ON THE TRANSPLANTATION OF THE GUINEA PIG SUPRARENAL AND THE FUNCTIONING OF THE GRAFTS.

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PLATE 19.

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In 1906 Elliott and Tuckett¹ reported their inability to transplant the guinea pig suprarenal homoplastically into the subcutaneous tissues of the abdominal wall. Within a few hours after grafting a half gland beneath the abdominal skin of a guinea pig the surrounding tissues swelled and appeared irritated. Next day the swelling increased, the skin over the transplant turned green, and a foul smelling, clear fluid transuded from between the skin sutures. The escape of the fluid relieved the irritation, the skin over the swollen area sloughed away, and the animal recovered. If the fluid did not escape, the irritation extended rapidly over the surface of the body, the animal’s temperature fell, and it became comatose and died in 30 hours. The only organ showing noteworthy pathology was the suprarenal, the cortex of which was congested, hemorrhagic, and without the brown granules, while the medulla showed the absence of chrome staining.

To our knowledge the successful transplantation of the guinea pig suprarenal has not been reported, due, we felt, to the failure to remove the medullary tissue from the grafts. Taking this precaution we sought to obtain positive and functioning autoplastic transplants in this animal. All previous experience has shown that medullary tissue never regenerates, so that its removal is of no consequence in a study of suprarenal transplants.

Methods.

Most of the guinea pigs used were born in the laboratory, and were between 2 and 3 months old at the beginning of the experiment, except for a few aged 5 or 6 months. Under ether anesthesia first the right suprarenal was completely removed through the dorsal route, and was kept in normal saline at 38°C. until the wound was closed. It was placed on a cork and cut longitudinally with a sharp razor blade into 4 or 8 segments. The medulla together with some of the adjacent cortex was removed from each segment with fine curved dissecting scissors, to insure extirpation of the epinephrine-containing tissues. Each segment of cortex was divided transversely, and the small pieces were washed for 15 minutes in the physiological saline. Meanwhile many pockets were prepared in the abdominal wall by puncturing the muscle with a cataract knife, and enlarging the openings by spreading an ordinary forceps in them. One fragment of suprarenal cortex was introduced into each pocket, the mouth of which was closed with a black silk marking suture. The skin was sewed with silk and covered with celluloid. 3 to 7 weeks later the left suprarenal was removed. All tissues were fixed in Zenker-formol, embedded in paraffin, serially sectioned, and stained with hematoxylin and eosin.

Data.

In 22 guinea pigs the right suprarenal was removed and auto-transplanted into the abdominal muscle. 3 to 7 weeks later the left suprarenal was removed from 17 of these. Some animals died from operative shock, others from suprarenal insufficiency, and still others were killed in good condition to terminate the experiment. There were no toxic deaths attributable to the transplants, although irritation and swelling of the abdominal muscle frequently occurred. 84 per cent of our animals had 1 or more takes varying from nests of a few cells to masses several mm. in diameter when examined after 3 weeks. Although 8 to 16 transplants were inserted in each animal, at autopsy usually only 3 or 4 were found. In the guinea pig the percentage of transplants that take compared with the number of transplants inserted is quite small because the severity of the reaction following the transplantation influences the course of the regeneration.

In spite of the gaps in our series, the successive histological changes in guinea pig suprarenal transplants can be followed. Stages of degeneration and regeneration, followed either by the absorption of the regenerated transplant or its marked growth, have been observed.

After 3 days transplants show marked necrosis, only a few supra-
renal cells remaining at the periphery. These surviving cells show cloudy protoplasm with indistinct cell walls. The necrotic area is edematous and is infiltrated by some polymorphonuclear leucocytes and round cells. At the periphery of the transplant new blood vessels have appeared. About the transplant the tissue has become edematous, some polymorphonuclear leucocytes and round cells have infiltrated, and sprouting fibroblasts appear.

By 11 days the transplant area is very cellular with little debris remaining. Great numbers of polyhedral cells are present having large vesicular nuclei, prominent nucleoli, and hazy, granular, pinkish staining cytoplasm. These cells sometimes occur in small groups appearing like multinuclear giant cells. Connective tissue cells are seen between the regenerating suprarenal cells, and new blood vessels penetrate the transplant. Some of the regenerating suprarenal cells are dividing mitotically. The muscle about the transplant shows an intense interstitial inflammation, and muscle giant cells and pigmented cells are seen.

By the 22nd day the transplants diminish in size and appear macroscopically as tiny yellow-white specks which fuse in color with the fascia of the abdominal muscle. Microscopically groups of newly regenerated suprarenal cortical cells in glomerular formation are seen. In addition considerable connective tissue has formed between these cells. Large numbers of lymphocytes appear in some transplants at this stage, probably in those destined for rapid absorption.

During the 4th and 5th weeks regeneration is active, and the suprarenal cells increase by hypertrophy and hyperplasia, and glomerular and fascicular formations appear. The transplant areas still show considerable interstitial connective tissue. At about this time transplants show tendencies either to absorption or rapid growth. Absorption progresses slowly, taking many weeks, and is characterized by shrinking of the suprarenal cells, progressive lymphocytic infiltration and interstitial fibrosis, and the appearance of many pigmented phagocytes. During the course of this slow absorption many of the animals die of suprarenal insufficiency, evidently due to the inability of the transplants to maintain life.

On the other hand the transplants which show active growth enlarge by the multiplication of suprarenal cells mostly through amitotic
division while the interstitial connective tissue diminishes. Dark, actively growing suprarenal cells are mingled with lighter larger cells, and as regeneration proceeds the cells become larger and more fatty. After 100 days some transplants show the glomerular, fascicular, and reticular layers while the connective tissue and lymphocytes have disappeared. With further growth the transplants sometimes appear as masses of very large closely packed polyhedral cells with markedly vacuolized protoplasm. Some of these cells are completely replaced by fat. Between 100 and 200 days transplants may reach the size of 3 or 4 mm. in diameter. The larger transplants may show adenomatous nodules at the periphery in which the typical cortical arrangement of the cells is seen. The oldest transplant we studied was 276 days, and it showed no signs of degeneration or exhaustion atrophy.

We studied homotransplants in 5 animals and found that during the first 2 weeks the changes are the same as in autoplastic transplants. During the 3rd week an occasional clump of regenerated suprarenal cells is seen, but foreign body reactions with giant cells and lymphocytes are already in evidence. By the 7th week most of the homotransplants are completely absorbed.

**Functioning of the Transplants.**

From these experiments we obtained evidence that autotransplastic transplants are capable of maintaining the life of guinea pigs in the absence of both main glands and suprarenal accessories. If the transplants are small or are undergoing absorption, they maintain the animal in a poor state, which may continue for weeks until the guinea pig dies of suprarenal insufficiency. In those instances where the transplants reach full development, guinea pigs are maintained normally for a sufficiently long time to warrant the assumption that they can live their normal life period. We have observed 1 transplanted guinea pig surviving suprarenalectomy in good condition for 9 months.

Of 17 doubly suprarenalectomized and transplanted animals 5 died from suprarenal insufficiency within 5 days after removal of the second gland. 1 death occurred on the 1st day, 1 on the 2nd, 2 on the 4th, and 1 on the 5th. The autopsies showed complete removal of both
main glands, and no macroscopic accessories. Transplants had been inserted from 24 to 59 days previously, and had been absorbed in all but 1 animal which showed some clumps of suprarenal cells in each of several transplants. They were apparently insufficient to maintain life. 1 guinea pig died from a transverse myelitis due to osteomyelitis of the thoracic spine on the 7th day after removal of the second gland; another died of a general infection following an abortion on the 15th day; a 3rd died from pneumonia on the 24th day. These 3 had been transplanted 29 to 59 days previously, and each had active regenerating transplants.

One guinea pig was sacrificed in good condition 13 days after removal of the left gland and 57 days after insertion of the transplants. The autopsy showed complete bilateral ablation of the suprarenals, no accessories, and numerous pin-head-sized positive transplant areas which when sectioned showed groups of vascularized cortical cells.

Following the removal of the second gland, 4 guinea pigs died of chronic suprarenal insufficiency, 1 each on the 41st, 46th, 52nd, and 57th day. The transplants had been inserted 85 to 104 days previously, and on histological examination were degenerated and fibrotic. No accessories were found in these animals.

One animal killed 220 days after the removal of the second gland showed a large cortical accessory. No transplants were present. The remaining 3 animals were killed, 2 on the 124th, and 1 on the 221st day after the removal of the second gland. Each had several large vascular transplants which were apparently physiologically active, maintaining the animals in good condition. 1 of these animals had a 3 mm. accessory.

In summary we can present 11 guinea pigs upon which we base our conclusions regarding the functioning of the transplants. These animals after suprarenalectomy and transplantation either died of suprarenal insufficiency or were killed to terminate the experiment months after transplantation. At autopsy no accessory cortical tissue was found in any of these animals. Of the 11 animals, 6 lived longer than 40 days after the removal of the second gland. These figures stand in marked contrast to the average survival time given in the literature for double suprarenalectomized guinea pigs, and which we ourselves have found, that is, 3 or 4 days. They indicate that
transplants are capable of prolonging the life of suprarenalectomized guinea pigs or of maintaining them indefinitely.

DISCUSSION.

Irritation phenomena occurred in our experiments even when suprarenal cortex alone was transplanted, but were different in their appearance, severity, and consequence from those described by Elliott and Tuckett who transplanted both cortex and medulla. The cortical fragments were definitely palpable as firm plaques for about 3 days after their insertion due to the absorption of water by the lipoid-rich tissue. By the 4th or 5th day serosanguineous fluid had collected underneath the abdominal wound stitches of nearly all the animals. Sometimes 10 cc. of a bloody fluid could be expressed. Frequently on the 6th or 7th day the wound opened spontaneously, the skin edges sloughed out, and the transplants were destroyed. The edema and inflammatory reactions around the remaining transplants were followed by connective tissue growth, which impaired the vitality of the transplant during its organization through pressure and interference with the circulation. The result is frequently absorption of the transplant, but even if the transplant is not absorbed its growth is usually interfered with.

The reactions that occur with suprarenal transplantation in the guinea pig have not been conclusively explained. Elliott and Tuckett believed that the edema and solution of the guinea pig tissues were caused by some substance in the medulla, not epinephrine, and not present in all mammalian suprarenales. They reported that the irritant substance was diffusible and destroyed by heating to 65°C. They excluded epinephrine as the toxic agent because the subcutaneous injection of 1 mg. in 1 cc. of salt solution caused a skin slough, but never the inflammatory reaction with edema and corrosion of the tissues such as is produced by the medulla. Even in lethal doses epinephrine did not cause an inflammatory reaction. It was further shown that this reaction did not follow the grafting of the whole gland on the peritoneum, or between the muscle and peritoneum. Neither do irritation phenomena follow the transplantation of the guinea pig suprarenal beneath the skin of the rabbit, rat, or cat. It
occurs only when the whole suprarenal is transplanted, auto-, homo-
or heteroplastically beneath the skin of a guinea pig. The appearance
of these changes is peculiar to the suprarenal, for transplantation of
the kidneys, spleen, thyroid, thymus, bone, and cartilage into the
subcutaneous tissues of the guinea pig is not followed by edema or
corrosion. Nor does transplantation either auto- or homoplastic of
the suprarenal of the dog, cat, rabbit, or rat produce this phenomenon.

We believe that the milder reactions we observed following the
transplantation of suprarenal cortex and the acute reactions described
by Elliott and Tuckett are identical and are induced by the same
irritating substance which however is present in greater concentration
in the medulla. We believe that this substance has entered the cortex
from the medulla through manipulation of the gland during its
removal. We cannot agree with Elliott and Tuckett that the sub-
stance is neither epinephrine nor related to it, for the injection of
epinephrine causes sloughing of the tissues. The difference between
the reaction which follows the introduction of epinephrine alone, and
that following the grafting of the entire suprarenal may be one of
degree, due to the poulticing effect exercised by the necrotic trans-
plant. Marine and Sandberg in some unpublished work concluded
that epinephrine was the substance responsible for the phenomenon
reported by Elliott and Tuckett. Some peculiarity of the guinea
pig's subcutaneous tissues and abdominal muscles may in part be
accountable for this idiosyncracy.

In our experiments the right gland was first removed and trans-
planted, and some weeks later the left gland was removed. This is
the satisfactory way to study transplantation in an animal so sus-
ceptible to double suprarenalectomy. We cannot say whether a
greater number of transplants would have taken and grown had a
suprarenal insufficiency been induced by the removal of more than
one gland at the initial operation, but it seems that the take of the
suprarenal transplant is not necessarily dependent upon an insuf-
fiency. 3 weeks elapse before the transplants regenerate, and at
about this time the second gland is removed in most cases. This
must be a stimulus for the growth of the transplants. Some respond
and grow, while others degenerate even when so stimulated.
CONCLUSIONS.

1. The suprarenal cortex of the guinea pig can be transplanted autoplastically into the abdominal wall and may remain for months, growing to fairly large size.

2. The suprarenal cortex of the guinea pig can be transplanted homoplastically but these transplants usually degenerate after a few months.

3. Small autoplastic transplants are capable of maintaining the life of a completely suprarenalectomized guinea pig for weeks and large transplants maintain the animal indefinitely in good condition.

4. Irritation phenomena follow the grafting of suprarenal cortex due to the entrance of epinephrine into this tissue during the manipulation involved in removal of the gland.

EXPLANATION OF PLATE 19.

Fig. 1. Low power of a 59 day autotransplant. Regeneration is progressing actively though there is considerable scarring in the center of the graft.

Fig. 2. High power of Fig. 1 showing cortical cells in glomerular formation.

Fig. 3. Large completely regenerated suprarenal transplant 142 days after insertion.

Fig. 4. High power of Fig. 3 showing closely packed, highly vacuolated cortical cells.
(Jaffe: Transplantation of guinea pig suprarenal.)