THE TRANSPLANTATION OF NORMAL TISSUES: WITH SPECIAL REFERENCE TO AUTO- AND HOMOTRANSPLANTS OF THYROID AND SPLEEN IN THE ANTERIOR CHAMBER OF THE EYE, AND SUBCUTANEOUSLY, IN GUINEA-PIGS

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The transplantation of tissues raises many yet unsolved problems of fundamental interest, and is of potentially great clinical importance, especially in endocrinology. The present investigation is concerned with three main problems: the immunological reactions associated with the growth of homografts in the anterior chamber of the eye and subcutaneously; the role of the reticulo-endothelial system in the resistance to homografts; and the applicability of Halsted's principle to auto- and homografts of endocrine tissue, and the mechanism involved.

Four groups of experiments are described. In group I, a preliminary study of auto- and homografts of thyroid in the guinea-pig, it is shown that, if a total thyroid deficiency is produced in the

host, autografts are uniformly successful both in the anterior chamber and subcutaneously, whereas homografts are usually successful in the anterior chamber but rarely so when made subcutaneously. Halsted's principle holds good for anterior chamber grafts whether auto- or homografts, i.e. the proportion of successful grafts increases *pari passu* with the degree of thyroid deficiency produced in the host, but appears to be less applicable to subcutaneous autografts. Some light is thrown on the mechanism of Halsted's principle by the observation that successful anterior chamber grafts can be obtained in non-thyroidectomized hosts if injections of pituitary thyrotrophic hormone are given.

The experiments of group II are concerned with the immunological phenomena relating to thyroid homografts. It is shown that a subcutaneous homograft of thyroid in the guinea-pig is demonstrably antigenic and the same is true of a homograft in the anterior chamber provided this is undergoing destruction. The state of immunity induced is general and extends to the anterior chamber. The existence of a state of immunity induced by a homograft is shown by the diminished chance of a subsequent homograft 'taking', but 'immunization' (by means of a subcutaneous graft) of an animal which already has a homograft established in the anterior chamber does not appear to modify the behaviour of the latter.

In group III it is shown that a homograft transferred after some months from the anterior chamber to a subcutaneous site is more likely to survive than a primary subcutaneous homograft. It thus appears that when a homograft is established in the anterior chamber for a sufficiently long time a process of adaptation occurs as between graft and host.

The experiments of group IV relate to the behaviour of auto- and homografts of spleen in the anterior chamber, and of simultaneous thyroid and splenic grafts. It is shown that the chances of success of a thyroid homograft in the anterior chamber are greatly reduced if the latter is provided with reticulo-endothelial tissue in the shape of a simultaneous autograft of spleen, but that a simultaneous homograft of spleen from the same donor has little or no deleterious effect. The mechanism of this phenomenon has not been fully elucidated but would appear to be either cellular, humoral or a combination of the two.

Introduction

The transplantation of tissues raises many yet unsolved problems of fundamental biological importance and is also of great clinical interest, particularly in the field of endocrinology.

The present investigation has been undertaken with the twofold object of further elucidating the fundamental laws governing the behaviour of tissue transplants and contributing to the ultimate solution of the clinical problem of obtaining functioning homotransplants of endocrine tissues for the treatment of such diseases as myxoedema, Addison's disease, and diabetes mellitus. It was, therefore, decided to work with transplants of an endocrine tissue despite the increased complexity which this would entail. As a result two groups of factors have had to be kept in mind, namely, immunological factors concerned with the behaviour of homotransplants in general and endocrinological factors concerned with the behaviour of transplants of endocrine tissue, whether auto-transplants or homo-transplants.

In planning new experiments it has been considered desirable to take account of the literature relating to the transplantation of embryonic and neoplastic as well as normal tissues. The relevant facts which have so far been established may be summarized as follows:

1. *Autotransplants*

Autotransplants (i.e. transplants to a new site in the same individual) of a wide variety of tissues commonly survive, and in many instances function.

Jaffe & Plavska (1926) obtained functioning subfascial autotransplants of suprarenal cortical tissue in rats and guinea-pigs, Loeb (1930) demonstrated that autotransplants of

thyroid and other tissues would survive in various situations, and Sochet (1929) found that autotransplants of guinea-pig endometrium made to the anterior chamber of the eye survived indefinitely and showed typical oestrus changes.

2. *Homotransplants*

Homotransplants (i.e. from one animal to another of the same species) do not ordinarily survive for long. Loeb (1930) and many others have observed that homotransplants of thyroid tissue are rapidly invaded by lymphocytes and destroyed, and more recently Medawar found that homotransplants of skin in both man (Medawar & Gibson 1943) and the rabbit (Medawar 1944, 1945) do not survive for more than 2 or 3 weeks.

3. *Conditions for survival of homotransplants*

There are a number of exceptions to the general rule that homotransplants are rapidly destroyed. Thus:

Transplantation between animals of a closely inbred strain is often successful. Ingle has reported survival of homotransplants of adrenal gland in an inbred strain of rats (Ingle, Higgins & Nilson 1938), and Loeb and his co-workers (Loeb, Burns, Suntzoff & Moskop 1937; Loeb & Kirtz 1939; Wolfe, Moskop, Kirtz & Loeb 1940) have obtained functioning subcutaneous homotransplants of anterior pituitary which persisted, however, only for 7 to 10 months, in inbred strains of mice. Furthermore, the homotransplantation of malignant tumours in pure-line strains of rats and mice is a familiar laboratory procedure (e.g. *vide* Furth, Boon & Kaliss 1944).

Certain types of tissue appear to be capable of surviving after homotransplantation for long periods of time. Homotransplants of cartilage in dogs (Young 1945) remained alive and unchanged for 18 months. Successful homotransplantation of human cornea—a well-established clinical procedure—has also been cited as an example, but probably depends on the site to which the transplants are made (*vide infra*) rather than on the nature of the tissue, since subcutaneous homotransplants of cornea in guinea-pigs evoke the same type of cellular reaction as homotransplants of other tissues, and are rapidly destroyed and replaced by fibrous tissue (Fleisher 1921).

Certain sites, notably the brain and the anterior chamber of the eye, appear to be peculiarly favourable for homotransplants, and also under certain circumstances for heterotransplants (i.e. transplants of tissue from an animal of different species to the recipient).

As regards the brain, Murphy & Sturm (1923) have reported successful intra-cerebral homo- and heterotransplantation of tumour tissue, and Willis (1935, 1936, 1939) successful homotransplantation of embryonic tissue.

As regards the anterior chamber, successful heterotransplantation of tumour and embryonic tissue, and successful homotransplantation of tumour tissue, embryonic tissue and certain normal adult tissues have been reported by various workers. The use of this site was first described by Van Dooremaal (1873), and shortly after this Zahn (1884) reported successful homo- and heterotransplantation of foetal and neoplastic tissues to the eyes of rabbits. In recent times, Greene and his associates (Greene 1938, 1941*a*, *b*, 1942*a*, 1943,

1946; Shrigley, Greene & Duran-Reynals 1945; Demins, Hovenanian & Greene 1947) have reported successful homo- and heterotransplantation of malignant tumour tissue and certain types of embryonic tissue. Despite numerous attempts, in no case were successful transplants of embryonic *endocrine* tissue obtained by Greene (1943).

The anterior chamber has also been used, though less extensively, as a site for homotransplants of normal tissues from both adult and immature animals. Markee (1932) has reported successful intra-ocular homotransplants of endometrium, and Turner (1938, 1939) functioning suprarenal gland, transplanted from immature rats into the eyes of adult hosts.

There is some evidence that tissue which has been cultivated for a time *in vitro* in a medium containing plasma from the prospective recipient may survive homotransplantation, either indefinitely or at any rate for a longer period than tissue not so treated. Stone, Owings & Gey (1934) have reported successful treatment of a patient with parathyroid tetany by means of homotransplants prepared in this way. More recently Lux, Higgins & Mann (1937*a*, *b*) have studied similar transplants of adrenal tissue in guinea-pigs, rabbits and rats, but the results obtained were too anomalous to allow them to draw definite conclusions.

Lastly, certain isolated instances of successful homotransplantation have been reported which do not at present appear to be explicable in terms of any general principle. For example, Broster & Gardiner Hill (1946) successfully treated a case of Addison's disease by transplanting to the sheath of the rectus abdominis muscle a whole suprarenal gland from a girl suffering from the adreno-genital syndrome. In this case a form of blood-vessel anastomosis was performed, but that alone does not explain the favourable result, since numerous laboratory attempts have been made to transplant whole organs by such means, and so far only autotransplants have proved successful (Markowitz 1937).

4. *Observations on multiple transplants*

Medawar (1944, 1945), working with skin homotransplants, found that small single transplants had a longer period of survival than large ones, but that if a set of transplants of differing sizes was made at one operation from a single donor to a single recipient the survival period was practically identical for all members of the set. Furthermore, 'second-set homografts' (i.e. transplants made to an animal which had received one or more transplants from the same donor on a previous occasion) survived for a much shorter period than the original transplants. Medawar (1946) also showed that the period of survival of skin homotransplants was reduced if the recipient received previous intradermal injections of a suspension of leucocytes from the prospective donor. He has therefore argued that the destruction of skin homografts is dependent on a systemic rather than on a local reaction, and that, in the rabbit at any rate, there is a close relationship between the antigens of the leucocytes and the skin.

Hesselberg & Loeb (1918) studied successive transplants of thyroid. Each recipient received a transplant from one donor followed later by a second transplant from a *different* donor. They found no significant difference between the reactions to the first and second grafts.

Investigations of successive tumour transplants, using the anterior chamber of the eye as the site for at least one of the transplants, have yielded very conflicting results. Saphir, Appel & Strauss (1941) transplanted Brown-Pearce carcinoma tissue to the skin in rabbits,

and found that after the transplant had retrogressed a further transplant to the anterior chamber would 'take' and grow, though its development was slower than normal.

Cheever & Morgan (1942), also working with the Brown-Pearce carcinoma, found that secondary intra-ocular transplantation was usually unsuccessful in animals 'immunized' by previous transplants to *other* sites, though usually successful following previous transplantation *to the opposite eye.* Greene (1942*b*) found that, following transplantation of a malignant rabbit tumour to the anterior chamber of an animal of the same strain, both the *testicle and the anterior chamber of the opposite eye* were resistant to re-inoculation.

There is thus disagreement, first as to whether transplants in the anterior chamber are effectively antigenic (i.e. give rise to a state of increased resistance to similar transplants made to other sites in the same animal), and secondly, whether the state of generally increased resistance induced by extra-ocular transplants extends to the anterior chamber.

5. *The role of the reticulo-endothelial system in the resistance to tissue transplants*

Murphy (1912, 1913, 1914*a*) has shown that while malignant tumours from animals of various species can normally be readily transplanted to the developing chick embryo, the embryo becomes completely resistant if a piece of adult chicken spleen or bone marrow is transplanted at the same time as the tumour. Similarly (Murphy & Sturm 1923) transplants of a number of mouse tumours grow actively when inoculated to the brain of rats, guinea-pigs and pigeons (whereas subcutaneous or intramuscular transplants to the same animals fail), but if the transplant comes in contact with a ventricle, or if a small piece of autologous—but not homologous—splenic tissue is transplanted with the tumour the transplant is rapidly destroyed. Conversely (Murphy 1914*b*), the resistance of an animal (the rat) to transplants of tumours from another species (Erlich mouse sarcoma) can be diminished by depressing the host's lymphoid tissue by subcutaneous injections of benzol or by exposure of the animal to X-rays.

The precise significance of these observations is difficult to assess. They all relate to heterotransplants of tumour tissue, and it is apparent that the reticulo-endothelial system plays a major part in the resistance to such transplants. The mechanism by which this resistance is effected, however, whether cellular or humoral or a combination of both, remains uncertain.

6. *Halsted's principle*

Halsted (1909) found that autotransplantation of parathyroid tissue in dogs was successful only if a deficiency 'greater than one-half' had previously been created in the recipient, and put forward the general hypothesis that a necessary condition for the success of any transplant of endocrine tissue is the prior existence in the recipient of a deficiency of the corresponding tissue.

Many experiments, including those of Lux *et al.* (1937*a*, *b*) referred to above, have been described which confirm Halsted's principle, though exceptions have also been reported, notably by Turner (1938, 1939), who found that homotransplants of adrenal tissue from immature rats to the eyes of adult recipients sometimes survived even when the latter had not been adrenalectomized.

Turner also found that the period of survival and rate of regeneration of adrenal transplants to the eyes of non-adrenalectomized animals could be increased by making serial

transplants of pituitary tissue to the same eye, and it thus appears probable that the behaviour of adrenal transplants is conditioned by pituitary corticotrophic hormone, and that the favourable effect of adrenalectomy is due to the increased secretion of this hormone which it causes.

Various workers have shown that both tissue transplants, and explants grown in tissue culture, may be influenced by administration of appropriate hormones. Silberberg (1934) showed that intraperitoneal injections of an acid extract of anterior pituitary induced hypertrophy and hyperplasia in autotransplants of guinea-pig thyroid, and slightly increased the survival time of subcutaneous homotransplants, and Gaillard (1942) found that when anterior pituitary tissue was present in the same culture it frequently influenced the behaviour of other tissues and cells, including embryonic testis and fibroblasts.

In the present investigation further experiments akin to those described have been performed, using, however, only normal adult tissue, with the object of obtaining fresh light on three main questions, viz.:

(*a*) The immunological reactions associated with the growth of homografts in the anterior chamber and subcutaneously.

(*b*) The role of the reticulo-endothelial system in the resistance to homografts.

(*c*) The applicability of Halsted's principle to auto- and homografts of endocrine tissue, and the mechanism involved.

Materials and methods

The experiments were performed on guinea-pigs weighing 400 to 500 g., though occasionally larger (up to 800 g.) or smaller (300 to 400 g.) animals were used. They were of mixed strain though mainly from a single source of supply. As far as possible the recipient of a graft was of the same sex as the donor but of a different colour.

Before describing the actual experiments certain technical procedures require brief mention.

1. *Thyroidectomy*

This operation was performed under ether anaesthesia through a mid-line longitudinal incision. In the majority of animals the thyroid was found to consist of two separate lobes, but in some an isthmus was present. If there was no isthmus each lobe was removed, starting at the upper pole which was freed by ligating and dividing the superior thyroid vessels, and dissecting in a caudal direction. If an isthmus was present, it was divided as the first step in the operation and each half of the gland then removed, as already described.

In a few cases the recurrent laryngeal nerve was injured on one or both sides, and such animals usually died or had to be destroyed.

In some cases inadvertently, and in others deliberately, a minute shred of thyroid tissue was left behind which subsequently grew into a nodule of considerable size, sometimes as large as a normal lobe. When this occurred the operation was classified as 'subtotal thyroidectomy', the term 'total thyroidectomy' being restricted to cases in which a careful post-mortem search at the end of the experiment failed to reveal any trace of thyroid tissue in the neck.

2. *Partial splenectomy*

This was performed under ether anaesthesia through a mid-line upper abdominal incision, the spleen being partially delivered and a small piece snipped off with scissors. Haemorrhage was arrested by applying a small piece of 'Gelfoam' soaked in a solution of ox thrombin.

3. *Anterior chamber grafts*

These were made under either general (ether) or local anaesthesia. Satisfactory local anaesthesia was obtained by instilling 2 drops of a saturated solution of cocaine into the eye about 5 min. before operation.

The majority of thyroid grafts consisted of between one-third and one-half of a normal lobe (i.e. between one-sixth and one-quarter of the whole gland). Fascia and fat were dissected away from the lobe and a graft of appropriate size cut by means of a pair of von Graefe cataract knives. Prior to insertion, the graft was moistened with a few drops of sterile normal saline containing a small quantity of penicillin (1000 units/ml.), preliminary experiments having shown that penicillin in this quantity did not affect the graft adversely. Splenic grafts were approximately the same size as the thyroid grafts and were prepared similarly.

For insertion of the graft the technique described by Greene (1938) was found to be rather unsatisfactory, and the following method was adopted instead:

The eyeball was steadied by grasping a fold of conjunctiva with a pair of curved Spencer Wells forceps. An incision was then made in the upper part of the cornea close to the corneo-scleral junction using a special glass instrument* made by drawing out a piece of glass tubing to an external diameter of about 1 mm., marking it on one side with a diamond and then breaking it by shearing—*not bending*—so that a sharp spike resulted.

The graft was placed in the wide end of a Pasteur pipette and pushed down towards the narrow end with a metal stilette; the narrow end of the pipette was then inserted into the anterior chamber through the corneal incision and the transplant expelled with the stilette. The pipette was withdrawn and the graft displaced downwards to a point diametrically opposite the incision by gentle pressure on the surface of the cornea with a blunt instrument as described by Greene. Some care was required to ensure that the graft remained in the anterior chamber and did not slip behind the iris.

Animals carrying anterior chamber grafts were examined every day or two for the first week, and thereafter at weekly intervals. At the end of the experiment the eye bearing the graft was excised and the lens removed. After fixation in 5 % formol-saline redundant tissue was trimmed away with scissors and the remainder, which consisted either of the graft together with a small portion of iris and cornea in its immediate vicinity, or the whole of the anterior chamber if the graft was not clearly recognizable, was embedded in paraffin. Serial sections were then cut and stained with haematoxylin and eosin.

4. *Subcutaneous grafts*

These were inserted by means of a pipette and stilette. Under ether anaesthesia a small incision was made in the skin of the abdomen with an ordinary scalpel, the pipette intro-

* The use of these instruments and the method of making them was first demonstrated to the writers by Dr I. Polunin in the laboratory of Dr I. Berenblum at the Sir William Dunn School of Pathology, University of Oxford.

duced for a distance of about 3 cm. and the graft expelled. The pipette was then withdrawn and the incision sutured. Only thyroid tissue was used and the majority of the grafts consisted of between one-half and one lobe, i.e. between one-quarter and one-half of the whole gland.

Changes in the size of the graft were estimated roughly by palpation. At the end of the experiment biopsy or autopsy was performed and histological sections made and stained with haematoxylin and eosin.

5. *Preparation of pituitary thyrotrophic hormone*

No attempt was made to obtain pure thyrotrophic hormone. Instead, we used an alkaline extract of anterior pituitary prepared for us by Professor F. G. Young, F.R.S., by the method he has described (Young, F. G. 1941). This was assayed by the method of Rowlands & Parkes (1934) and found to contain between 1 and 2 Parkes-Rowlands units/ml.

6. *Statistical procedure*

The data have been treated as a series of 2×2 tables and analyzed by straightforward χ^2 tests. In a few instances the number of cases in a cell of one of the tables was less than five, and here either Yates's correction has been applied (Fisher 1936) or the probability of the result having arisen from random sampling has been calculated from first principles. The usual biological convention has been followed of labelling a result 'significant' if the probability of its occurring as an error of random sampling were less than 1 in 20.

Experiments and results

Group I. Preliminary study of auto- and homografts of thyroid tissue

The object of this study was to compare the behaviour of auto- and homografts of thyroid tissue in two particular situations, namely, the anterior chamber of the eye and a small subcutaneous pocket prepared beneath the skin of the abdomen, and to investigate the applicability of Halsted's law to such grafts.

The experiments performed were as follows:

1. *Autografts*
   - (i) To anterior chamber.
     - (*a*) After total thyroidectomy.
     - (*b*) After subtotal thyroidectomy.
     - (*c*) After hemi-thyroidectomy.
   - (ii) To subcutaneous site.
     - (*a*) After total thyroidectomy.
     - (*b*) After hemi-thyroidectomy.
2. *Homografts*
   - (i) To anterior chamber.
     - (*a*) After total thyroidectomy.
     - (*b*) After subtotal thyroidectomy.
     - (*c*) After hemi-thyroidectomy.
     - (*d*) In intact animals receiving no special treatment.
     - (*e*) In intact animals receiving subcutaneous injections of pituitary thyrotrophic hormone.
   - (ii) To subcutaneous site—after total thyroidectomy.

Anterior chamber grafts. These were left *in situ* for periods varying from 28 days to over a year. Initially the grafts were observed for relatively long periods, but it was found that after about 28 days the subsequent fate of an anterior chamber graft could be predicted accurately from its appearance.

It has therefore seemed reasonable to compare grafts which had been grown for different periods provided these were never less than 28 days. Grafts grown for shorter periods could not be validly compared unless the periods were the same in each case, and all such 'short-term' grafts have therefore been excluded from the experiments.

For the purpose of comparison grafts were classified as 'successful' or 'unsuccessful' on the basis of the histological findings at the termination of the experiment. These conformed to one of the following four types:

A. Graft consisted entirely of normal-looking vascular thyroid and the surrounding tissues showed little or no reaction (see figures 17, 19, 23, 26, 30, 32, plates 28 to 30).

B. Graft consisted for the most part of normal-looking vascular thyroid, but a small portion had been replaced by connective tissue. The surrounding tissue showed little or no reaction.

C. Graft contained some recognizable thyroid acini but showed disintegration or actual necrosis accompanied by gross infiltration with connective tissue and/or lymphocytes (see figures 25, 29, plates 29 and 30).

D. Graft absorbed completely or contained no recognizable thyroid tissue (see figures 22, 27, 31, plates 29 and 30).

Grafts of types A and B were counted as successful, those of types C and D as unsuccessful.

Grafts which were later found to be successful gradually deepened in colour, becoming bright red due to vascularization from the iris within a few weeks (usually 2 to 3 weeks, occasionally up to 7 weeks) (see figures 1, 2, 5, 7, 9, 11, plate 28). Change in the size o a graft appeared to depend on the amount of circulating thyrotrophic hormone, as determined by the degree of thyroid deficiency created in the host or by giving injections of this substance. Thus auto- and homografts to totally thyroidectomized animals usually showed little change, though a few long-term grafts increased up to double their original size, and a few decreased though never to less than half their original size. Such grafts as were successful in non-thyroidectomized or partially thyroidectomized hosts nearly all decreased considerably, some to as little as one-tenth of their original size, unless injections of pituitary extract were given, when the grafts remained unchanged in size or decreased very slightly.

Unsuccessful grafts nearly all became progressively more pale (see figures 3, 4, 6, 8, 10, plate 28), though a few became pink for a week or two and then faded. The majority decreased markedly in size, many becoming completely absorbed before the end of the experiment, but a few showed little or no change.

Subcutaneous grafts. The same histological criteria of success or failure were applied as in the case of anterior chamber grafts, and again grafts which remained *in situ* for less than 28 days were excluded from the experiment.

The progress of a subcutaneous graft could not be judged with the same accuracy as an anterior chamber graft, but it was observed that unsuccessful grafts usually appeared to enlarge, as estimated by palpation, for a week or two (apparently as a result of an

inflammatory reaction provoked by the graft) and then gradually decreased in size until they became impalpable. The majority of successful grafts, on the other hand, decreased slightly for a week or two and thereafter showed little or no change.

Again it was found that the histological distinction between successful and non-successful grafts was quite sharp after 28 days, and it seemed that the fate of a subcutaneous graft was settled by this time. Autografts examined at various periods from 28 days to over 6 months were all successful. On the other hand, out of thirty-three homografts examined after 28 to 35 days, twenty showed no trace of thyroid, nine showed merely scattered remnants, and only four were classed as successful. Four homografts were observed for a little longer (up to 56 days), but no trace of thyroid was found in any of these at the end of the experiment. It has therefore seemed reasonable once again to compare grafts grown for different periods provided this was never less than 28 days. It is just possible, though unlikely, that one or more of the four successful homografts might have regressed if observed for a longer period, but it seems quite certain that none of the grafts reckoned as unsuccessful would have recovered however long the experiment had been prolonged.

Sections of successful grafts are shown in figures 20, 21 and 33, and of unsuccessful grafts in figures 24 and 34, plates 29 to 30. It will be seen that lymphatic infiltration occurred to a much greater degree in the case of unsuccessful subcutaneous grafts than in unsuccessful grafts to the anterior chamber.

A general summary of the results is given in table 1, but complete protocols are not included owing to limitations of space.

The findings support the following conclusions:

(1) If a total thyroid deficiency is produced in the host autografts are uniformly successful, both in the anterior chamber and subcutaneously, whereas homografts are successful in the anterior chamber in the great majority of cases (78 %) but are only occasionally successful subcutaneously (11 %).

(2) Halsted's principle holds good for anterior chamber grafts, in that with both autografts and homografts the chance of success decreases very considerably if even a small amount of the host's thyroid is left *in situ*. In the case of homografts, where four levels of thyroid deficiency were studied, it was found that the proportion of successful grafts increased *pari passu* with the proportion of host thyroid removed. With autografts, where the number of cases was small, the same regularity was not observed, i.e. the proportion of successful grafts was actually higher after hemithyroidectomy than after subtotal thyroidectomy.

(3) Halsted's principle is less applicable to subcutaneous autografts than to anterior chamber grafts. It is obviously impossible to make an autograft without first removing some tissue, but it can at least be said that subcutaneous autografts succeed just as well in hosts with only 50 % thyroid deficiency as in those with a total deficiency.

(4) Some light is thrown on the mechanism of Halsted's principle, and the explanation mentioned in the introduction appears to be confirmed, namely, that it is not the thyroid deficiency *per se* which is important but the increased production of pituitary thyrotrophic hormone which this deficiency evokes, since anterior chamber homografts to animals without thyroid deficiency, but which received injections of pituitary thyrotrophic hormone, were uniformly successful. It also appears that the existence of a thyroid deficiency or the

administration of thyrotrophic hormone, though important for the establishment of a graft, is not necessary to ensure its continued survival when once established, since cessation of injections of pituitary extract after four weeks in experiment 2 (i) (*e*) did not appear to have any deleterious effect on the grafts (figure 23, plate 29).

Group II. Immunological studies with thyroid homografts

The object of these studies was twofold. First, to determine whether a subcutaneous homograft induces a state of immunity which extends to the anterior chamber of the eye, and, if so, to determine the degree of specificity of this reaction. Secondly, to determine whether an anterior chamber graft is itself effectively antigenic.

The experiments performed are set out below. For the subcutaneous grafts a piece of tissue about half the size of a normal thyroid lobe was used.

1. *Simultaneous grafts to anterior chamber and subcutaneously*
   - (i) Autografts, after total thyroidectomy (control experiments).
   - (ii) Homografts, both grafts being taken from the same donor.
     - (*a*) After total thyroidectomy.
     - (*b*) After subtotal thyroidectomy.
   - (iii) Homografts, subcutaneously from one donor and to the anterior chamber from another, after total thyroidectomy.

2. *Homografts to anterior chamber following an earlier subcutaneous homograft*
   - (i) Both grafts from the same donor.
   - (ii) Grafts from different donors.

   The sequence of operation was as follows:

   First stage:

   Donor: hemithyroidectomy (or total thyroidectomy in a proportion of the cases in which a different donor was to be used for the second stage).

   Host: total thyroidectomy, followed immediately by subcutaneous graft.

   Second stage, approximately 4 weeks later:

   Donor: hemithyroidectomy or total thyroidectomy.

   Host: biopsy, with removal of subcutaneous graft if not already completely absorbed, followed immediately by graft to anterior chamber.

3. *Homograft to anterior chamber following an earlier homograft to the opposite anterior chamber*
   - (i) Both grafts from the same donor.
   - (ii) Grafts from different donors.

   The sequence of operation was as follows:

   First stage:

   Donor: hemithyroidectomy or total thyroidectomy.

   Host: graft to right anterior chamber.

   Second stage:

   Donor: hemithyroidectomy or total thyroidectomy.

   Host: total thyroidectomy, followed immediately by graft to the left anterior chamber.

TABLE 1. EXPERIMENTS OF GROUP I

no. of exp.	nature of exp.	total no. of animals used	period for which grafts observed (days)			classification of animals in terms of histological findings at end of exp. (see text)				no. of 'successful' grafts (i.e. A+B)
			longest	shortest	av.	no. showing type A	no. showing type B	no. showing type C	no. showing type D	
1 (i) (*a*)	autograft to anterior chamber after total thyroidectomy	5	445	102	353	5	0	0	0	5 (100 %)
1 (i) (*b*)	autograft to anterior chamber after subtotal thyroidectomy	3	63	28	48	0	0	2	1	0
1 (i) (*c*)	autograft to anterior chamber after hemi-thyroidectomy	9	106	35	70	3	1	1	4	4 (44 %)
1 (ii) (*a*)	autograft subcutaneously after total thyroidectomy	6	216	28	108	6	0	0	0	6 (100 %)
1 (ii) (*b*)	autograft subcutaneously after hemi-thyroidectomy	6	112	29	61	6	0	0	0	6 (100 %)
2 (i) (*a*)	homograft to anterior chamber after total thyroidectomy	51	424	30	105	38	2	2	9	40 (78 %)
2 (i) (*b*)	homograft to anterior chamber after subtotal thyroidectomy	14	157	38	96	4	0	5	5	4 (29 %)
2 (i) (*c*)	homograft to anterior chamber after hemi-thyroidectomy	10	216	28	82	1	1	3	5	2 (20 %)
2 (i) (*d*)	homograft to anterior chamber in intact animals receiving no special treatment	41	94	28	39	4	3	7	27	7 (17 %)
2 (i) (*e*)	homograft to anterior chamber in intact animals receiving injections of pituitary extract daily for 30 days	6	81	30	55	5	1	0	0	6 (100 %)
2 (ii)	homograft subcutaneously after total thyroidectomy	37	54	28	32	2	2	9	24	4 (11 %)

TABLE 2. EXPERIMENTS OF GROUP II

no. of exp.	nature of exp.	total no. of animals used	period for which grafts observed (days)			interval between 1st and 2nd grafts (days)			no. of successful anterior chamber grafts	average value of ratio of area at end of exp. to original area for *successful* anterior chamber grafts	no. of successful subcutaneous grafts
			longest	shortest	av.	longest	shortest	av.			
	anterior chamber and subcutaneous grafts										
1 (i)	autografts to anterior chamber and subcutaneously at the same time after total thyroidectomy	6	227	30	133	—	—	—	5 (83 %)	0·8	6 (100 %)
1 (ii) (*a*)	homografts to anterior chamber and subcutaneously at the same time and from the same donor, after total thyroidectomy	18	134	28	65	—	—	—	8 (44 %)	1·0	3 (17 %)
1 (ii) (*b*)	homografts to anterior chamber and subcutaneously at the same time and from the same donor, after subtotal thyroidectomy	7	90	42	68	—	—	—	3 (43 %)	0·8	1 (14 %)
1 (iii)	homografts to anterior chamber and subcutaneously at the same time from different donors, after total thyroidectomy	15	188	28	65	—	—	—	10 (67 %)	0·9	3 (20 %)
	anterior chamber grafts										
2 (i)	homograft to anterior chamber following total thyroidectomy and a previous subcutaneous homograft from the same donor	12	134	28	56	49	28	30	4 (33 %)	0·9	—
2 (ii)	homograft to anterior chamber following total thyroidectomy and a previous subcutaneous homograft from a different donor	15	179	28	87	51	28	35	7 (47 %)	0·9	—
	2nd anterior chamber grafts										
3 (i)	homograft to anterior chamber following a previous homograft to the opposite anterior chamber from the same donor, thyroidectomy being performed immediately prior to the second graft	10	100	29	56	35	29	30	0	—	—
3 (ii)	homograft to anterior chamber following a previous homograft to the opposite anterior chamber from a different donor, thyroidectomy being performed immediately prior to the second graft	9	168	36	84	35	29	31	3 (33 %)	0·9	—
	anterior chamber grafts after subcutaneous grafts made										
4 (i)	homograft to anterior chamber after total thyroidectomy; when well established, subcutaneous homograft of refrigerated thyroid from the same donor	4	118	28	82	39	35	38	4 (100 %)	—	0
4 (ii)	homograft to anterior chamber after total thyroidectomy; when well established, subcutaneous homograft of fresh thyroid from the same donor	9	190	35	83	33	21	26	9 (100 %)	—	0
4 (iii)	homograft to anterior chamber after total thyroidectomy; when well established, subcutaneous homograft of fresh thyroid from a different donor	2	117	35	76	26	23	25	2 (100 %)	—	0

It was desirable that the host should be in a state of *total* thyroid deficiency at the time of making the second graft. The ideal way to ensure this would have been to proceed along the lines of the previous experiment and remove the first graft immediately prior to making the second. This would have involved removing one eye and then grafting to the remaining one, and on humanitarian grounds permission to do this was not sought. The alternative possibility was to find some means of causing the first graft to degenerate, and preliminary experiments (see group I) showed that this could be achieved in the majority of cases by postponing thyroidectomy of the host for a few weeks after the first graft was made. The procedure described above was therefore adopted, thyroidectomy being performed immediately prior to making the *second* graft.

4. *Homografts to anterior chamber followed by a subcutaneous graft*

These experiments were designed to determine the effect, if any, of a subcutaneous graft on a homograft already well established in the anterior chamber.

(i) Using refrigerated thyroid from the same donor for the subcutaneous graft.

The sequence of operation was as follows:

First stage:

Donor: total thyroidectomy; half of gland used immediately and rest placed in sterile normal saline and kept in the refrigerator at 0° C.

Host: total thyroidectomy, followed immediately by homograft to anterior chamber.

Second stage, performed when anterior chamber graft well established (5 to 6 weeks after first stage):

Host received a subcutaneous graft of the thyroid stored in the refrigerator.

(ii) Using fresh thyroid from the same donor for the subcutaneous graft.

The sequence of operation was as follows:

First stage:

Donor: hemithyroidectomy.

Host: total thyroidectomy, followed immediately by homograft to anterior chamber.

Second stage, performed when anterior chamber graft well established (3 to 6 weeks after first stage):

Donor: remaining lobe of thyroid removed.

Host: received subcutaneous homograft.

(iii) Using fresh thyroid from a different donor for the subcutaneous graft.

Procedure similar to the previous experiment except that a different donor was used for the second graft.

A summary of the results of this group of experiments is given in table 2, and typical macroscopic and microscopic appearances are illustrated in figures 4 to 11 and 25 to 32 inclusive (plates 28 to 30).

It will be seen that a subcutaneous homograft of thyroid from the same donor, whether made at the same time as an anterior chamber graft or a month earlier, significantly reduced the chances of success of the latter.

Thus comparing experiment I, 2 (i) (*a*) (uncomplicated anterior chamber homografts following total thyroidectomy) with experiment II, 1 (ii) (*a*) (simultaneous subcutaneous

and anterior chamber grafts from the same donor following total thyroidectomy) we have 40 successes out of 51 as against 8 out of 18, for which $\chi^2=7{\cdot}28$ and $P<0{\cdot}01$.

Again, comparing experiment I, 2 (i) (*a*) with experiment II, 2 (i) (homografts to anterior chamber preceded by total thyroidectomy and a subcutaneous graft from the same donor) we have 40 successes out of 51 as against 4 out of 12, for which χ^2, calculated after applying Yates's correction, $=6{\cdot}34$ and $P\doteqdot 0{\cdot}01$.

When, instead of taking the subcutaneous and anterior chamber grafts from the same donor, these were taken from different donors, it was found that a subcutaneous graft significantly reduced the chance of success of a subsequent graft to the anterior chamber, as in the previous experiment, but had no significant effect on an anterior chamber graft made at the same time.

Thus, comparing experiment I, 2 (i) (*a*) and experiment II, 2 (ii) (anterior chamber grafts following previous total thyroidectomy and subcutaneous graft from a different donor) we have 40 successes out of 51 as against 7 out of 15, for which $\chi^2=5{\cdot}70$ and $P<0{\cdot}02$.

Again, comparing experiment I, 2 (i) (*a*) (uncomplicated anterior chamber grafts following total thyroidectomy) and experiment II, 1 (iii) (simultaneous subcutaneous and anterior chamber grafts from different donors, following total thyroidectomy), we have 40 successes out of 51, as against 10 out of 15, for which $\chi^2=0{\cdot}87$, $P\doteqdot 0{\cdot}3$.

It seems clear from these results that a subcutaneous homograft induces a state of general immunity to like tissue, which extends to the anterior chamber of the eye. As regards the effect of a subcutaneous graft on a subsequent graft to the anterior chamber no other explanation seems possible. As regards the effect on an anterior chamber graft of a simultaneous subcutaneous graft in experiment II, 2 (i), it was at first thought that this might be explained by supposing that the latter actively functioned for a short time and thereby adversely affected the former in accordance with Halsted's principle. This hypothesis appeared to gain some support from the fact that the effect of a simultaneous homograft on an anterior chamber graft from the same donor was much the same whether the recipient was subjected to total or to subtotal thyroidectomy (cf. experiments II, 1 (ii) (*a*) and II, 1 (ii) (*b*)), but would appear to be ruled out by the following considerations:

First, in experiment II, 1 (i), when simultaneous subcutaneous and anterior chamber *autografts* were made, after total thyroidectomy, the anterior chamber graft was successful in five out of six cases, despite the survival of the subcutaneous graft in every case, whereas uncomplicated autografts to the anterior chamber following hemi-thyroidectomy succeeded in only 4 out of 9 cases.

Secondly, in experiment II, 1 (iii), where simultaneous subcutaneous and anterior chamber grafts were made from *different* donors, the subcutaneous graft had no significant effect on the chances of survival of the graft in the anterior chamber.

It will be seen that the proportion of surviving anterior chamber homografts occurring when either a preceding or a simultaneous subcutaneous homograft was made was somewhat less when the two grafts were from the same donor than when they were taken from different donors, but the difference is much too small to be accepted as statistically significant.

Thus comparing experiments II, 1 (ii) (*a*) and II, 1 (iii) we have 8 successes out of 18 as against 10 out of 15, for which $\chi^2 = 1{\cdot}63$, $P \doteqdot 0{\cdot}2$, i.e. non-significant; and comparing experiment II, 2 (i) and II, 2 (ii), we have 4 successes out of 12, as against 7 out of 15, for which $\chi^2 = 0{\cdot}49$, $P \doteqdot 0{\cdot}5$, i.e. non-significant, and still more obviously so if Yates's correction is applied.

Similar but even more striking results were obtained when an initial 'immunizing' graft to one anterior chamber was followed about a month later by a graft to the opposite anterior chamber; viz. when both grafts were from the same donor the second was unsuccessful in every case, and when the grafts were from different donors the chances of success of the second graft were less dramatically but still significantly reduced.

Thus comparing experiment I, 2 (i) (*a*) (uncomplicated anterior chamber grafts following total thyroidectomy) with experiment II, 3 (i) (anterior chamber grafts following total thyroidectomy, preceded by a graft to the opposite anterior chamber from the same donor), we have 40 successes out of 51 as compared with 0 out of 10, a result which is obviously significant.

Again, comparing experiment I, 2 (i) (*a*) with experiment II, 3 (ii) (anterior chamber grafts, following total thyroidectomy, preceded by a graft to the opposite anterior chamber from a different donor), we have 40 successes out of 51 as against 3 out of 9, for which χ^2, calculated after applying Yates's correction, $= 5{\cdot}60$, $P < 0{\cdot}02$.

Once again it seems clear that the initial graft has had an immunizing effect, and this time the results obtained when both grafts were taken from the same donor differ considerably from those obtained by using two different donors, though still not quite sufficiently to satisfy the conventional criterion of significance.

Thus comparing experiments II, 3 (i) and II, 3 (ii) we have 0 successes out of 10 as against 3 out of 9. Owing to the small numbers exact treatment is preferable to a χ^2 test. The appropriate value of P is equivalent to the probability that a random selection of 10 cases from 16 unsuccessful and 3 successful ones shall contain no successful case. From first principles this is $\dfrac{16 \times 15 \times 14 \times \ldots \times 7}{19 \times 18 \times 17 \times \ldots \times 10} = 0{\cdot}086$.

As an alternative to the immunological hypothesis it could perhaps be urged that since the initial graft was not removed it might have continued to function and thereby reduce the animal's demand for thyroid, thus prejudicing the chances of the second graft. It seems, however, reasonable to exclude this latter hypothesis on the following grounds:

First, in no case did the original graft appear really healthy when the second graft was made. In six animals in experiment II, 3 (i) and in eight in experiment II, 3 (ii), it appeared to be quite avascular, and in the remainder, with one exception, there was only slight evidence of vascularization as judged by the colour of the graft. The one exceptional graft referred to was bright red in colour but only 2 sq.mm. in area (animal no. 402).

Secondly, the opinion formed by inspection of the original graft was subsequently confirmed by histological examination at the end of the experiment, healthy thyroid tissue being demonstrated in only 2 out of 10 cases in experiment II, 3 (i) and 2 out of 9 cases in experiment II, 3 (ii). It seems most unlikely that functioning thyroid tissue was present at the time of the second graft except in the few cases where healthy-looking thyroid was demonstrated subsequently, since, had it been, it would presumably have

survived and probably even become more active as a result of the thyroidectomy performed when the second graft was made.

Thirdly, in experiment II, 3 (i) the total failure of the second graft differs significantly from the partial success obtained with uncomplicated anterior chamber homografts after subtotal thyroidectomy (experiment I, 2 (i) (*b*), 4 out of 14 successes), and it seems most unlikely that the remnants of the first graft in the former experiment exceeded in secretory capacity the thyroid tissue deliberately left *in situ* in the latter.

The experiments of this group which have been discussed so far were concerned mainly with immunological factors interfering with the 'taking' of a homograft in the anterior chamber. The remaining experiments of the group (experiments II, 4 (i), (ii) and (iii)), performed to determine whether the subsequent behaviour of a homograft already well established could be modified by similar means, all yielded completely negative results. The appearance of the anterior chamber graft remained unchanged after the subcutaneous graft was made, and histological examination at the end of the experiment revealed healthy vascular thyroid tissue in the anterior chamber in every case. It thus appears that the procedure previously shown to diminish the chances of an anterior chamber graft 'taking' has no observable effect on a graft already well established.

From the whole group of experiments the following conclusions regarding thyroid homografts in the guinea-pig may thus be drawn:

(1) A subcutaneous homograft is demonstrably antigenic, and the same is true of a homograft in the anterior chamber provided that this is undergoing destruction. It has not been determined whether a surviving homograft in the anterior chamber is antigenic, but, if it is, the corresponding state of immunity must take a considerable time to develop—certainly longer than the graft requires to become well established.

(2) The state of immunity induced by a homograft is general and extends to the anterior chamber, i.e. when the immunizing graft is subcutaneous the state of immunity is demonstrable in either anterior chamber, and when the immunizing graft is in one anterior chamber the state of immunity is demonstrable in the opposite one.

(3) There is some suggestive, though not conclusive, evidence that an immunizing homograft renders the recipient more resistant to a subsequent homograft from the same donor than to one from another individual.

(4) Immunization of an animal by means of a subcutaneous or anterior chamber graft diminishes the chance of a subsequent homograft 'taking', but 'immunization' (by means of a subcutaneous graft) of an animal which already has a homograft established in the anterior chamber does not (within the limits of the experiment) modify the behaviour of the latter in any way.

Group III. Experiments to determine whether a homograft growing in the anterior chamber undergoes a process of adaptation which will enable it to take and survive when subsequently transferred to a subcutaneous site in the same animal

Experiment 1. *Graft transferred after a relatively short period* (*viz.* 28 *days or less*) *in the anterior chamber*

Animals for this experiment were taken from stock, thyroidectomized, and given a homograft of thyroid to the anterior chamber. The eye was subsequently removed at a pre-

determined time, irrespective of the appearance of the graft, the graft dissected out aseptically, a small portion kept for histological examination and the remainder re-implanted subcutaneously. Subsequent changes in the size of the graft were estimated at weekly intervals by palpation, and 4 to 6 weeks after the transfer the animal was killed and the graft, if any remained, removed for histological examination.

Experiment 2. *Graft transferred after a relatively long period* (*viz.* 3 *months or more*) *in the anterior chamber*

For this experiment twenty animals were chosen by a process of randomization from among those used in experiment I, 2 (i) (*a*) (homografts of thyroid to anterior chamber following thyroidectomy),* subject to the proviso that animals which had been used also in experiment II, 4 were excluded.

Five of the selected animals died, either before graft transfer was undertaken or within a few days of this operation, and were excluded from the experiment. In four others the graft was absorbed before 3 months had elapsed, and these were counted as failures from the point of view of the present experiment though transfer of the graft was not actually carried out.

The remaining eleven showed bright red healthy-looking grafts at the end of 3 months, and transference of the graft was performed, either at once or after waiting for a further period, by the method described in experiment I. In each of these animals the piece of tissue kept for histological examination at the time of transfer was found to consist of healthy vascular thyroid.

A summary of the results of both experiments is shown in table 3, and sections illustrating a successful and an unsuccessful transfer are shown in figures 33 and 34, plate 30.

TABLE 3. EXPERIMENTS OF GROUP III

Behaviour of homografts of thyroid, following total thyroidectomy, grown for periods of 1 day to 6 months in the anterior chamber and then transferred to a subcutaneous site in the same animal.

age of anterior chamber graft when transferred to subcutaneous site	no. of animals in group	no. of animals in which graft became successfully established in subcutaneous site
1 day	3	0
4 days	2	0
7 days	3	0
14 days	4	0
21 days	4	0
28 days	3	0
3 to 6 months	15†	6

† Includes four animals in which the anterior chamber graft was unsuccessful and the intended transfer was not performed.

It will be seen that in experiment I, in every case, the graft failed to survive after transfer, but that in experiment 2 the graft became re-established after transfer in six cases. Comparing this latter result with that of experiment I, 2 (ii) (primary subcutaneous

* One of these animals (no. 135) originally classified as experiment I, 2 (i) (*a*) was found at post-mortem to have a nodule of regenerating thyroid in the neck and was therefore reclassified as experiment I, 2 (i) (*b*) (homograft of thyroid to anterior chamber following *subtotal* thyroidectomy).

homografts of thyroid following total thyroidectomy) it appears that the chances of obtaining a successful subcutaneous homograft in a given recipient from a given donor are significantly greater if the graft is made first to the recipient's anterior chamber and 3 months or more later transferred to a subcutaneous site, than if a subcutaneous graft is made direct.

Thus in experiment III, 2 we have 6 successes out of 15 (counting as failures the four animals in which the graft failed to become established in the anterior chamber), as against 4 successes out of 37 in experiment I, 2 (ii), for which χ^2, after applying Yates's correction, $=4{\cdot}09$, $P<0{\cdot}05$.

This would seem to imply that when a homograft of thyroid is established in the anterior chamber for a sufficiently long time a process of adaptation occurs between graft and host, though there is nothing to indicate whether this process occurs exclusively in the graft, exclusively in the host, or in both graft and host.

Group IV. Study of auto- and homografts of splenic tissue to the anterior chamber, and of combined thyroid and splenic grafts

The experiments performed were as follows:

1. Autografts of spleen to the anterior chamber, after partial splenectomy.
2. Homografts of spleen to the anterior chamber.
3. Simultaneous autografts of thyroid and spleen to the same anterior chamber, after total thyroidectomy and partial splenectomy.
4. Simultaneous homograft of thyroid and autograft of spleen to the same anterior chamber, after total thyroidectomy and partial splenectomy.
5. Simultaneous homografts of thyroid and spleen from the same donor to the same anterior chamber, after total thyroidectomy.

Splenic grafts. Auto- and homografts of spleen alone behaved similarly. Four auto- and six homografts were performed and observed for periods of from 50 to 210 days. In every case the outline of the graft became somewhat indefinite after 2 to 12 weeks (figures 12 and 13, plate 28). In one animal in each experiment the graft was completely absorbed; in the remainder the grafts decreased in size (usually to between half and a quarter of the original size), and histological examination at the end of the experiment showed vascular connective tissue with clusters of lymphocytes, many red blood cells, and in some cases a little smooth muscle—apparently splenic tissue in a state of partial disintegration (figures 35 and 36, plate 30).

The temporary clouding of the cornea which follows nearly all grafts to the anterior chamber was rather more prolonged with splenic than with uncomplicated thyroid grafts (usually 5 to 7 days as against 3 to 4 days), but in no case did any severe reaction occur in the eye.

Simultaneous thyroid and splenic grafts. A summary of the results of experiments 3, 4 and 5 is shown in table 4.

Autografts of thyroid and spleen. The grafts appeared to become fused after 4 to 7 days, and the whole mass became bright red in colour within 2 to 6 weeks (figure 14, plate 28). In three cases the mass increased considerably in size; in the remainder it decreased slightly.

Histological examination at the end of the experiment showed, in every case, healthy vascular thyroid with splenic tissue in various stages of disintegration adjacent to but sharply demarcated from it (figure 37, plate 30).

Homografts of thyroid with autografts of spleen. In two cases suppuration occurred in the anterior chamber within a few days of grafting and the experiment had to be terminated. In the remainder considerable clouding of the cornea which obscured the grafts, accompanied by a variable degree of iritis, occurred for 4 to 21 days. When this cleared the grafts were seen to be fused.

Thereafter in eight cases the composite mass gradually decreased in size and its outline became progressively less definite (figure 15, plate 28). Histological examination at the end of the experiment showed splenic tissue in various stages of disintegration with either no trace of thyroid (two cases), scattered thyroid acini with lymphocytes in connective tissue (four cases, figure 38, plate 30) or a small piece of thyroid grossly infiltrated with lymphocytes (two cases).

TABLE 4. EXPERIMENTS OF GROUP IV (EXPERIMENTS 3, 4 AND 5)

Behaviour of simultaneous grafts of thyroid and spleen to the anterior chamber.

no. of of exp.	nature of grafts	total no. of animals used	period for which grafts observed (days) longest	shortest	av.	no. of cases in which healthy vascular thyroid found on histological examination at end of experiment
IV, 3	thyroid / spleen } autografts	5	219	56	130	5 (100 %)
IV, 4	thyroid, homograft spleen, autograft	11*	96	28	66	3 (27 %)
IV, 5	thyroid / spleen } homografts from same donor	15*	96	28	53	9 (60 %)

* Excluding animals in which suppuration occurred in the anterior chamber.

In the remaining three cases the mass became bright red in colour, the outline remained sharp and subsequent histological examination showed healthy vascular thyroid with splenic tissue adjacent to but sharply demarcated from it.

The significance of the suppuration which occurred in two cases, and which occurred also in three animals in the last experiment of this group but in no other experiments, is not clear, but even if it is accepted as due to accidental introduction of infection and the animals concerned are excluded from the experiment we have only three out of eleven successes as far as the thyroid grafts are concerned, and the result still differs significantly from that of experiment I, 2 (i) (*a*) (uncomplicated thyroid homografts to anterior chamber following total thyroidectomy), but not from that of experiment I, 2 (ii) (subcutaneous homografts of thyroid following total thyroidectomy).

Thus comparing the present result with experiment I, 2 (i) (*a*) we have 3 successes out of 11 as against 40 out of 51, for which χ^2, after applying Yates's correction, $=9{\cdot}90$, $P<0{\cdot}01$. On the other hand, comparing the present experiment with experiment I, 2 (ii) we have 3 successes out of 11, as against 4 out of 37, for which $\chi^2=1{\cdot}85$, $P\div 0{\cdot}18$, i.e. non-significant, and still more obviously so if Yates's correction is applied.

It thus appears that as a site for a homograft of thyroid an anterior chamber supplied with an autograft of splenic tissue is much less favourable than a normal anterior chamber and is not significantly better than a subcutaneous pocket on the abdominal wall. This finding accords with that of Murphy (1914*a*), to which reference was made in the introduction.

Homografts of thyroid and spleen from the same donor. In three cases suppuration occurred in the anterior chamber. In all except three of the remainder clouding of the cornea and iritis occurred, as in the previous experiment, but were not quite so marked. When the cloud cleared the grafts appeared to be fused.

In nine cases the mass became bright red, the outline remained definite (figure 16, plate 28), and healthy vascular thyroid with splenic tissue was found on subsequent histological examination (figure 18, plate 28).

In the remaining six cases the mass gradually decreased in size and its outline became progressively less definite. Subsequent histological examination showed disintegrating splenic tissue with either no trace of thyroid (five cases) or a few thyroid acini embedded in connective tissue (one case).

The proportion of successful thyroid grafts was thus considerably higher than in the previous experiment and, in fact, does not differ significantly from that found in experiment I, 2 (i) (*a*) (uncomplicated thyroid homografts to the anterior chamber after total thyroidectomy).

Thus making this comparison we have 9 successes out of 15 as against 40 out of 51, for which $\chi^2 = 2{\cdot}16$, $P \div 0{\cdot}15$.

The explanation of these results seems reasonably clear.

It appears from experiments IV, 3 and IV, 5 that splenic and thyroid grafts from the same donor are not intrinsically incompatible, i.e. they are able to survive side by side without either affecting the other adversely, and the low survival rate of the thyroid grafts in experiment IV, 4 must therefore be related to the fact that in this experiment the thyroid and splenic grafts were from different donors, the former being a homograft and the latter an autograft. The precise mechanism involved has not been established but there would seem to be three possibilities, viz. that the splenic autograft functioned as a source of lymphocytes and possibly other cells directly inimical to the thyroid homograft, as a local source of antibodies or in both capacities.

Discussion

There are two important questions on which the investigation throws some light, but to which no complete answer can as yet be given, namely: What is the mechanism by which subcutaneous homografts are destroyed? and Why is the anterior chamber so much more favourable to homografts than a subcutaneous site?

As regards the first question, it would seem that the answer must be a cellular process, a humoral process or a combination of the two. It has been assumed by most workers in this field that the lymphocytes which accumulate round, and eventually permeate, a subcutaneous homograft are responsible for its destruction, presumably by a process of phagocytosis. This may well be true, but there would seem to be two other possible

explanations, namely, that the graft undergoes necrosis as the result of a humoral mechanism and the lymphocytes appear as a reaction to the necrotic graft, or that the lymphocytes are not phagocytic but are the source of antibodies which are the proximate cause of the destruction of the graft.

The answer to the second question will inevitably depend on the answer to the first.

In so far as humoral factors are responsible for the destruction of homografts there would seem to be two possible reasons why the anterior chamber should be a favourable site, namely:

A graft in the anterior chamber survives as a tissue culture until it has become vascularized. During this initial phase it is not effectively antigenic, and later when vascularized is no longer vulnerable, or

A graft in the anterior chamber is antigenic *ab initio*, but the corresponding immune state takes a considerable time to develop and before it is established the graft has become vascularized and hence no longer vulnerable.

Both these explanations assume that once vascularized an anterior chamber graft is no longer vulnerable to humoral attack, and the investigation (especially experiment II, 4 (ii)) provides strong evidence in support of this assumption. This is at variance, however, with the observation of Medawar (1944, 1945) that in man and the rabbit a homograft of skin to a raw area normally becomes vascularized but is subsequently destroyed, apparently as the result of a general immunological reaction, since it would seem likely that a graft in the anterior chamber when once vascularized would be just as open to humoral attack as a vascularized graft in any other situation.

In so far as cellular attack is responsible for the destruction of homografts the favourable properties of the anterior chamber can only be explained by supposing that for some unknown reason lymphocytes do not accumulate so readily in this situation as elsewhere. This seems at first sight unlikely, but it gains some support from the fact that, even when an anterior chamber graft of thyroid degenerated because the recipient was not subjected to thyroidectomy (experiment I, 2 (i) (*d*)), large accumulations of lymphocytes were rarely seen.

It is apparent that much more work must be undertaken before any final solution of the many problems relating to tissue transplantation is reached. Various future experiments have been planned and the nature of some of these has been indicated already, but one further matter requires mention.

In all the experiments described above the animals were taken at random from a mixed stock and relatively large numbers of animals had to be used to obtain significant results, presumably because of a variable genetic relationship between donor and host. In future it is proposed, if possible, to work with two pure-line strains (the donors being all of one strain and the recipients of the other), and under such conditions, save for technical errors, any given result should be capable of being reproduced indefinitely.

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Description of plates 28 to 30

Plate 28

Figures 1 to 16 are all at a magnification of $\times 1\frac{1}{2}$.

Figure 1. Animal no. 77. Experiment I, 1 (i) (*a*). Eye with successful thyroid autograft 372 days old. For section see figure 19.

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Figure 19. Animal no. 77. Experiment I, 1 (i) (*a*). Section ($\times 46$) through anterior chamber showing successful thyroid autograft 422 days old.

Figure 20. Animal no. 125. Experiment I, 1 (ii) (*a*). Section ($\times 50$) of successful subcutaneous thyroid autograft 216 days old.

FIGURE 21. Animal no. 307. Experiment I, 1 (ii) (*b*). Section (×46) of successful subcutaneous autograft 112 days old.

FIGURE 22. Animal no. 520. Experiment I, 2 (i) (*b*). Section (×107) of part of unsuccessful thyroid homograft in anterior chamber 61 days old, showing connective tissue and lymphocytes but no recognizable thyroid.

FIGURE 23. Animal no. 296. Experiment I, 2 (i) (*e*). Section (×43) through anterior chamber showing successful thyroid homograft 81 days old (i.e. 51 days after injections of pituitary extract had ceased). The graft is attached to the iris and partly enclosed in a capsule of connective tissue.

FIGURE 24. Animal no. 116. Experiment I, 2 (ii). Section (×43) of unsuccessful subcutaneous thyroid homograft 42 days old, showing gross lymphocytic infiltration and a few just recognizable thyroid acini.

FIGURE 25. Animal no. 503. Experiment II, 1 (ii) (*a*). Section (×50) through anterior chamber showing unsuccessful thryoid homograft 98 days old, consisting of connective tissue attached to iris and cornea and containing a few thyroid acini and some lymphocytes.

FIGURE 26. Animal no. 496. Experiment II, 1 (iii). Section (×46) through anterior chamber showing successful thyroid homograft 99 days old.

FIGURE 27. Animal no. 467. Experiment II, 2 (i). Section (×64) through anterior chamber showing unsuccessful thyroid homograft 134 days old, consisting of a small piece of connective tissue attached to iris and cornea.

FIGURE 28. Animal no. 484. Experiment II, 2 (ii). Section (×50) through anterior chamber showing successful thyroid homograft 117 days old.

PLATE 30

FIGURE 29. Animal no. 442. Experiment II, 3 (i). Section (×86) of part of unsuccessful thyroid homograft in left anterior chamber 100 days old, showing connective tissue with a few recognizable thyroid acini.

FIGURE 30. Animal no. 415. Experiment II, 3 (ii). Section (×75) of successful thyroid homograft in left anterior chamber 168 days old.

FIGURE 31. Animal no. 450. Experiment II, 3 (ii). Section (×57) through left anterior chamber showing unsuccessful thyroid homograft 135 days old, consisting of connective tissue with a few lymphocytes.

FIGURE 32. Animal no. 365. Experiment II, 4 (ii). Section (×57) through anterior chamber showing successful thyroid homograft 216 days old (i.e. 193 days after this animal received a subcutaneous homograft).

FIGURE 33. Animal no. 86. Experiment III, 2. Section (×50) of thyroid homograft 44 days after successful transfer from anterior chamber to a subcutaneous site.

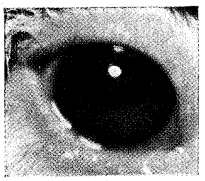
FIGURE 34. Animal no. 515. Experiment III, 2. Section (×57) of thyroid homograft 30 days after unsuccessful transfer from anterior chamber to a subcutaneous site, showing connective tissue infiltrated with lymphocytes but no recognizable thyroid.

FIGURE 35. Animal no. 388. Experiment IV, 1. Section (×86) through anterior chamber showing splenic autograft 210 days old.

FIGURE 36. Animal no 389. Experiment IV, 2. Section (×91) through anterior chamber showing splenic homograft 210 days old.

FIGURE 37. Animal no. 374. Experiment IV, 3. Section (×64) through anterior chamber showing autografts of spleen and thyroid 201 days old. The grafts are in contact but sharply demarcated.

FIGURE 38. Animal no. 459. Experiment IV, 4. Section (×53) through anterior chamber showing disintegrating thyroid homograft 96 days old, with gross lymphocytic infiltration. The adjacent splenic autograft is not included in the section.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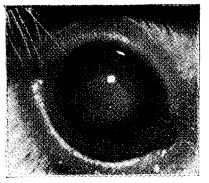
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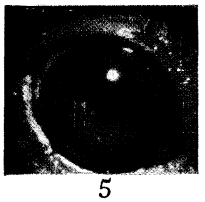
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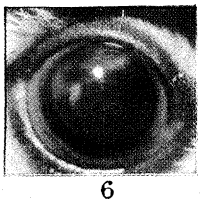
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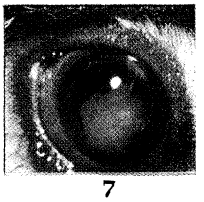
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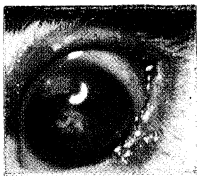
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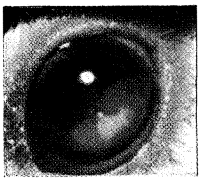
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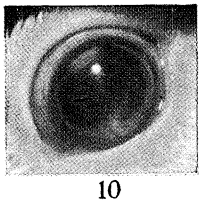
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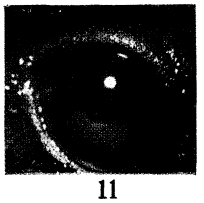
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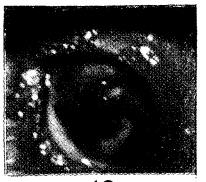
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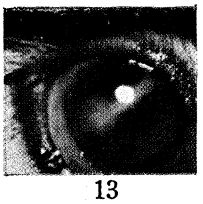
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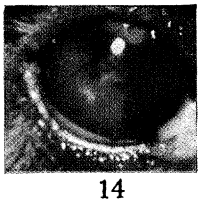
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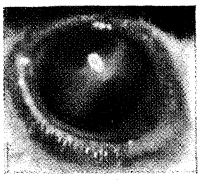
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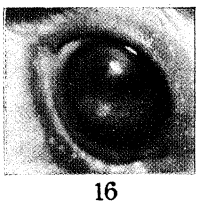
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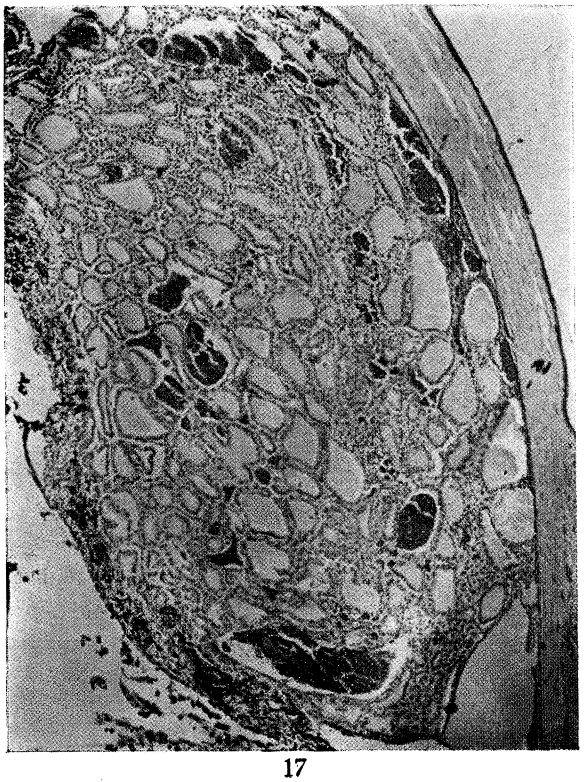
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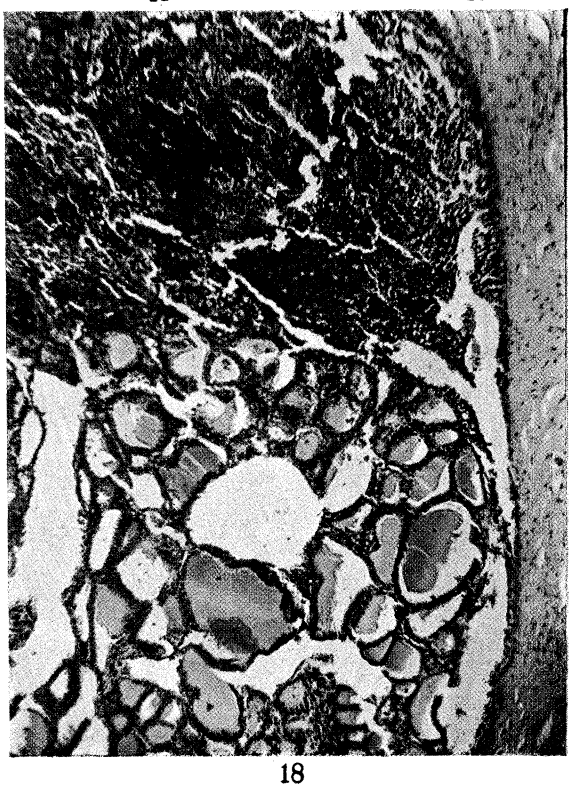
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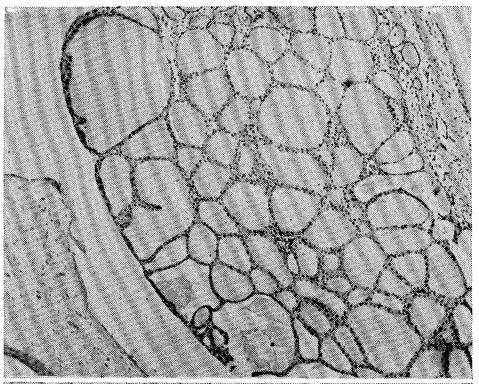

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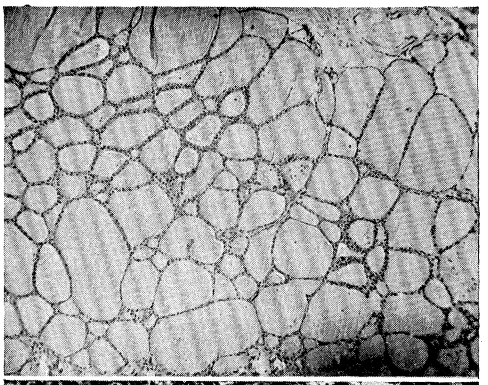

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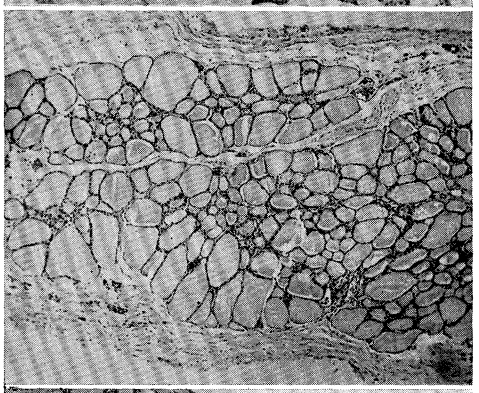

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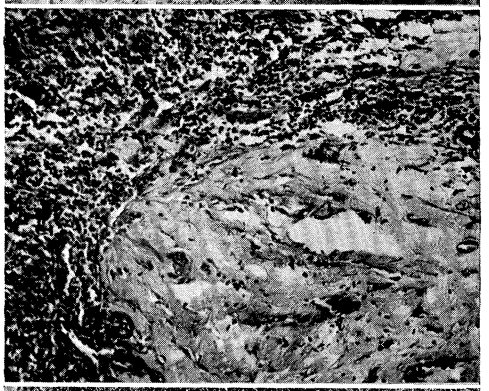

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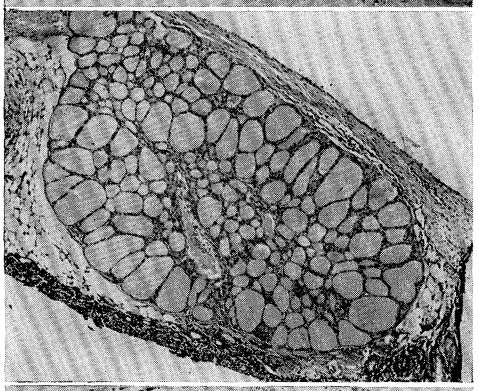

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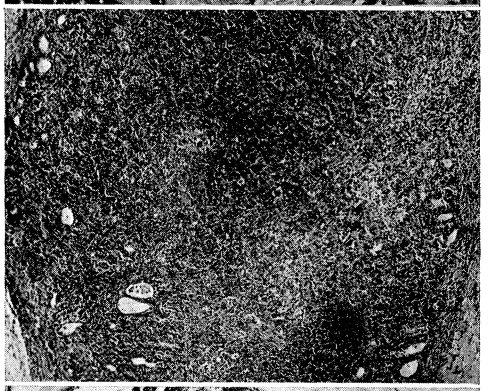

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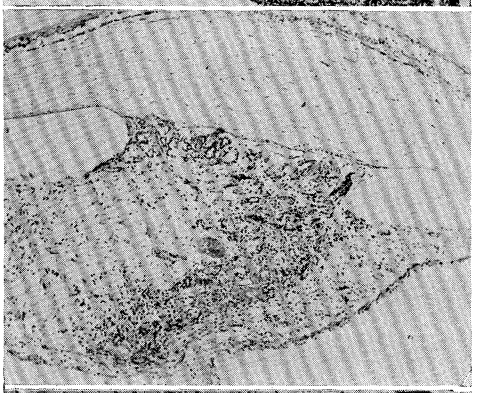

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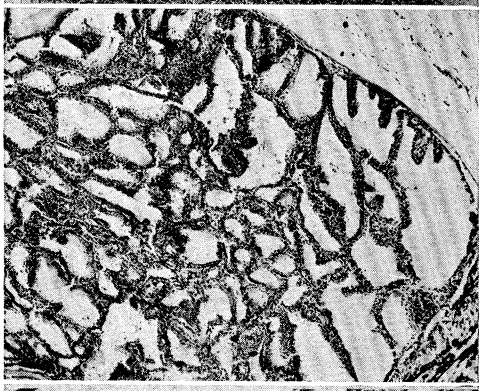

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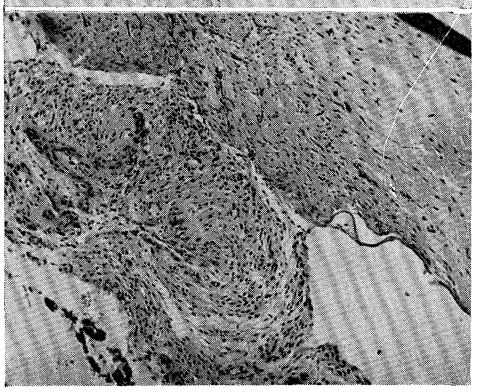

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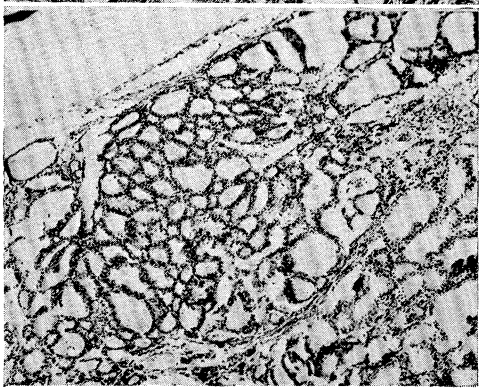

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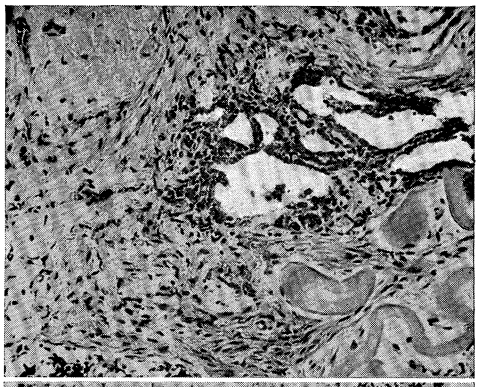

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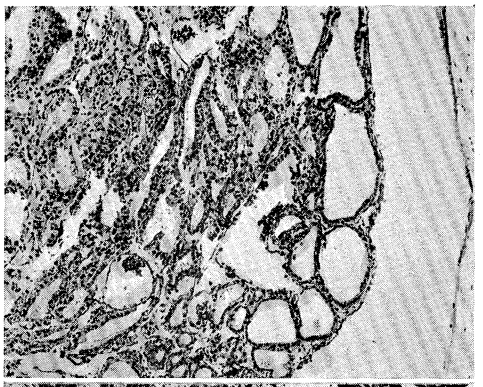

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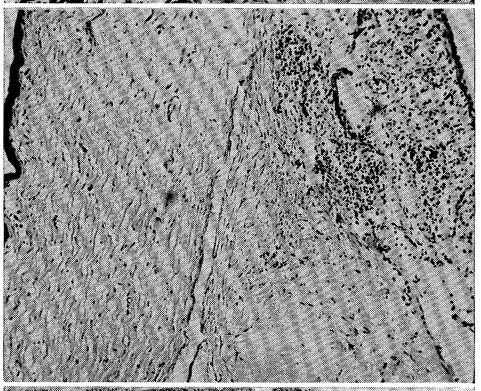

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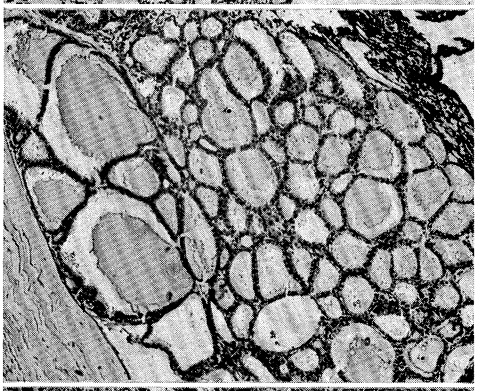

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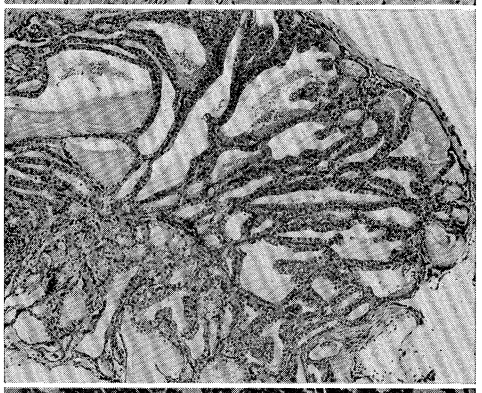

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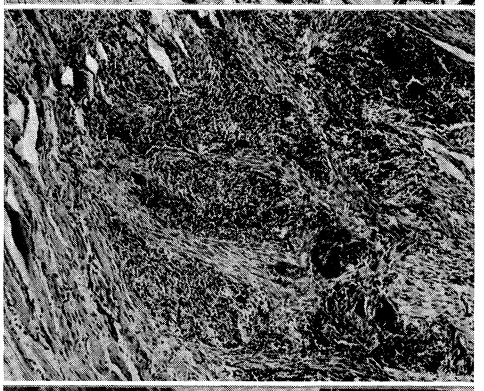

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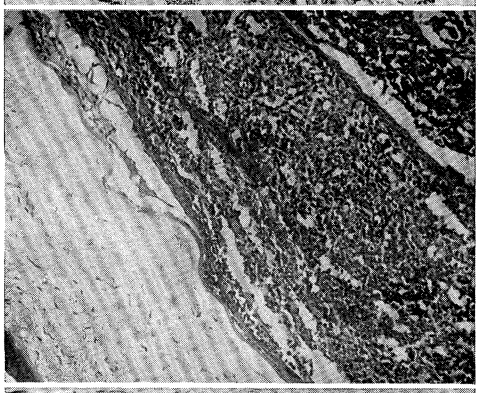

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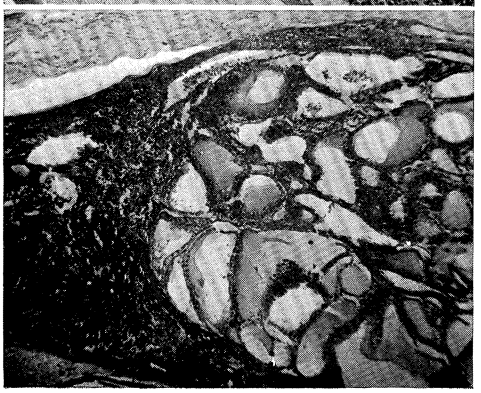

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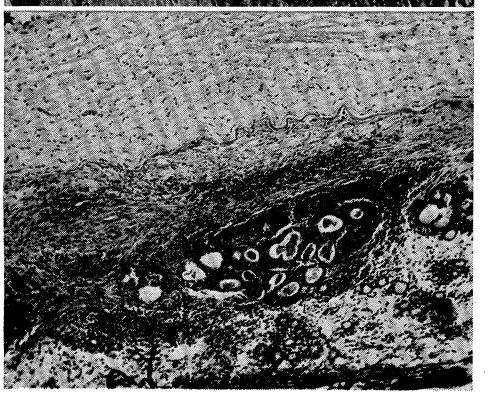

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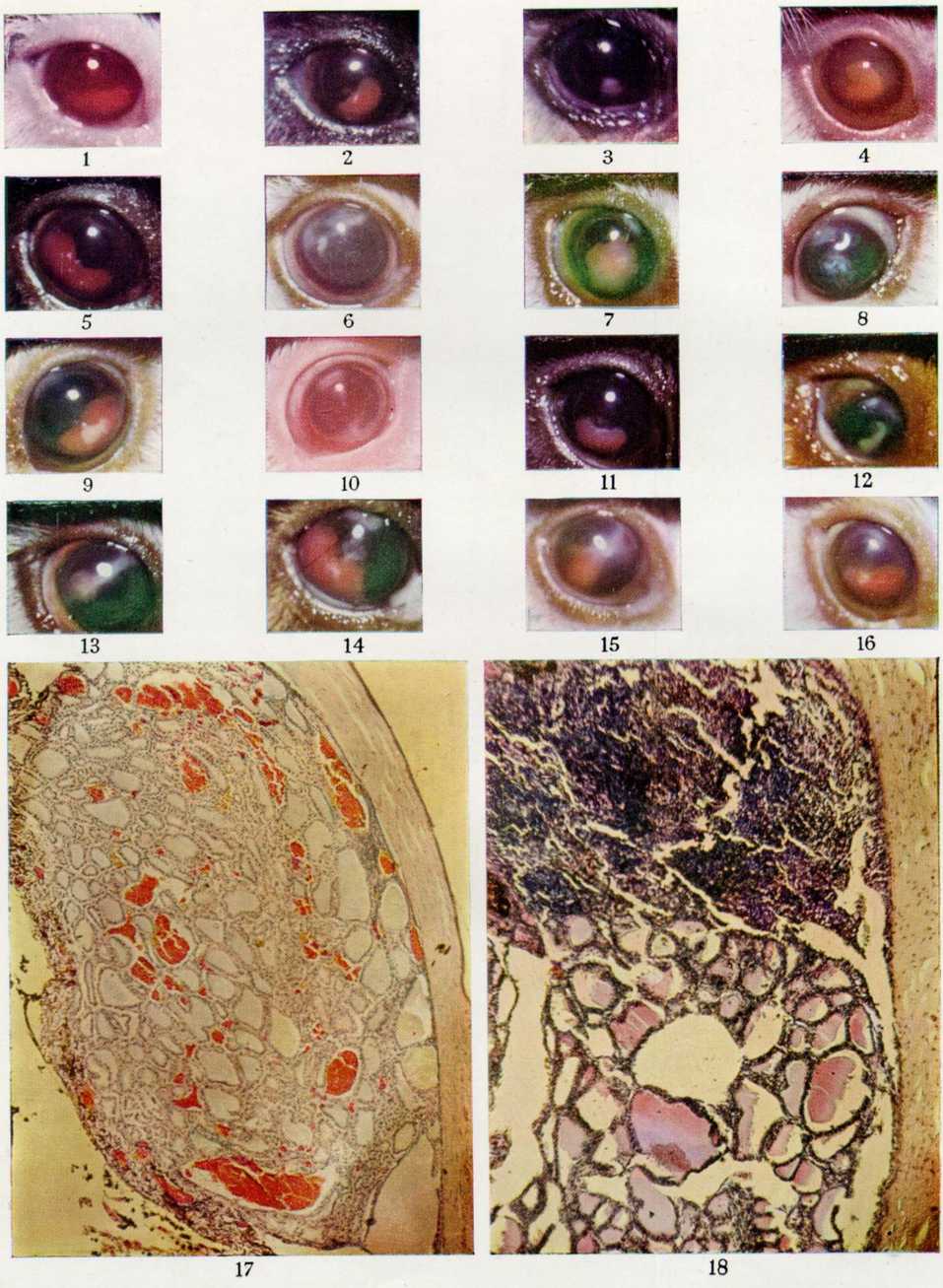

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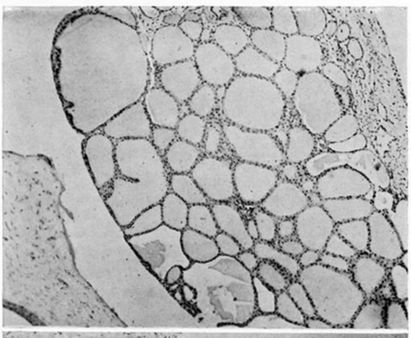

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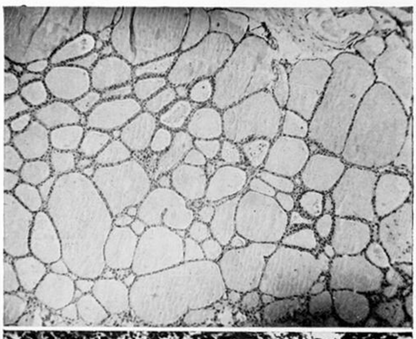

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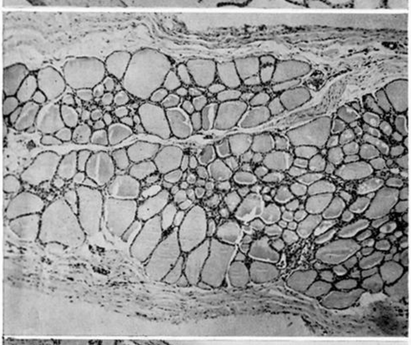

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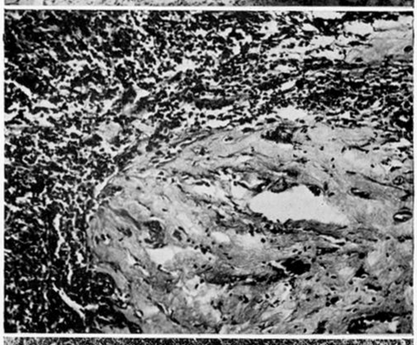

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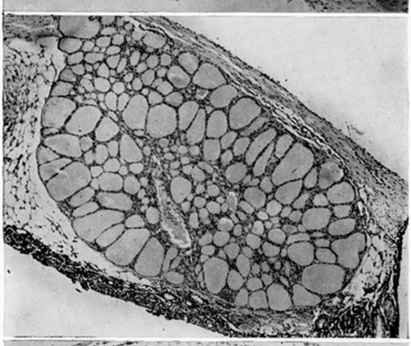

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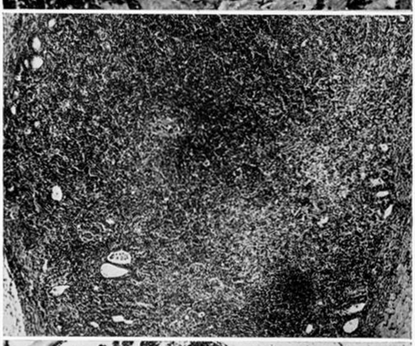

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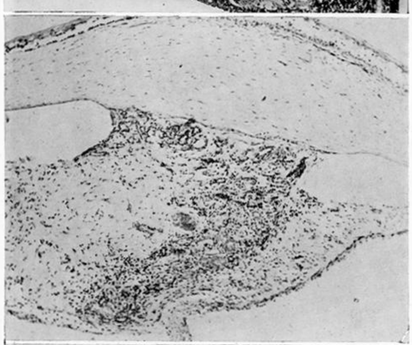

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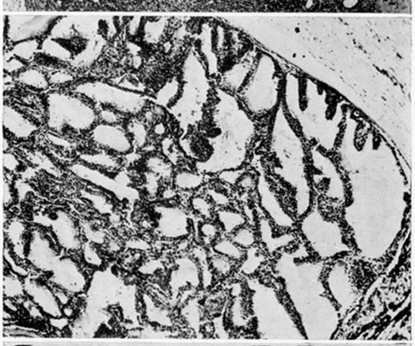

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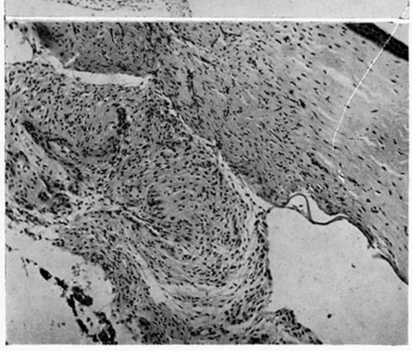

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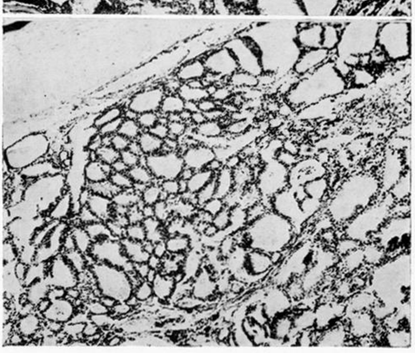

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Plate 29

Figure 19. Animal no. 77. Experiment I, 1 (i) (*a*). Section (× 46) through anterior chamber showing successful thyroid autograft 422 days old.

Figure 20. Animal no. 125. Experiment I, 1 (ii) (*a*). Section (× 50) of successful subcutaneous thyroid autograft 216 days old.

Figure 21. Animal no. 307. Experiment I, 1 (ii) (*b*). Section (× 46) of successful subcutaneous autograft 112 days old.

Figure 22. Animal no. 520. Experiment I, 2 (i) (*b*). Section (× 107) of part of unsuccessful thyroid homograft in anterior chamber 61 days old, showing connective tissue and lymphocytes but no recognizable thyroid.

Figure 23. Animal no. 296. Experiment I, 2 (i) (*e*). Section (× 43) through anterior chamber showing successful thyroid homograft 81 days old (i.e. 51 days after injections of pituitary extract had ceased). The graft is attached to the iris and partly enclosed in a capsule of connective tissue.

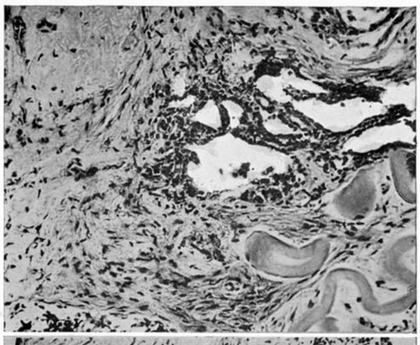
Figure 24. Animal no. 116. Experiment I, 2 (ii). Section (× 43) of unsuccessful subcutaneous thyroid homograft 42 days old, showing gross lymphocytic infiltration and a few just recognizable thyroid acini.

Figure 25. Animal no. 503. Experiment II, 1 (ii) (*a*). Section (× 50) through anterior chamber showing unsuccessful thryoid homograft 98 days old, consisting of connective tissue attached to iris and cornea and containing a few thyroid acini and some lymphocytes.

Figure 26. Animal no. 496. Experiment II, 1 (iii). Section (× 46) through anterior chamber showing successful thyroid homograft 99 days old.

Figure 27. Animal no. 467. Experiment II, 2 (i). Section (× 64) through anterior chamber showing unsuccessful thyroid homograft 134 days old, consisting of a small piece of connective tissue attached to iris and cornea.

Figure 28. Animal no. 484. Experiment II, 2 (ii). Section (× 50) through anterior chamber showing successful thyroid homograft 117 days old.



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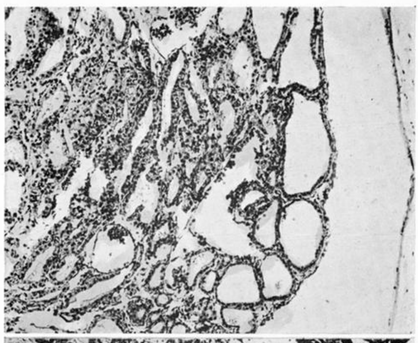

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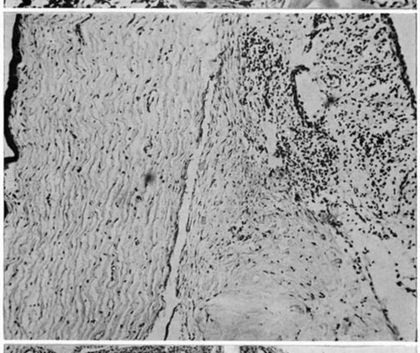

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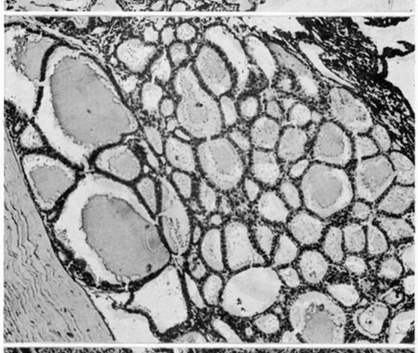

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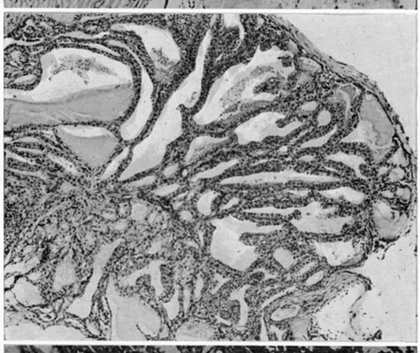

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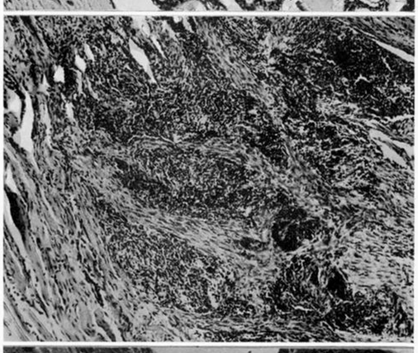

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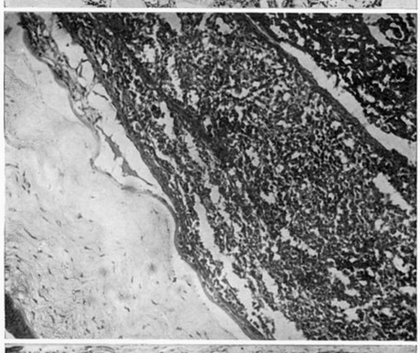

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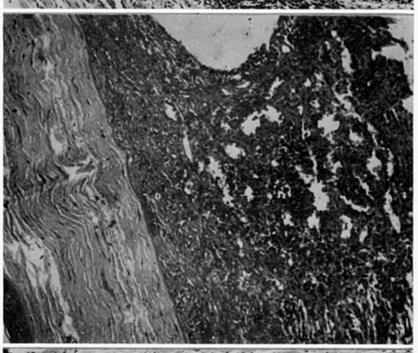

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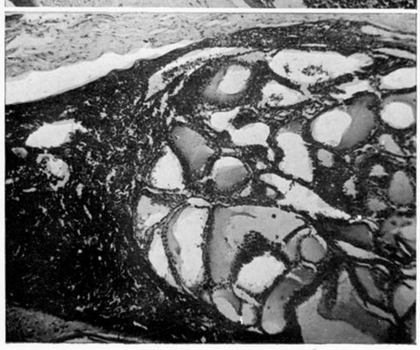

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Plate 30

Figure 29. Animal no. 442. Experiment II, 3 (i). Section (×86) of part of unsuccessful thyroid homograft in left anterior chamber 100 days old, showing connective tissue with a few recognizable thyroid acini.

Figure 30. Animal no. 415. Experiment II, 3 (ii). Section (×75) of successful thyroid homograft in left anterior chamber 168 days old.

Figure 31. Animal no. 450. Experiment II, 3 (ii). Section (×57) through left anterior chamber showing unsuccessful thyroid homograft 135 days old, consisting of connective tissue with a few lymphocytes.

Figure 32. Animal no. 365. Experiment II, 4 (ii). Section (×57) through anterior chamber showing successful thyroid homograft 216 days old (i.e. 193 days after this animal received a subcutaneous homograft).

Figure 33. Animal no. 86. Experiment III, 2. Section (×50) of thyroid homograft 44 days after successful transfer from anterior chamber to a subcutaneous site.

Figure 34. Animal no. 515. Experiment III, 2. Section (×57) of thyroid homograft 30 days after unsuccessful transfer from anterior chamber to a subcutaneous site, showing connective tissue infiltrated with lymphocytes but no recognizable thyroid.

Figure 35. Animal no. 388. Experiment IV, 1. Section (×86) through anterior chamber showing splenic autograft 210 days old.

Figure 36. Animal no 389. Experiment IV, 2. Section (×91) through anterior chamber showing splenic homograft 210 days old.

Figure 37. Animal no. 374. Experiment IV, 3. Section (×64) through anterior chamber showing autografts of spleen and thyroid 201 days old. The grafts are in contact but sharply demarcated.

Figure 38. Animal no. 459. Experiment IV, 4. Section (×53) through anterior chamber showing disintegrating thyroid homograft 96 days old, with gross lymphocytic infiltration. The adjacent splenic autograft is not included in the section.