THE EFFECT OF CORTISONE AND ACTH ON ADRENAL TRANSPLANTS IN THE RAT

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SUMMARY

The effect of cortisone and ACTH on auto- and homotransplants of adrenal tissue has been studied in adrenalectomized rats. If the two hormones are administered simultaneously in appropriate dosage the regeneration of autotransplants is not inhibited, but neither ACTH alone nor cortisone and ACTH combined, in various dosages, have any decisive beneficial effect on the survival of homotransplants.

A positive Thorn test is of value as an index of the presence of a healthy transplant in the strain of rats employed, but a negative result does not necessarily exclude this.

The significance of these findings is discussed.

Homotransplants, with a few exceptions, do not survive permanently, and there is strong evidence that they are destroyed as the result of an actively acquired immunity in the host [Medawar & Gibson, 1943; Medawar, 1944, 1945, 1946; Woodruff & Woodruff, 1950]. Studies of the effect on homotransplants of procedures which are known to delay or inhibit the development of immunity to antigens of various kinds might therefore throw some light on the nature of the resistance to homologous tissue; they might also lead to results of clinical importance since there is some reason to suppose that permanent survival might be obtained if the development of immunity could be delayed beyond a certain critical period [Woodruff, 1952].

During the last 3 years the effect of one such procedure—administration of cortisone or ACTH—on homotransplants of skin has been investigated both clinically and in experimental animals. The results are, however, difficult to interpret because they differ in different species and sometimes with different observers using the same species. In man the early reports were very optimistic, and it was suggested that permanent survival might be achieved by administration of ACTH [Whitelaw, 1951]. This has not been confirmed, however, and in several recent papers it has been reported that the period of survival is unaffected by either ACTH or cortisone [Ellison, Martin, Williams, Clatworthy, Hamwi & Zollinger, 1951; Weisman, Quinby, Wight & Cannon, 1951; Baxter, 1951]. Weisman et al. have also reported negative results with skin homotransplants in guinea-pigs and young hogs. In rabbits, on the other hand, Medawar and his colleagues have shown that the period of survival may be at least doubled by administering cortisone systemically or by applying it locally to the transplants [Billingham, Krohn & Medawar, 1951a, b].

Cortisone and ACTH have also been used in an attempt to prolong the survival of homotransplants of whole kidneys in dogs [Persky & Jacob, 1951], but without success.

The effect of cortisone on transplants of endocrine tissues does not appear to have
been investigated; it seemed, however, to provide a useful field for research because, in addition to being of interest from the point of view of possible clinical application, endocrine transplants offer several important advantages to the experimenter, viz. (1) they may be made in a variety of sites, and no special provision is needed for maintaining them in place or for drainage of an external secretion; (2) the characteristic epithelium can be easily recognized and distinguished from host tissue; (3) specific hormonal stimuli can often be applied; (4) tests of functional activity can be employed.

A disadvantage is that the behaviour of an endocrine transplant may be influenced by the extent to which the host is deficient in the endocrine in question; this, however, can be overcome by creating a total deficiency prior to transplantation in every case, or by administering appropriate hormones [Woodruff & Woodruff, 1950].

The experiments with adrenal transplants, which form the subject of this paper, were performed on rats, partly because adrenalectomy is an easy and well-tolerated operation in this species and partly because suitable animals were readily available. By using Wistar rats, of a strain known to possess little or no accessory cortical tissue, as recipients, and hooded rats as donors, it was hoped to avoid the risk of failure of a transplant due to the presence of functioning adrenal tissue in the host, and also the risk of obtaining successful homotransplants merely as the result of accidental genetic relationships between donors and hosts.

It seemed desirable to study the behaviour of transplants from both immature and adult donors. In each case the glands were transplanted whole because it was felt that, owing to the complex structure of the adrenal, more uniform results would be obtained with whole glands than with small fragments. Preliminary experiments confirmed the statement of Ingle & Higgins [1938] that whole glands could be successfully transplanted autologously to the ovary, and this site was therefore used for all transplants from adult donors. Transplants from day-old rats could not be readily attached to the recipient's ovary and were, instead, placed beneath the femoral vessels in the thigh. This site has also been tried for adult glands, but in our experience is not as satisfactory as the ovary.

It seemed likely that cortisone, whatever its effect on the development of immunity in the host, might prove deleterious to the transplant, since atrophy of the adrenals occurs in normal rats treated with cortisone for some weeks [Winter, Silver & Stoerk, 1950], probably owing to inhibition of the animal's own secretion of ACTH. To guard against errors due to this cause three precautions were taken: (1) Some animals received a large dose of cortisone, others a small dose. (2) All animals which received cortisone received also ACTH, beginning at the same time as the cortisone and continuing for 1 week longer. (3) Whenever possible, the behaviour of homotransplants was compared with that of autotransplants in animals receiving the same treatment.

**Experiment I. Transplantation of adrenals from day-old rats to the thighs of adrenalectomized adult recipients**

Sixteen female Wistar rats (each weighing about 170 g) were randomized into four groups of four. Each animal was adrenalectomized under ether anaesthesia and then received a pair of homologous adrenals from a day-old black and white rat, the glands being placed whole in pockets prepared beneath the superficial femoral vessels.
about the middle of each thigh. The donors were killed immediately prior to removal of the transplants.

After operation all the recipients were kept at a temperature of 70° F, and received a liberal diet of rat cubes, fresh greens, and bread and milk, to which was added 5 mg calcium pantothenate per rat per day, as recommended by Ralli [1946].

Hormones were administered as follows:
Group 2. ACTH 7.5 mg (equivalent of Armour standard LA-1-A) twice daily.
Group 3. Cortisone 2 mg (Cortisone acetate, Merck and Co. Inc.) daily and ACTH 7.5 mg twice daily.
Group 4. Cortisone 10 mg daily and ACTH 7.5 mg twice daily.

Both hormones were given by subcutaneous injection. ACTH was given for 5 weeks starting on the day of operation, cortisone for 29 days* starting the day before operation. The animals of groups 1 and 2 were given 1% NaCl instead of water to drink; those of groups 3 and 4 were not given saline in view of the danger of salt retention.

Two animals, one in group 1 and the other in group 4, died within 2 weeks after operation and were replaced. After 4 weeks a biopsy was performed and the right transplant, if it could be found, was removed and serially sectioned. If the right transplant could not be identified the animal was killed and a search was made for the left transplant. After 3 months there were three survivors, two in group 1 and one in group 4. To investigate the presence of functioning adrenocortical tissue eosinophil counts were made before, and 4 hr. after, a test injection of 10 mg ACTH, as recommended by Thorn, Forsham, Prunty & Hills [1948]. The animals were then killed and the remaining transplants removed for histological examination.

Results

Transplants removed after 28 days showed either a small area of hydropic cortical epithelial cells or no recognizable adrenal tissue. The host reaction was predominantly fibroblastic, mononuclear infiltration being very slight and polymorphs almost entirely absent.

The periods of survival of the animals of the various groups were as follows:
Group 1. 3 months (killed), 12 days (replaced), 3 months (killed), 38 days, 28 days (killed).
Group 2. 73 days, 49 days, 76 days, 28 days (killed).
Group 3. 48 days, 41 days, 39 days, 45 days.
Group 4. 3 months (killed), 28 days (killed), 10 days (replaced), 29 days, 35 days.

When death occurred within a week or two after the 28-day biopsy, the histological picture was as already described; in the remaining animals there was no trace of the second transplant post mortem. No fall in circulating eosinophils occurred in any of the three Thorn tests performed in animals surviving for 3 months.

It is thus apparent that neither ACTH alone, nor cortisone and ACTH together, facilitated transplant survival or demonstrably modified the host reaction.

* Not all the animals survived long enough to receive the full course.
Experiment II. Transplantation of adrenals from adult rats to the ovaries of adult recipients

Autotransplants. Adult female Wistar rats (weighing about 180 g) were adrenalectomized and received their own adrenals back as autotransplants, one whole gland being sutured to each ovary with a single fine linen stitch. The ovaries were approached by slightly extending the dorsal incisions used for the adrenalectomies.

Homotransplants. Adult female Wistar rats were used as recipients. Each received a pair of homologous adrenals from an adult female black and white hooded rat (weighing about 200 g), the glands being transplanted whole to the ovaries by the technique used for autotransplants. All the recipients except those in group 8 (see below) were adrenalectomized.

All the operations were performed under ether anaesthesia.

Initially, twenty recipients were used, subdivided into four groups of five. Two animals in each group received autotransplants, three received homotransplants. The animals of group 1 received no hormones; those of group 2 received ACTH only, and those of groups 3 and 4 received both cortisone and ACTH. The dose schedules and general after-treatment were the same as in Expt. I. Subsequently, owing to the fact that homotransplants in two of the group-3 animals survived for the duration of the experiment, the number of animals in this group was increased, and the study of homotransplants was further extended by creating four additional groups. Group 5 animals received the same dose of cortisone as those of group 3, but a single daily injection of 'Acthar gel' was given instead of two daily injections of ordinary ACTH. In groups 6 and 7 the dose of cortisone was reduced in successive weeks while the dose of ACTH (group 6) or gel (group 7) remained constant; this scheme was tried because it seemed likely that if a transplant became established it would itself begin to secrete cortisone or closely related steroids. The animals of group 8 received ACTH only, but differed from those of group 2 in not being adrenalectomized.

As in Expt. I cortisone was given for 28 days, starting the day before operation, ACTH (or gel) for 35 days from the time of operation. After 4 weeks a biopsy was performed on all surviving animals. This involved laparotomy with removal of the left ovary and attached transplant, and was less well tolerated than the simpler procedure required in Expt. I. After fixation in formol acetate the stitch uniting transplant and ovary was removed, and serial sections were then cut and stained with haematoxylin and eosin. At the end of 3 months, Thorn tests were performed on all the animals which still survived. These animals were then killed and the remaining transplants removed for histological examination.

Results

The results are summarized in Table 1.

The histological findings in animals surviving 28 days or longer conformed to one of the following four types:

Type A. There was complete regeneration of cortical tissue with total disappearance of the medulla. The transplant consisted of healthy vascular cortical tissue enclosed in a thin capsule. Sometimes two or three distinct zones could be seen; more often the structure of the whole transplant resembled that of the fascicular zone of a normal
gland. There was no accumulation of inflammatory cells and no increase in interstitial connective tissue.

Table 1. Results of experiment II

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Type of transplant</th>
<th>No. of animal (days)</th>
<th>Period of survival (days)</th>
<th>Histological findings</th>
<th>At end of experiment 28 days</th>
<th>Thorn test (on animals surviving 90 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Controls; no hormones</td>
<td>Auto</td>
<td>1 90*</td>
<td>A</td>
<td>A</td>
<td>55% fall</td>
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<td></td>
<td></td>
<td>Homo</td>
<td>2 90*</td>
<td>A</td>
<td>A</td>
<td>26% fall</td>
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</tr>
<tr>
<td>2</td>
<td>ACTH 7.5 mg twice daily</td>
<td>Auto</td>
<td>3 30</td>
<td>C-</td>
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<td>Homo</td>
<td>5 30</td>
<td>D</td>
<td>D</td>
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<tr>
<td>3</td>
<td>Cortisone 2 mg daily plus ACTH 7.5 mg twice daily</td>
<td>Auto</td>
<td>6 90*</td>
<td>A</td>
<td>B</td>
<td>42% fall</td>
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<td></td>
<td></td>
<td>Homo</td>
<td>7 90*</td>
<td>B</td>
<td>B</td>
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<td>Homo</td>
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<td>Homo</td>
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<td>D</td>
<td>D</td>
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<td>4</td>
<td>Cortisone 10 mg daily plus ACTH 7.5 mg twice daily</td>
<td>Auto</td>
<td>11 90*</td>
<td>B</td>
<td>B</td>
<td>79% fall</td>
<td></td>
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<td></td>
<td></td>
<td>Homo</td>
<td>12 90*</td>
<td>B</td>
<td>A</td>
<td>56% fall</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Homo</td>
<td>13 90*</td>
<td>B</td>
<td>B</td>
<td>No fall</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Homo</td>
<td>14 90*</td>
<td>D</td>
<td>D</td>
<td>27% fall</td>
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<td></td>
<td></td>
<td>Homo</td>
<td>15 90*</td>
<td>B</td>
<td>B</td>
<td>48% fall</td>
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<td></td>
<td></td>
<td>Homo</td>
<td>16 49</td>
<td>B</td>
<td>C</td>
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<td></td>
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<td>Homo</td>
<td>17 51</td>
<td>C</td>
<td>C</td>
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<td></td>
<td></td>
<td>Homo</td>
<td>18 48</td>
<td>C</td>
<td>C</td>
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<td>Homo</td>
<td>19 26</td>
<td>90*</td>
<td>C</td>
<td>C</td>
<td>No fall</td>
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<td>Homo</td>
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<td>C</td>
<td>D</td>
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<td>Homo</td>
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<td>C</td>
<td>C</td>
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<td></td>
<td>Homo</td>
<td>22 28</td>
<td>C</td>
<td>C</td>
<td>—</td>
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<tr>
<td>5</td>
<td>Cortisone 2 mg plus ACTH gel 15 mg daily</td>
<td>Homo</td>
<td>30 49</td>
<td>C-</td>
<td>D</td>
<td>—</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Homo</td>
<td>31 62</td>
<td>C-</td>
<td>D</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Homo</td>
<td>32 90*</td>
<td>C</td>
<td>D</td>
<td>No fall</td>
<td></td>
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<tr>
<td>6</td>
<td>Cortisone 3 mg daily for the 1st week</td>
<td>Homo</td>
<td>33 45</td>
<td>C</td>
<td>D</td>
<td>—</td>
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<td></td>
<td>2 mg daily for the 2nd week</td>
<td>Homo</td>
<td>34 44</td>
<td>C</td>
<td>D</td>
<td>—</td>
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<tr>
<td></td>
<td>1 mg daily for the 3rd week</td>
<td>Homo</td>
<td>35 45</td>
<td>C-</td>
<td>D</td>
<td>—</td>
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<tr>
<td></td>
<td>0.5 mg daily for the 4th week</td>
<td>Homo</td>
<td>36 45</td>
<td>C-</td>
<td>D</td>
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<tr>
<td></td>
<td>ACTH 7.5 mg twice daily</td>
<td>Homo</td>
<td>37 45</td>
<td>C-</td>
<td>D</td>
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<tr>
<td></td>
<td>Homo</td>
<td>38 45</td>
<td>C-</td>
<td>D</td>
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<tr>
<td>7</td>
<td>Cortisone as in group 6</td>
<td>Homo</td>
<td>39 28</td>
<td>D</td>
<td>D</td>
<td>—</td>
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<tr>
<td></td>
<td>ACTH gel 15 mg daily</td>
<td>Homo</td>
<td>40 90*</td>
<td>C-</td>
<td>C-</td>
<td>No fall</td>
<td></td>
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<tr>
<td></td>
<td>Homo</td>
<td>41 41</td>
<td>C</td>
<td>D</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>ACTH 5 mg three times a day</td>
<td>Homo</td>
<td>42 90*</td>
<td>C</td>
<td>D</td>
<td>Not tested</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Recipients not adrenalectomized</td>
<td>Homo</td>
<td>43 90*</td>
<td>C</td>
<td>D</td>
<td>Not tested</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Homo</td>
<td>44 90*</td>
<td>C</td>
<td>D</td>
<td>Not tested</td>
<td></td>
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</tr>
</tbody>
</table>

* Killed.

Type B. There was extensive regeneration of cortical tissue with disappearance of the medulla, but the sections differed from those of type A in one or more of the
following respects: (a) some epithelial cells showed a slight degree of hydropic degeneration; (b) the amount of interstitial connective tissue was slightly increased; (c) islets of fibrous tissue or necrotic material were present, usually in the centre of the transplant. Patchy calcification was often observed in necrotic areas.

*Type C.* The transplant contained some viable-looking cortical tissue, but the epithelial cells usually showed marked hydropic degeneration, and the amount of connective tissue was markedly increased. In some transplants there were areas containing numerous round cells or polymorphs.

The mark C− (read as ‘C minus’) in the table indicates that the transplant was of type C and that only a very small amount of viable cortical tissue was present.

*Type D.* The transplant had disappeared completely or contained no viable cortical tissue.

Photomicrographs of the various types of transplant are shown in Pl. 1.

Four animals died before 28 days had elapsed. In two the histological features were those of type C; in the other two no viable cortical tissue remained.

Two criteria of success were applied:

1. **Initial success.** The animal survived at least 28 days and the histological findings on biopsy after this period were those of type A or type B.

2. **Complete success.** The animal survived 3 months and the histological findings on biopsy after 28 days and again at the end of the experiment were those of type A or type B.

The large dose of cortisone used in group 4 was not well tolerated. All the animals in this group lost weight; one died during the course of treatment and three shortly after this had ended.

*Autotransplants.* In groups 1, 2 and 3 the autotransplants were all completely successful, and in four of the seven animals subjected to the Thorn test there was a fall of 40% or more in the circulating eosinophils.

*Homotransplants.* The homotransplants were completely successful in two out of the first twelve recipients. The animals bearing successful transplants were both in group 3. In one of them the Thorn test showed a 46% fall in eosinophils.

Subsequently, a further twenty-two animals received homotransplants; of these seven were in group 3 and three (in group 5) received similar treatment to the members of group 3, except that a single daily injection of ACTH gel was given in place of two daily injections of ordinary ACTH. Only one further success was obtained, and that according to the first criterion only. For all the animals which received 2 mg cortisone, we thus have finally three successes and ten failures by the first criterion; two successes and eleven failures by the second criterion.

**DISCUSSION**

The findings would seem to warrant the following conclusions:

1. **Autotransplants** of whole adrenals to the ovaries of adult Wistar rats after adrenalectomy are usually successful. The medulla and part of the cortex become necrotic, but cortical tissue regenerates extensively in a few weeks.

2. In Wistar rats of the strain used in these experiments a positive Thorn test is good evidence of a successful transplant, but a negative test does not exclude this.

3. Prolonged administration of cortisone in a dosage of 10 mg daily is not well
tolerated by rats, and exerts a deleterious effect on adrenal autotransplants. In smaller dosage cortisone is well tolerated, and administration of 2 mg cortisone daily together with 7.5 mg ACTH twice daily has at most a slight and temporary deleterious effect on adrenal autotransplants.

(4) Homotransplants of whole adrenals from both new-born and adult hooded rats to adrenalectomized adult Wistar rats are usually unsuccessful.

(5) Neither ACTH alone, nor cortisone and ACTH together in the dosages employed in the experiments, have a consistent and decisive beneficial effect on the survival of adrenal homotransplants.

The cause of the two successful homotransplants in the second experiment is uncertain. Administration of cortisone may have played some part, but can scarcely have been the sole cause because in all the other cortisone-treated animals the homotransplants failed; it seems necessary, therefore, to postulate some fortuitous innate compatibility between donor and host. This suggestion is not one to be made lightly because all the experimental and clinical work on homotransplants—and, in particular, the work of Ingle, Higgins & Nilson [1938] on adrenal transplants in rats—points to the conclusion that donor-host compatibility is, to say the least, rare, unless the donor and host happen to be identical twins or members of a closely inbred strain. In some species, however, homotransplants between animals which differ genetically occasionally survive unexpectedly. In the guinea-pig, for example, subcutaneous homotransplants of thyroid between animals drawn from a mixed population sometimes survive [Woodruff & Woodruff, 1950], and it appears unlikely that all the reports of successful homotransplants of skin between unrelated human beings are false [see Rogers, 1950; Woodruff & Allan, 1953]. It would seem unreasonable, therefore, to deny the possibility of inter-strain compatibility in rats, and the findings in the present study provide evidence that this does sometimes occur.

From a clinical point of view the conclusions reached are discouraging.

From the wider viewpoint of experimental biology they provide further evidence in support of the belief that the dramatic effect of cortisone on the behaviour of homotransplants in the rabbit is peculiar to this species.

REFERENCES

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Ralli, E. P. [1946]. Endocrinology, 39, 225.
DESCRIPTION OF PLATE

Photomicrographs of adrenal transplants (×100). After 4 weeks: figs. 1, 2, 3, 7 and 8. After 4 months: figs. 4–6.

Fig. 1. Autograft (type A), showing complete cortical regeneration with a wide fascicular zone. Rat no. 2 (Expt. II, group 1).

Fig. 2. Autograft (type B), showing partial regeneration of cortex. Rat no. 11 (Expt. II, Group 3).

Fig. 3. Homograft (type B), showing partial regeneration of cortex. Rat no. 13 (Expt. II, group 3).

Fig. 4. Autograft (type A), showing complete cortical regeneration with three clearly defined zones. Rat no. 1 (Expt. II, group 1).

Fig. 5. Autograft (type B). The whole graft consists of viable-looking cortical tissue, but the fascicular zone is not well developed and the cells stain unevenly. Rat no. 11 (Expt. II, group 3).

Fig. 6. Homograft (type B). Apart from a small area of fibrosis in the centre (not shown in the figure) the graft consists of viable-looking cortical tissue resembling the reticular zone of a normal gland. Rat no. 15 (Expt. II, group 3).

Fig. 7. Homograft (type C). There is a narrow outer zone containing fairly normal-looking cortical cells, a middle zone containing some hydropic cortical cells, and a large central area of necrosis. Rat no. 34 (Expt. II, group 6).

Fig. 8. Autograft (type C), showing an outer zone of connective tissue, a narrow middle zone containing a few hydropic cortical cells, and a large central area of necrosis. Rat no. 16 (Expt. II, group 4).