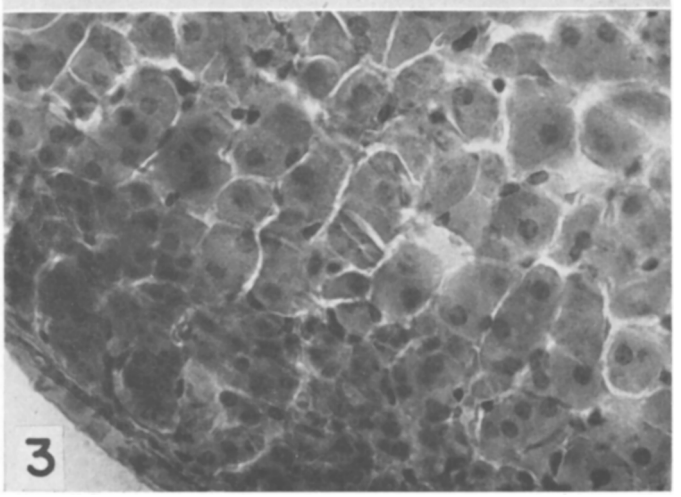

FIG. 3. Zona glomerulosa and part of zona fasciculata of adrenal cortex of adult female golden hamster × 430. Compare with Fig. 2.

Healthy cortical cells provided the main bulk of all transplants, including those of Hamsters 3 and 4. The cortical cells were arranged in small clusters or in curved cords of varying lengths. Two cell types were seen, one type having rather eosinophobic cytoplasm and believed to be cells of the zona glomerulosa, the other type being larger, having smoothly eosinophilic cytoplasm and believed to be cells of the zona fasciculata. In 3 transplants these cell types were segregated into zones and were arranged in fairly long cords (Fig. 2), somewhat similar to the arrangement in a typical adult adrenal (Fig. 3), with the glomerulosa cells lying in a narrow, compact layer directly under a definite capsule. (The adrenal cortex of the adult hamster has been well described in several recent papers including that of Meyers and Charipper(4)). No zona reticularis or medullary tissue was found, however, in the transplants. In the other 3 transplants the 2 cell types were arranged randomly and irregularly, and a discrete capsule was lacking. Small areas of dead or dying cortical cells and white blood cells were occasionally seen. A minor invasion by fibroblasts and connective tissue fibers from surrounding cheek pouch tissue or from the capsule occurred in most of the transplants. All transplants were well vascularized. No cellular connection was seen microscopically between the 2 original transplants in Hamster 5; the separate glands were equal in size and differentiation, and were bound together closely by a connective tissue sheath. The relation of the 2 glands in the transplant of Hamster 3 could not be determined, owing to the very small volume.

To explain the deaths of Hamsters 3 and 4 soon after cessation of postoperative therapy despite the presence of healthy adrenocortical cells in their transplants, we propose that the volume of functioning tissue was too small to maintain life.

All transplants showed marked cellular differentiation as well as increase in size when compared to the 14-day fetal adrenals fixed on the day of transplantation. The 14-day fetal adrenal (Fig. 1) is a very small, spherical mass of closely-packed, highly undifferentiated cells, recognizable as adrenal only by its unmistakable position in relation to the kidney. Cells of the transplants, although not always organized in radially-arranged rows or in segregated zones, were similar in appearance to those of an adult adrenal cortex.

Discussion. In a previous study in this laboratory, homotransplantation of fetal adrenals to 4 intact adult hamsters, with no subsequent adrenalectomy, resulted unexpectedly (in view of Halsted's principle) in clear histological evidence of growth and differentiation of transplants after 14 to 28 days in the cheek pouch, but provided no physiological evidence of their function; this result suggested the present study wherein adrenalectomy was delayed until 3 weeks after transplantation. Previously, homotransplantation of fetal adrenals to 2 adult hamsters on the day following adrenalectomy failed, although in the rat Martinovitch(5) has successfully homotransplanted infantile adrenal cortex to adrenalectomized hosts. Autotransplantation of half-glands at the time of complete adrenalectomy failed previously in 3 adult hamsters, the animals dying within a month despite post-operative therapy, the transplants showing mostly dead cortical cells. Auto-transplantation of partial glands at the time of incomplete (unintentional) adrenalectomy again resulted in complete failure of transplants to regenerate in 3 hamsters, although host survival was increased.

Successful autologous and homologous transplantation of adult adrenal cortex in the hamster may depend upon further studies of delayed or partial adrenalectomy and upon revision of the type and schedule of post-operative therapy.

Fetal hearts have also been successfully homotransplanted to the hamster cheek pouch (6), visible evidence for functional survival of cardiac tissue being regular contractions observed in the transplants up to 10 months after transplantation.

Summary. 1. Adrenal glands from golden hamster fetuses, transplanted homologously into cheek pouches of adult hamsters which were adrenalectomized 22 days after transplantation, maintained the untreated hosts for at least 4 times the expected survival time of adrenalectomized and untreated hamsters

without transplants. 2. When the adrenal transplants were excised from surviving hosts, after 67 to 154 days in the cheek pouch, the hosts died within 6 days. 3. Microscopic appearance of healthy cortical cells with some indication of adult arrangement in zones and cords, supported the physiological evidence for growth, differentiation and function of the adrenal transplants. This is the first known instance of successful adrenocortical transplantation in the hamster.

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Human Parotid Gland Secretion: Flow Rate and Interrelationships of pH and Inorganic Components.*† (23798)

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Limited studies by Hildes and Ferguson (1) and Thaysen, Thorn, and Swartz(2) have indicated that pH, sodium, bicarbonate, and chloride levels of parotid saliva are directly related to rate of secretion, in contrast to potassium and phosphorus concentrations which were independent of flow-rate. The secretory rate is influenced by numerous local and systemic factors which include: method of stimulation, diet, sleep, dehydration, infectious diseases, emotional factors, psychological abnormalities, nervous disorders, and presence of various drugs. Thus any attempt to comprehend metabolic processes and secretion-mechanism of the salivary glands demands that the effect of all possible affecting variables be predetermined and controlled.

In a previous study(3), analysis of parotid saliva from 50 individuals permitted a preliminary establishment of normal mean and dispersion values for sodium, potassium, calcium, bicarbonate, chloride, phosphorus, and pH. The present publication reports the effect of various stimuli on secretory rate of the parotid gland and the interrelationships between rate of secretion and salivary composition.

Materials and methods. Parotid saliva was collected using a vacuum cup(4). Graduated collection tubes were immersed in iced water and mean flow-rate (ml/min) calculated by recording the time required for secretion of a fixed volume. The saliva was analyzed for sodium, potassium, calcium, chloride, and phosphorus. Samples for determination of pH and bicarbonate were collected under paraffin oil. Paraffin (masticatory), lemon flavor‡ (gustatory), and flavored chicle§ (masticatory and gustatory) were used to study effect of different stimulatory agents. *Analytical procedures*: Sodium and potassium values were determined with an internal standard (Li) flame photometer; calcium, by the Clark-Collip(5) modification of the Kramer-Tisdall principle; chloride, using Benotti's(6) modification of the Van Slyke prin-

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‡ Lemon Life Savers were used without any sucking or masticatory action.

§ Dentyne Gum.