PARATHYROID TRANSPLANTS IN RATS

A Comparison of Their Survival Time with That of Skin Grafts*

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The specific reaction generated by an animal against a graft of living tissue received from another member of its species is now generally agreed to be on an immunologic basis. Although the anterior chamber of the eye and those areas within the meninges of the central nervous system are probably examples of exceptions (1, 2) the homograft reaction is an anatomically generalized phenomenon ordinarily (3). The substances appreciated as antigens by the recipient of a homograft have not been finally identified (4), but considerable evidence is at hand which indicates that the genetic difference between donor and recipient can be sensitively revealed by the rejection of a graft transplanted between them (5, 6).

Since genetic control of tissue antigenicity seems so well established it is logical to expect that various tissues of the same individual might contain closely similar complements of antigens and that all would be rejected in roughly the same way. For practical reasons skin has been the tissue most intensively studied in grafting experiments, and the timing and vigor of the reaction have been most thoroughly described in such superficial grafts. The technical problems surrounding the proper assessment of viability of buried grafts have raised obstacles to obtaining comparable information for other tissues. This was recognized as a relative limitation, for example, in the adrenal grafting experiments of Medawar and Russell (7). One specific means of determining the functional status of buried grafts was described by Billingham and Parkes (8), who effectively used the state of vaginal cornification of the ovariectomized recipient as a test of ovarian graft survival in rats.

Much of the presently available information concerning parathyroid grafts derives from clinical experience in humans which has repeatedly provided suggestive evi-

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dence of successful homologous grafts (9–13). Christiani and Christiani (14) have reported successes with rats. Pinkus, Maddock, and Coller (15), also using rats of uncertain genetic relationship, reached similar conclusions on the basis of the ability of parathyroid grafts to prevent tetany. Halsted's experience (16) with parathyroid grafts in the dog, unlike that of some others since (17, 18), was one of consistent homograft failure.

The experiments to be reported here were designed to take advantage of the powerful effect of functioning parathyroid tissue on calcium metabolism, an effect which readily lends itself to measurement. Using this functional criterion of graft viability we have sought critical information about the survival of parathyroid grafts between rats and have compared their longevity to that of skin.

Plan and Methods of Experiments

Two groups of rats were used in these experiments. One consisted of adult members of the partially inbred Wistar strain[1] weighing 250 to 300 gm. The other group of animals were adult males of a widely diverse brown and white hooded strain[2] weighing 300 to 450 gm.

Parathyroidectomized Wistar female rats were used throughout as graft recipients. All recipients were maintained on a diet extremely low in calcium but deficient in no other respect (see below).

Three classes of experiments were done:

(*a*) Autografts (Wistar). (*b*) Homografts between rats having the greatest genetic diversity available (hooded to Wistar). (*c*) Homografts between rats having extensive genetic overlap (Wistar to Wistar).

Parathyroidectomy and Its Effects.—With intraperitoneal nembutal anesthesia supplemented by ether, the two parathyroid glands were exposed through a ventral midline cervical incision, according to the method first described by Erdheim (19). After separating the strap muscles the dark red thyroid lobes became visible just posterior to the larynx. On the anteroventral surface of each a discrete pearly-white parathyroid was usually visible under the dissecting microscope. Those animals in which two distinct parathyroids could not be readily identified (about 20 per cent) were excluded from the experiment. Removal was accomplished by sharp dissection, sparing all but a very small piece of thyroid. Our experience with the strains of animals we have used confirms that of Hoskins and Chandler (20) who reported accessory parathyroid tissue in less than 10 per cent of rats. The normal serum calcium of adult rats on a regular laboratory diet ranges from 9 to 10 mg. per 100 ml. We have also confirmed the observation of Davies, Gordon, and Mussett (21) that although parathyroidectomized animals exhibit little disability even on several months of observation, the serum calcium falls moderately to levels of about 7.5 to 8 mg. per cent in less than a week. As Erdheim indicated, however, over a period of several weeks there may be some softening and breakage of the incisor teeth which grow continually in the usual rodent fashion.

Munson's work (22) has elegantly revealed that maintenance on a very low calcium diet

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[1] Our Huntington Laboratory Wistar rats have been maintained for 4 years with no attempt at inbreeding since they were obtained from Dr. F. Hisaw's Harvard strain which has been under his observation as a closed colony, with no lengthy periods of inbreeding, for over 30 years. This strain was originally developed at the Wistar Institute, Philadelphia.

[2] Obtained from the White Animal Farm, Pine Point, Maine, where they have been maintained for some 30 years without inbreeding and with repeated introduction of new genetic material into the colony.

greatly enhances the changes brought about by parathyroidectomy, particularly in young (50 to 60 day old) rats. In as short a time as 4 hours the serum calcium falls precipitously to about 4.5 mg. per cent where it remains through the rapid onset of tetany which soon ends in death. Munson's low calcium diet, modified from Shaw (23) by the substitution of calcium-deficient salts, is essentially free of calcium, but includes ample amounts of all other necessary ingredients.[3]

We have found that by the age of 6 to 10 months normal rats tolerate this low calcium diet for many weeks with no detectable symptoms, probably because of their heavier and more stable bones, which can meet the demands of the activated parathyroids with no change in serum calcium levels. Furthermore, rats of this age which have been on an initial low calcium diet for 5 to 8 days show a much slower serum calcium response to parathyroidectomy than younger animals. In about 4 days the serum calcium reaches a stable plateau at values of 3 to 5 mg. per cent. Death rarely results from these strikingly low levels, and frank tetany is unusual. Nevertheless these parathyroidectomized rats have a distinct early loss of as much as 15 per cent of body weight which then stabilizes in the first few days after operation. Some degree of muscular hyperexcitability can also be demonstrated. One convenient test for this consists of lifting the animal off the top of its cage so that its legs are suspended. The normal rat holds its legs quietly in a somewhat flexed attitude, whereas the adult parathyroidectomized rat wildly bats the air in simulated swimming movements. The weight changes and "tail test" have been helpful adjuncts in evaluating the status of newly parathyroidectomized rats.

Calcium Determinations.—Approximately 1.5 ml. of blood is collected for each set of calcium determinations from the transected tip of the tail. Storage of serum in siliconed tubes completely prevents the prompt fall in measurable calcium ion concentration which has been observed with storage in untreated glassware (24). Freezing in tightly stoppered siliconed tubes allows indefinite storage.

Calcium determinations are done in duplicate by titration of a 0.2 ml. serum sample with ethylenediaminetetraacetate (EDTA) directly in a cuvette in place in a spectrophotometer using ammonium purpurate as indicator according to the method of Munson, Iseri, Kenny, Cohn, and Sheps (25). This method has been eminently satisfactory in our hands. In 270 consecutive pairs of assays on 270 different sera the mean difference of the duplicate values was 0.16 mg. per cent with a standard deviation of 0.12 mg. per cent. Values expressed below are the mean of duplicate determinations.

Parathyroid Grafting.—Following removal in the manner described above, each parathyroid gland was isolated as well as possible from surrounding bits of connective tissue and thyroid substance and was kept for a few minutes in Hanks' solution until used in grafting.

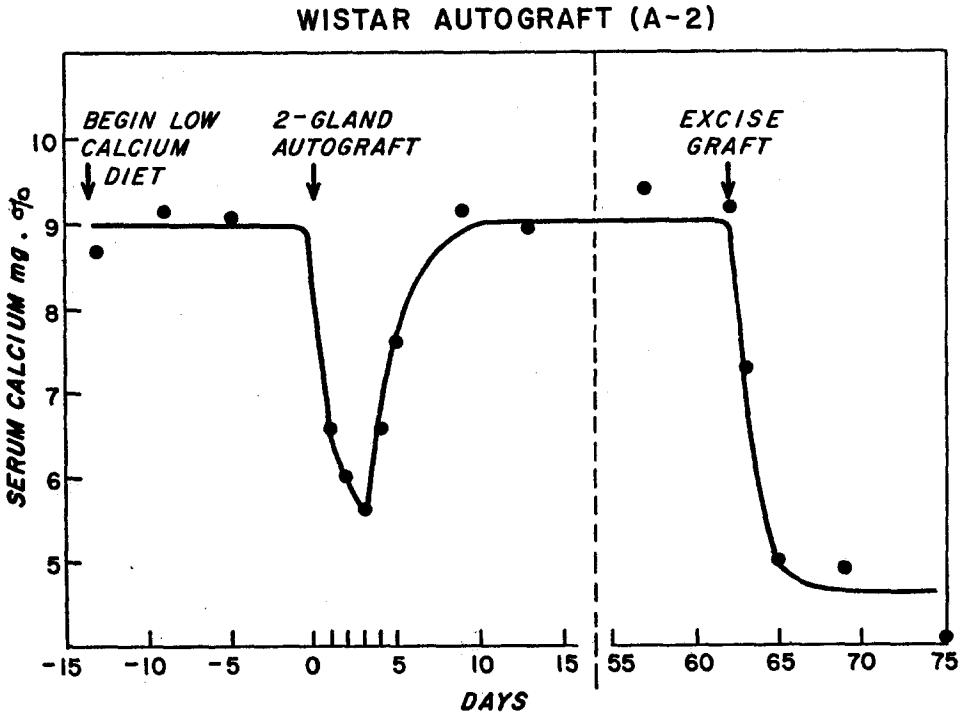
Whole parathyroids were employed as grafts since their minute size allowed sufficiently rapid vascularization to prevent ischemic necrosis (see Fig. 1). One or two parathyroids were grafted at a single time according to the plan of the individual experiment. Each graft was placed into a small pocket either just beneath the muscle fascia or in the substance of the hamstring muscles prepared with sharp forceps through a 1 cm. longitudinal skin incision on the posterior thigh in a fashion similar to that used in adrenal grafting by Medawar and Russell (7). The incision was closed with a single fine silk suture in the fascia and another in the skin, the deeper suture serving as a guide for the later recovery of the graft.

Histologic preparation of all tissues was carried out by fixing in 10 per cent formalin, embedding in paraffin, sectioning at 8 to 10 μ and staining with hematoxylin and eosin.

Skin grafts.—Skin grafts were done according to a modification of the technique designed for mice by Billingham and Medawar (26). With intraperitoneal nembutal anesthesia sup-

[3] We gratefully acknowledge receipt of the low calcium diet for our rats from Dr. Paul L. Munson of the School of Dental Medicine, Harvard University, Boston.

plemented by ether, skin was removed from the amputated ear of the donor by peeling both dorsal and ventral cutaneous surfaces from the cartilage as two half circular pieces. Residual connective tissue and cartilage were removed as well as possible by gently scraping the under surface of the graft, inverted on saline-moistened filter paper, to give two roughly hemicircular pieces of thin, anatomically full-thickness skin. The two pieces from a single ear were placed together as a single oval graft in open style upon a bed prepared on the lateral thoracic wall of the recipient by removing the integument down to, but not including,



TEXT-FIG. 1. Serum calcium changes in a Wistar rat which accompany total parathyroidectomy and immediate transplantation of the glands to the hamstring muscles as autografts. Later excision of the grafts is followed by a prompt fall in calcium to base line levels.

the panniculus carnosus layer. A similar autograft was usually placed on the same bed as a technical and histologic control. Grafts were maintained under dressings. These consisted of tulle gras and gauze roller bandage surmounted by a light plaster sheath. Inspections were carried out at regular intervals under anesthesia and similar dressings replaced except when the grafts were solidly healed in place and no open areas remained. Photographic records and histologic studies were obtained at representative times after grafting.

RESULTS

Autografts (Wistar).—

Five Wistar parathyroid autografts were made. Two weeks after the institution of the low calcium diet, during which time the normal calcium base line

level was established, both parathyroids were removed and immediately transplanted to the thigh muscles. Thereafter the serum calcium level of each animal was closely followed. There were no technical failures in this group. Text-fig. 1 portrays the results obtained in a single animal representative of the group. Graft function becomes evident between the 3rd and 4th postoperative day, corresponding to the time of vascularization in the morphologic studies of skin grafts reported by Taylor and Lehrfeld (27) and of skin and thyroid grafts by Edgerton and Edgerton (28). Excision of the grafted tissue (at 62 days in the case represented by Text-fig. 1) results in a typical parathyroidectomy curve and proves that the autograft was indeed responsible for maintaining the normal serum calcium level.

Histologically an established autograft appears as a thinly encapsulated gland, sometimes with a few thyroid follicles attached (Fig. 1). It is well approximated to surrounding structures, is richly vascularized, and is devoid of any ischemic necrosis or inflammatory cell infiltration. Prominent connective tissue septa sharply define lobules of parenchymal cells with clear basophilic cytoplasm.

Homografts (Hooded-to-Wistar).—

Twenty standard skin grafts were transplanted from male hooded donors to female Wistar recipients, all selected at random. They were uniformly rejected between the 13th and 16th day, the criterion for rejection being complete loss or necrosis of epithelium histologically. Five had been transferred in the presence of the low calcium levels of parathyroid insufficiency which appeared to have no influence on the skin survival time. Six "second set" grafts between the same donor and recipient pairs reached the end point in 6 to 9 days in a characteristically violent inflammatory reaction. These data are in close agreement with those of Woodruff and Simpson (29) who used rats of strains very similar to ours. They attest to the antigenic disparity between donors and recipients.

Nineteen Wistar females were prepared as prospective parathyroid graft recipients by parathyroidectomy and maintenance on the low calcium diet. After establishing a satisfactory low serum calcium base line level each of these animals received either one or two parathyroid gland grafts from a male hooded donor into the hamstring muscles.

Table I contains the results obtained in this group. Four animals failed to show an appreciable rise in serum calcium after grafting. These were considered technical failures,—one probably on the basis of local infection,—and hence they were excluded from further consideration. In all of the other fifteen the serum calcium rose significantly by the 4th day and continued to climb sharply thereafter to the normal range. This calcium rise was invariably accompanied by a gain in body weight and a change of "tail test" from positive to negative. The use of only a single parathyroid for transplantation, which was done in ten instances, appeared to influence neither the likelihood of a successful graft

nor its functional effectiveness. Following this early evidence of functional activity, the grafts fell into two distinct groups. Four were rejected between the 9th and 14th day after grafting with a rapid decline in serum calcium over a 3 to 4 day period quite analogous to that expected after surgical parathyroidectomy. Text-fig. 2 illustrates the course of the serum calcium changes in one of these. The time of parathyroid rejection has been defined as that at which the sharp downward inflection of serum calcium concentration begins. The remaining eleven animals differed strikingly in that they maintained persistently normal serum calcium levels and appeared generally to be entirely normal.

The four animals which spontaneously rejected their parathyroid grafts were used to gain some insight into the immunizing capacity of parathyroid tissue since it might have been expected to be maximal in this group.

TABLE I

The Fate of Hooded-to-Wistar Parathyroid Homografts

	Spontaneous rejection	Rejection induced by skin homograft	Random group for long term survival	Technical failures	Total
No. of animals	4	6	5	4	19
Time of functional failure, *days*	9, 14, 14, 14	40, 40, 42, 50, 55, 57	52,* 81,* 120,‡ 120,§ 141*		

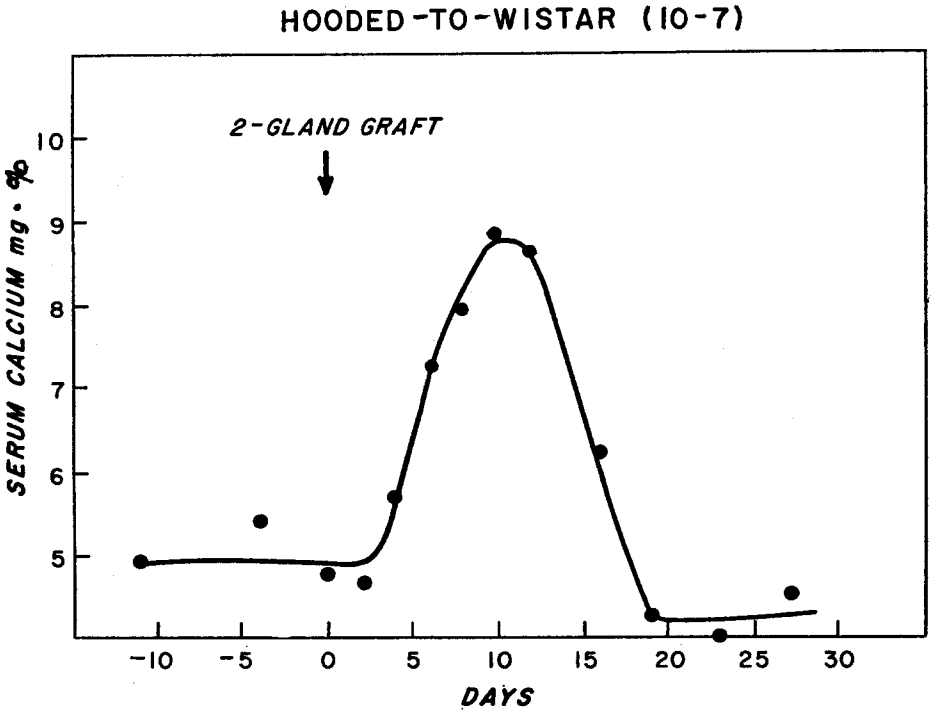
* Died with normal serum calcium concentration.

‡ Graft excised.

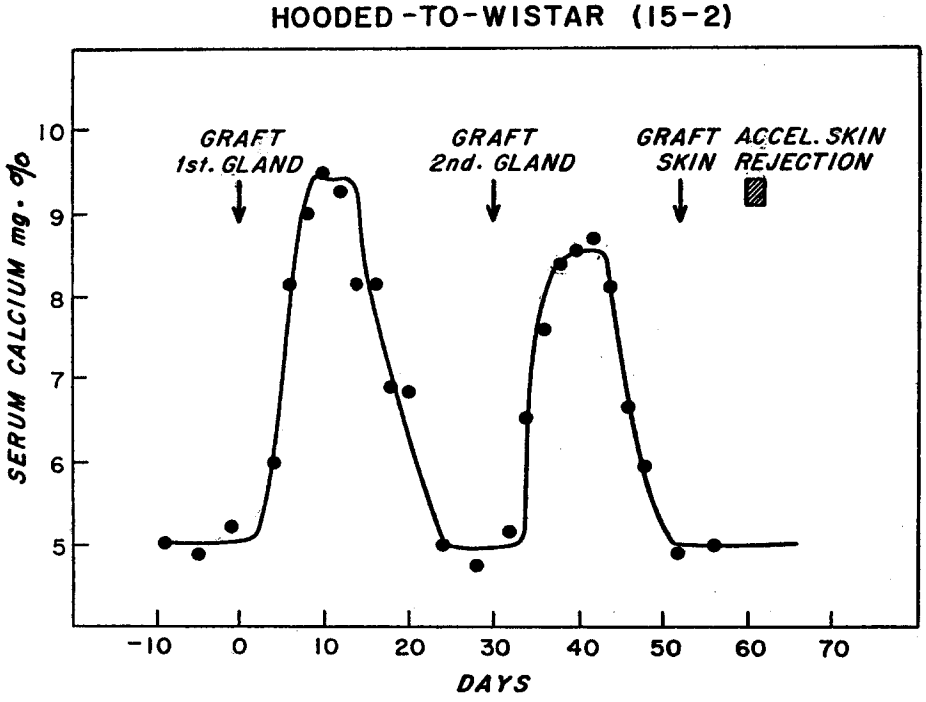
§ Graft ceased functioning with rejection of skin homograft.

Two of the rats had rejected grafts of both of the donor's parathyroids simultaneously. Fifteen days after this rejection was complete, as indicated by the return of the serum calcium to the previous low base line level, they were grafted in the standard fashion with ear skin from the original gland donors. One skin graft was rejected in 12 and one in 14 days, both showing changes not definitely distinguishable either in the gross or histologically from a brisk first set reaction. The other two animals, having rejected grafts of a single parathyroid, were grafted with the second gland from the same donor in the contralateral thigh. Only one proved technically successful, but the findings were significant and are accordingly presented in Text-fig. 3. The second set parathyroid graft did not show signs of functional failure until 14 days after grafting, as did the first, although it was less vigorous by a significant degree during its functional period. No definite indication, therefore, was seen of an accelerated response at this time. Finally this same animal (No. 15-2) received a standard skin graft from its parathyroid donor. This test skin graft suffered a distinctly accelerated rejection, in the gross and microscopically, with total sloughing of epithelium at 9 days.

It appears that active immunity to skin *can* be induced by parathyroid grafts in this experimental arrangement.



TEXT-FIG. 2. Spontaneous rejection of a hooded parathyroid graft by an untreated Wistar host.

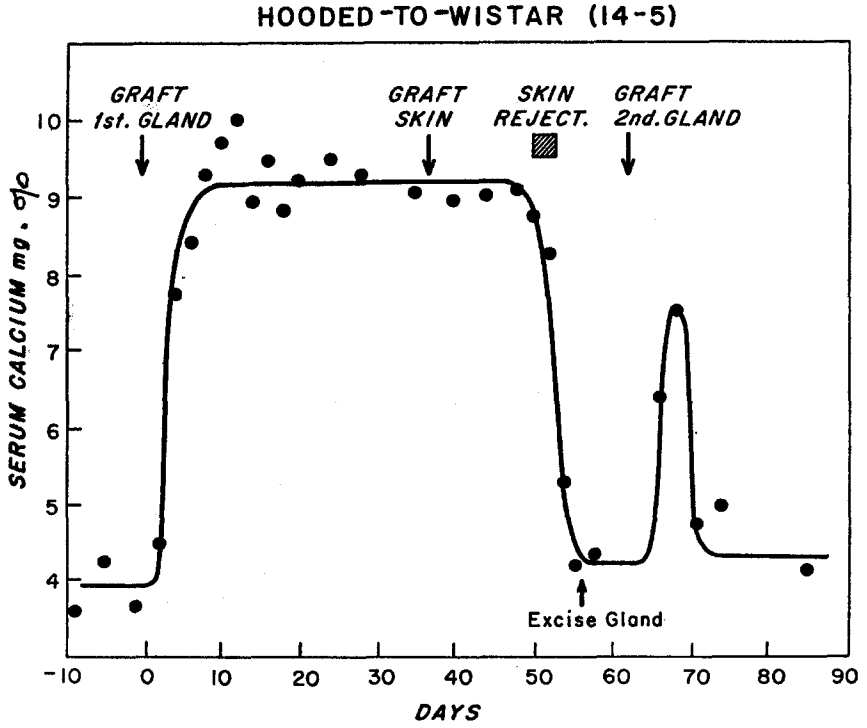


TEXT-FIG. 3. Results of independent first and second parathyroid grafts from same hooded donor to Wistar recipient, with later accelerated skin graft rejection.

The larger group of eleven animals in which parathyroid grafts had not been spontaneously rejected were treated as follows:—

(*a*) Six were strongly immunized to the skin of their appropriate parathyroid donors by means of a standard skin homograft. (*b*) Five were chosen at random for long term observation, the observation being terminated as detailed below.

In every instance those animals receiving skin grafts promptly rejected them in typical fashion in 13 to 16 days. At the time of rejection of the skin the parathyroid



TEXT-FIG. 4. Results of rejection of surviving hooded parathyroid graft by Wistar recipient, induced by skin graft from same donor. Subsequent accelerated reaction to second parathyroid of same donor. Histologic appearance of excised first parathyroid graft can be seen in Fig. 2.

grafts, which had been surviving for between 40 and 57 days, promptly ceased functioning as indicated by a fall in serum calcium levels in each animal, similar to that after surgical parathyroidectomy. A fully representative example of this group of rats is illustrated in Text-fig. 4.

It is evident that a graft of hooded parathyroid tissue which fails to succumb to a spontaneous homograft reaction will nevertheless succumb promptly in the presence of skin induced immunity. Since three of the animals had originally

been grafted with only a single gland it was possible to follow the immunizing skin graft with a graft of the second parathyroid, all from the appropriate donor animal. In all three the second parathyroid functioned well for a short period of time, the longest survival being 9 days as determined by the sharp downward inflection of the serum calcium curve (again see Text-fig. 4). The inflammatory reaction responsible for the destruction of the first parathyroid graft in this animal, removed as indicated in the text-figure, is seen in Fig. 2.

In a separate experiment three parathyroidectomized Wistar females were first grafted with standard ear skin grafts. Ten days following rejection of these grafts, a process which again was completed by the 13th to 16th day, they received parathyroid grafts from the same donors. All showed a sharp curtailment of incipient function by 5 or 6 days. Text-fig. 5 illustrates the accelerated response which occurred in one of these recipients. The survival times of parathyroid grafts to rats previously sensitized specifically to the skin of their parathyroid donors are recorded in Table II.

All of a group of 5 animals with functioning parathyroid grafts selected for longer observation gave every indication of continuing graft function as long as they were

TABLE II

Survival Times of Hooded Parathyroid Grafts in Previously Immunized Wistar Recipients

Previous graft rejected	Skin only	Skin plus one parathyroid
Parathyroid rejection time, *days*.....	4, 6, 6	6, 6, 9

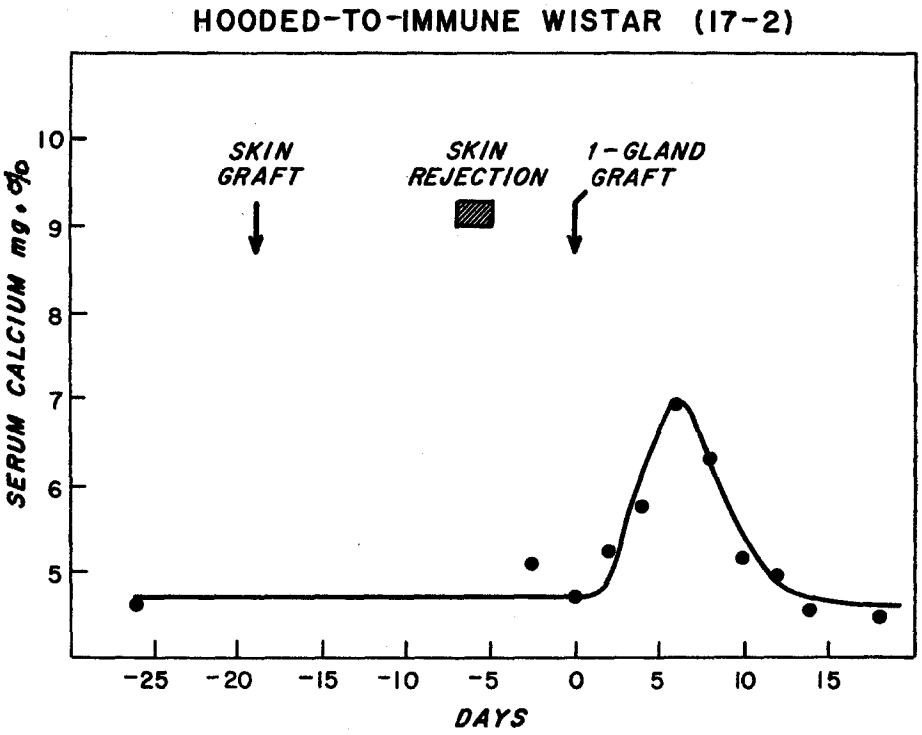
allowed. Three died while under observation (see Table I), the last 141 days after receiving its parathyroid graft. Histologic examination of these grafts showed that some may elicit no detectable inflammatory response whatever, as in the case represented in Fig. 3, a gland which was removed 120 days after grafting. In some, however, in spite of apparently quite normal parathyroid function, a very mild inflammatory reaction can be seen. As Fig. 4 demonstrates, this may consist of a relatively sparse collection of lymphoid cells about the capsule of the grafted parathyroid with virtually no cellular infiltration into the substance of the graft.

At 120 days persistent function of one of the long-surviving grafts was confirmed by a typical parathyroidectomy serum calcium curve after its removal. The final animal of this group, also bearing a functional graft for 120 days, served to show that its graft had continued to produce specific antigens since it promptly succumbed to the immunity induced by a skin graft from its parathyroid donor in the same manner observed in those animals receiving skin grafts at an earlier time.

Homografts (Wistar-to-Wistar).—

Thirteen parathyroid grafts, of fifteen attempts, proved technically successful in this experiment. Recipients were parathyroidectomized Wistar females throughout. Donors were Wistar adults selected at random. Eleven of these received skin grafts

from their parathyroid donors, either simultaneously with parathyroid grafting (in nine animals) or at a time when the parathyroid graft had long been functioning (two animals). The remaining two parathyroid recipients of the group of thirteen received no skin grafts. In one of these latter two, the parathyroid tissue ceased functioning spontaneously at 35 days. In the other the parathyroid grafts continued with full evidence of functional survival until removed at 110 days after grafting. Function of



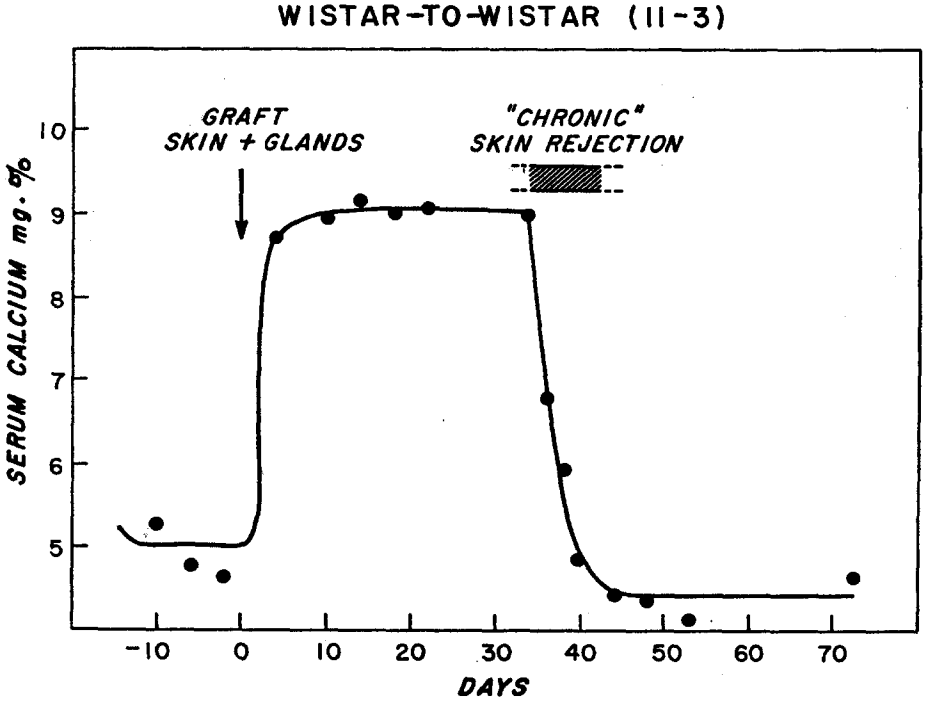
TEXT-FIG. 5. Results of accelerated destruction of a parathyroid graft in an animal which had previously received sensitizing skin graft.

the grafted tissue through this extended period was confirmed by a prompt fall in serum calcium values to the previously established base line after its removal.

The remaining eleven animals with coexisting skin and parathyroid grafts form the bulk of our experience with this genetic combination. All the skin grafts were eventually rejected, although there was a considerable range in both the vigor and timing of the response. The earliest rejection, at 14 days, was accompanied by erythema, swelling, and necrosis, observable in the gross, of epithelium with sloughing over a period of 2 days. The spectrum of response extended from this acute reaction to a slow, almost "chronic" rejection at about 60 days requiring as long as 2 weeks for the progression of mild erythema, alopecia, and punctate loss of epithelium to progress by gradual contraction of the graft to its final end stage as a small, mummified scab. The

point at which definite reduction of the graft area occurs, as determined by serial tracings of its outline, has been arbitrarily taken as a fairly well defined phase of rejection to be designated as the "end point." The progress of such a rejection phenomenon is shown in Fig. 5.

Eight recipients showed sharp cessation of parathyroid function with a rapid decline in serum calcium at the time that skin graft rejection became apparent, in a manner quite similar to that found throughout the hooded-to-Wistar group. Nevertheless functional rejection of parathyroid tissue has in no case been of the "chronic" type



TEXT-FIG. 6. Evidence of rapid cessation of parathyroid function concurrently with early evidence of "chronic" skin graft rejection (see Fig. 5 for appearance of skin graft).

often seen in skin in these experiments. The data from one of these instances are portrayed in Text-fig. 6 which records the rapid serum calcium changes which occurred with the slow rejection of the skin homograft photographed in the gross in Fig. 5.

In the remaining three recipients of both parathyroid and skin, the skin grafts were considered rejected entirely in typical fashion at 18, 36, and 60 days after grafting (see Table III). In all of these, however, the parathyroid grafts continued to function, after complete skin rejection, in sharp contrast to all previous experience. One of these animals died 10 days after final sloughing of the skin graft, having maintained a normal serum calcium. The other two received secondary skin grafts from their appropriate donors. Both responded with a typical accelerated or "second set" response.

Nevertheless, in both instances the parathyroid grafts continued to function actively. Finally removal of the glands from their hamstring muscle sites at 18 and 30 days after completion of the accelerated skin graft rejection resulted in a prompt fall of serum calcium levels to the base line figure. The course of one of these animals is depicted in Text-fig. 7.

These findings indicate, therefore, as Billingham and Hildeman have recently reemphasized (30), that when the genetic difference between donor and recipient is small the survival of skin grafts is correspondingly increased, the longer survival times probably occurring when more closely related animals are selected from a partially inbred, and therefore still heterogeneous, population. These experiments show that reaction to parathyroid grafts can be so debilitated

TABLE III

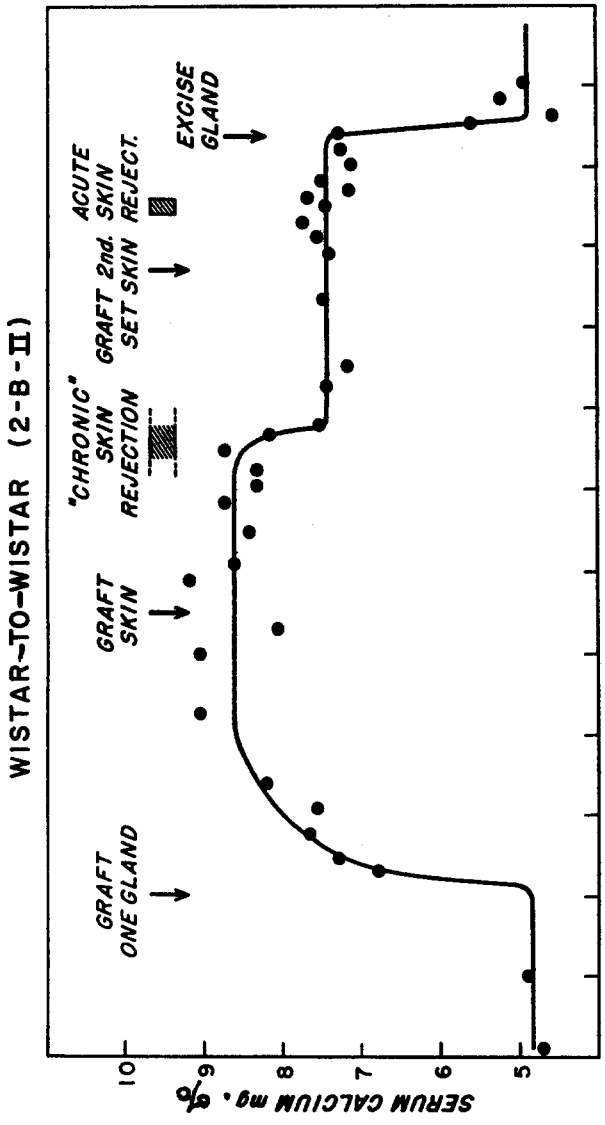
Concurrent Skin and Parathyroid Grafts (Wistar-to-Wistar)

	Simultaneous rejection of skin and parathyroid	Skin rejection with parathyroid persisting
No. of animals	8	3
Skin rejection time, *days*	14, 15, 16, 18, 18, 28, 35, 36	18 (1st set only) 38 (1st set) 16 (2nd set) 60 (1st set) 25 (2nd set)

as to allow their functional survival in the presence of the state of heightened reactivity entailed in a second set response to skin from the same donor.

Histologic study of parathyroid grafts in this group of experiments reveals that functional failure of grafted tissue is again accompanied by an active local inflammatory reaction with central necrosis and hemorrhage into the graft. Fig. 6 demonstrates such an active response to a Wistar parathyroid graft by its Wistar recipient 18 days after simultaneous grafting with both parathyroid and skin from the same donor. At this time the active skin graft rejection had just reached the point of complete epithelial necrosis while the parathyroid graft demonstrated in the figure had begun to show early signs of functional failure. The serum calcium at the time it was removed had fallen from 9.6 mg. per cent to 8.2 mg. per cent in 2 days.

In those cases in which the parathyroid outlived both first and second set skin grafts the reaction, although present, was much more subdued. Fig. 7 shows such a parathyroid graft removed from animal 2-B-II at a time indicated appropriately in Text-fig. 7, which outlines the course of the serum calcium



TEXT-FIG. 7. Evidence of functional survival of Wistar parathyroid graft in spite of accelerated rejection of skin. Gland removed 18 days after completion of second set skin graft rejection is shown histologically in Fig. 7.

changes in this animal. The graft was functioning, although at less than optimal efficiency in spite of the visible inflammatory reaction to it. The reaction consisted of a heavy lymphocytic infiltration about the graft and along the connective tissue septa of the gland, largely sparing the glandular acini. A biopsy of the second set response to grafted skin, which had occurred 18 days before removal of this parathyroid graft from the animal, is seen in Fig. 8. This demonstrates the concurrent appreciable degree of sensitivity to skin in the same animal.

DISCUSSION

Although the hooded and Wistar rats used in these experiments were of demonstrated genetic disparity by virtue of the Wistar animals' rapid and uniform rejection of hooded skin grafts, parathyroid tissue provoked a similar reaction in a minority of animals. Even those grafts which survived vigorously for a long period, however, could be destroyed by a sufficient level of immunity brought about by a skin graft from the same donor source. Thus it seems inescapable that all of the parathyroid grafts from hooded donors released some antigens foreign to their Wistar recipients, and that at least some of these antigens were shared by the donor skin.

What could explain the striking fact that two distinctly different types of response to parathyroid grafts occurred; *i.e.*, prompt rejection or long term survival? It might be supposed that the behavior of these small bits of grafted tissue depended markedly on the vagaries of the local anatomic situation of the graft in the recipient, particularly its state of vascularization. This is a point which has been particularly investigated by Merwin and Hill (31). On removal from its hamstring muscle site each graft was carefully examined. All were found in the gross to be vascularized, a fact which was later confirmed histologically. Although it is difficult to completely exclude the influence of the local anatomic state we have found nothing to substantiate its importance in these experiments.

Broadly, therefore, the possible explanations for the extended survival time of some parathyroid grafts should lie between:—

(*a*) Host inability to respond in some cases. (*b*) A high degree of graft resistance to the host response. (*c*) Weak antigenicity of the graft.

Combinations of these possibilities might also occur. As an independent explanation the first of those cited can be dismissed because of the uniform and active response on the part of every recipient to grafted skin. The second is made unlikely by virtue of the rapid and complete loss of parathyroid graft function in the presence of skin-induced immunity. Although long surviving grafts may be focal points for the collection of small numbers of lymphoid inflammatory cells, they also may elicit no detectable reaction whatever. High resistance of these grafts to an existing level of immunity, therefore, can hardly be a sufficient explanation for their survival.

Of the three choices, the third would appear to be the most likely. Weak antigenicity might, of course, be assumed on the basis of merely the amount of the grafted tissue as measured by gross weight, since parathyroid grafts are minute in size. This would not account for spontaneous parathyroid rejection in some cases, a thing which has occurred with grafts of only a single parathyroid. Alternatively weak antigenicity could be understood on the basis of a relative deficiency of effective transplantation antigens in parathyroid tissue as compared with skin. This might be a reflection of a deficiency in production, availability, or content of antigens.

Thus parathyroid, possibly as a consequence of its specialized character, might be limited as to its effective quota of the total content of transplantation antigens possessed by the animal in question, antigens more widely displayed, for example, in skin. A few "strong" transplantation antigens amongst the presumptive limited number could be responsible for a clearly demonstrable reaction to grafts of this tissue by a host not similarly endowed. Parathyroid tissue devoid of a sufficient number of such strong antigens would provoke, at best, a very weak response.[4] This concept gains some support from the fact that a detectably accelerated response to skin was not seen in any of the six Wistar animals with long surviving hooded parathyroid grafts even though the later rejection of the parathyroid tissue in the face of skin-induced immunity proved that it had contained antigens shared by skin. The converse of this, namely the accelerated rejection of hooded skin by a Wistar animal which had previously received, and reacted against, parathyroid tissue from the same donor was observed in one instance.

In the hooded-to-Wistar group the skin grafts were all rejected with maximal intensity even though the genetic relationship between the animals must have varied widely. Skin graft survival time, therefore, is an insensitive measure of genetic relationship at this dosage and range of genetic diversity. Parathyroid grafts, however, acting as they do at very small dosage, might conceivably reveal such closer genetic relationships by more prolonged survival. This explanation, which depends on the *combined* action of dosage with the genetic relationship of donor and recipient would not require the existence of any antigenic weakness on the part of parathyroid as a tissue.

The further evidence gained from grafts between members of the moderately inbred Wistar strain weighs against this last alternative. Here again considerable difference in the genetic dissimilarity between random pairs of animals is to be expected, just as it is between the two strains used. That the gap is considerably less, however, is shown by the more extended survival time of skin grafts between Wistar rats than in the hooded-to-Wistar combination. In this series of experiments, it was significant that in three instances parathyroid

[4] The terms "strong" and "weak" have been applied to transplantation antigens in mice by Counce, Smith, Barth, and Snell (32).

grafts outlived skin grafts from the same donor animal, and continued to function in spite of the presence of a state of sensitivity sufficient to dispose completely of skin. Longer survival of grafts of all tissues with less violent reactions might be expected between these more closely related animals, but if parathyroid and skin grafts of the same animal were entirely equivalent antigenically, except for dosage, no case of parathyroid survival after skin graft rejection should occur. Since parathyroid and skin share at least some antigens in common perhaps a higher level of immunity than that achieved by two sets of skin grafts,—if it could be achieved in this relatively inbred group, —would result in the destruction of these parathyroid grafts. If so, this event would not overturn the reasoning presented.

A relative weakness in antigenicity of parathyroid as a tissue would, of course, provide this tissue with an apparent resistance to existing immunity against it, since its effectiveness in localizing the elements of response would be correspondingly limited. As stated above, however, some antigenic material was certainly issuing from the Wistar parathyroid grafts, as from those of hooded parathyroids, in order to call forth the collection of lymphoid inflammatory cells visible histologically. The concentration of inflammatory cells about the periphery of the parathyroid substance and along the connective tissue septa between epithelial lobules raises the possibility that the active antigenic material may have been situated preponderantly in the non-epithelial components of the graft.

Although these findings hold out some hope of occasional clinical success for parathyroid grafting in human beings, they would argue against the method, otherwise attractive, of attempting parathyroid grafting with vascular anastomoses. Since this method requires transferring the entire thyroid and pretracheal tissues, the non-parathyroid portions of such composite grafts might well bring about the downfall of the parathyroids themselves.

CONCLUSIONS

1. A reliable method of parathyroid grafting in rats, and of assessing the functional state of such grafts in terms of blood calcium has been devised.

2. Parathyroid homografts between rats of proven genetic diversity may be rejected as promptly as skin; but the majority survive for an indefinite period, there being no intermediate class.

3. Surviving parathyroid grafts in this genetically diverse combination invariably succumb in the presence of the immunity induced by a later skin graft from the same donor.

4. Parathyroid grafts between rats of less genetic diversity have usually responded to skin graft–induced immunity in the same manner as those between more dissimilar rats. The less divergent group, however, revealed an occasional persistent functioning of parathyroid grafts even in the face of the sensitivity incidental to an accelerated reaction to skin.

5. The hypothesis is presented that the parathyroid tissue of the rats studied was deficient in effective transplantation antigens as compared with those of their skin. This weakness in apparent antigenicity may be a reflection of a deficiency in production, availability, or content of antigens in the tissue transferred.

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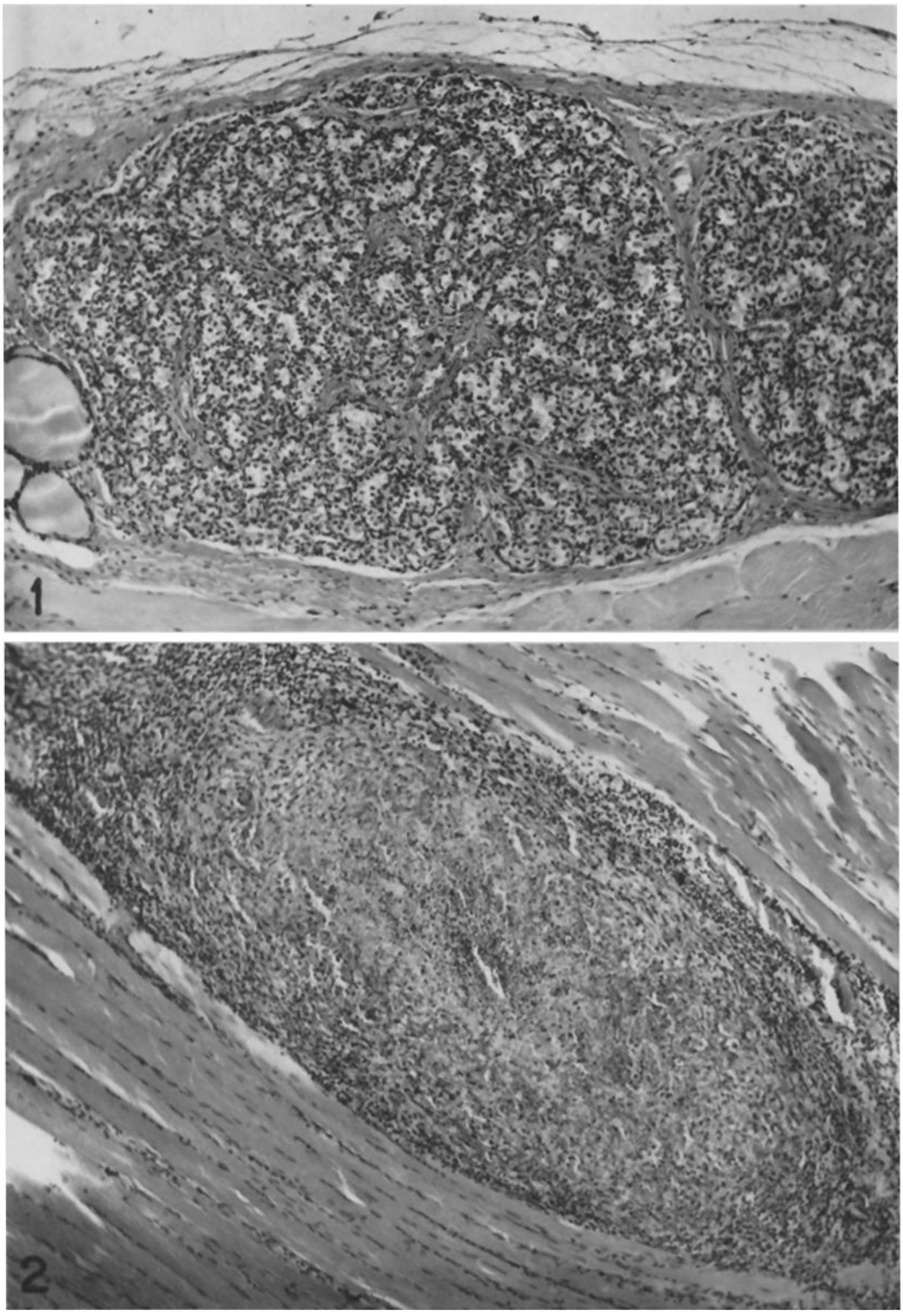
EXPLANATION OF PLATES

PLATE 65

FIG. 1. A functioning Wistar parathyroid autograft removed 62 days after grafting. Prominent connective tissue septa between glandular acini are seen. A small tag of thyroid tissue is present. The gland is well vascularized and shows no indication of ischemic changes. (Animal A-2). Hematoxylin and eosin. × 116.

FIG. 2. A non-functioning hooded parathyroid homograft removed from its Wistar recipient 14 days after skin grafting from the same donor. There is a vigorous lymphoid inflammatory cell reaction with complete destruction of normal glandular architecture. (Animal 14-5). Hematoxylin and eosin. × 116.

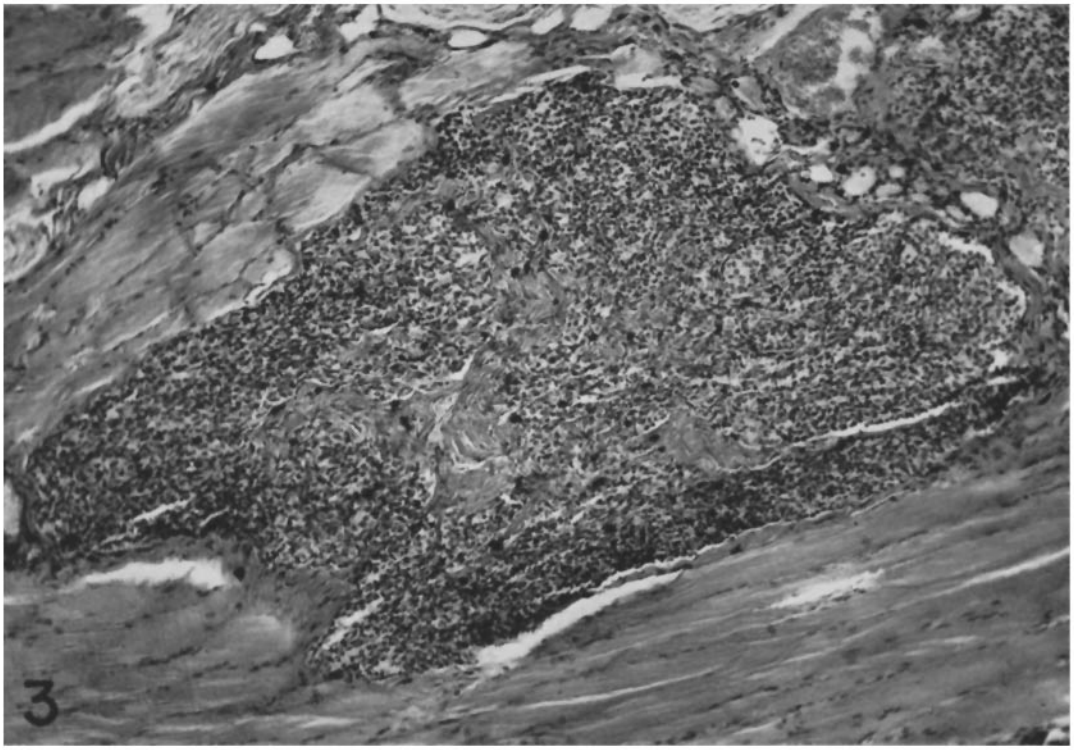


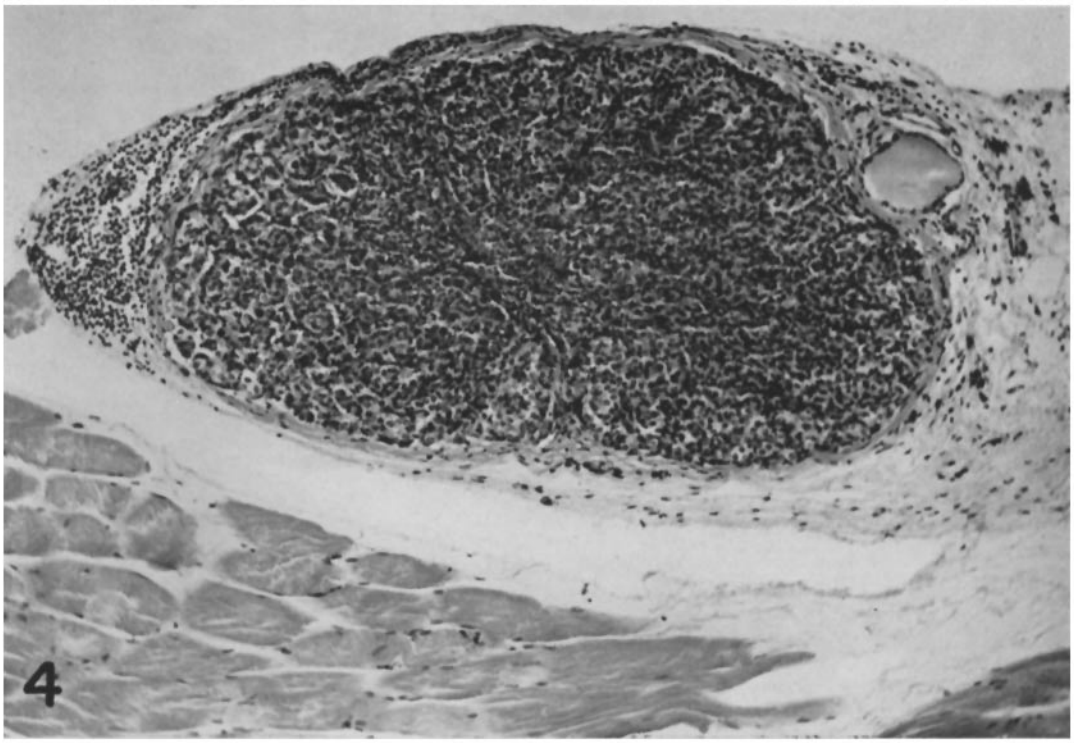
(Gittes and Russell: Parathyroid transplants)

PLATE 66

FIG. 3. A normally functioning hooded-to-Wistar parathyroid homograft 120 days after transplantation. There is no evidence of inflammatory response. (Animal 14-1). Hematoxylin and eosin. × 122.

FIG. 4. A fully functional hooded to Wistar parathyroid homograft 81 days after transplantation showing slight evidence of inflammatory cell mobilization about the capsule with no appreciable penetration into the substance of the gland. (Animal 15-5). Hematoxylin and eosin. × 122.





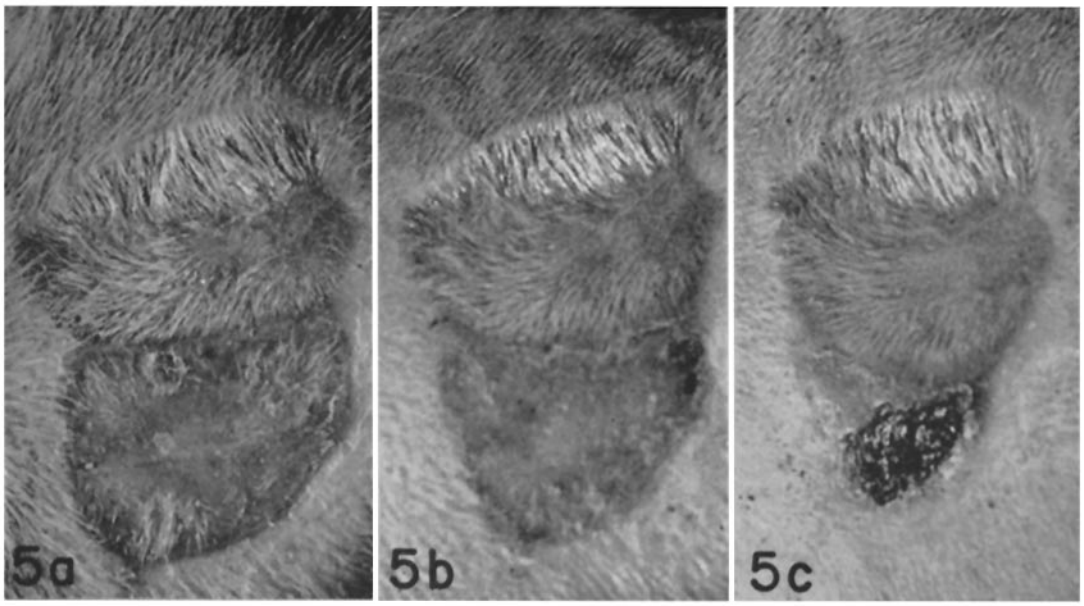
(Gittes and Russell: Parathyroid transplants)

PLATE 67

FIG. 5 *a* to 5 *c*. An example of the "chronic" rejection often observed in skin grafts between members of the Wistar strain. The rejection time of this graft was placed at 38 days on the basis of distinct shrinkage in area, which began at that time. By comparison with the control autograft just dorsal to it the alopecia, punctate loss of epithelium, and reduction in area are evident. (*a*) 38 days, (*b*) 42 days, (*c*) 53 days after grafting.

The acute functional failure in this animal of a parathyroid graft from the same donor is illustrated in Text-fig. 6. (Animal 11-3). × $2\frac{1}{5}$.

FIG. 6. Wistar parathyroid removed from Wistar host 18 days after grafting simultaneously with skin from the same donor. The skin graft was completely rejected at 18 days; serum calcium levels indicated early functional failure of this parathyroid tissue (see text). (Animal 11-2). Hematoxylin and eosin. × 108.



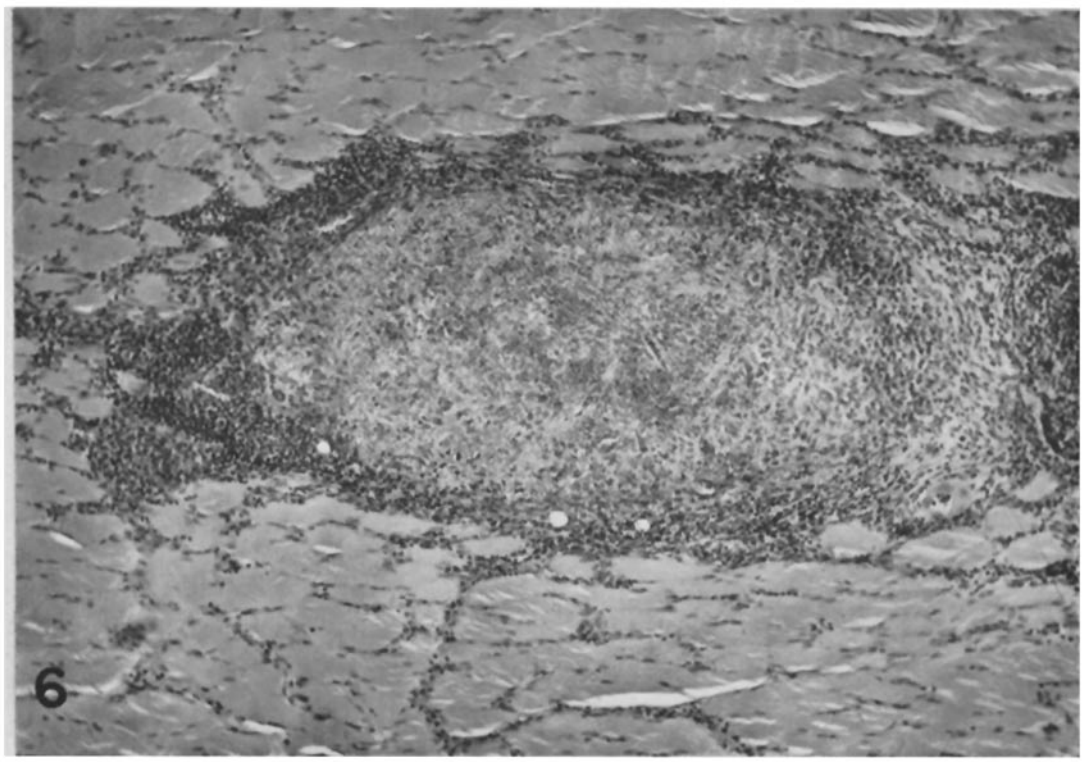


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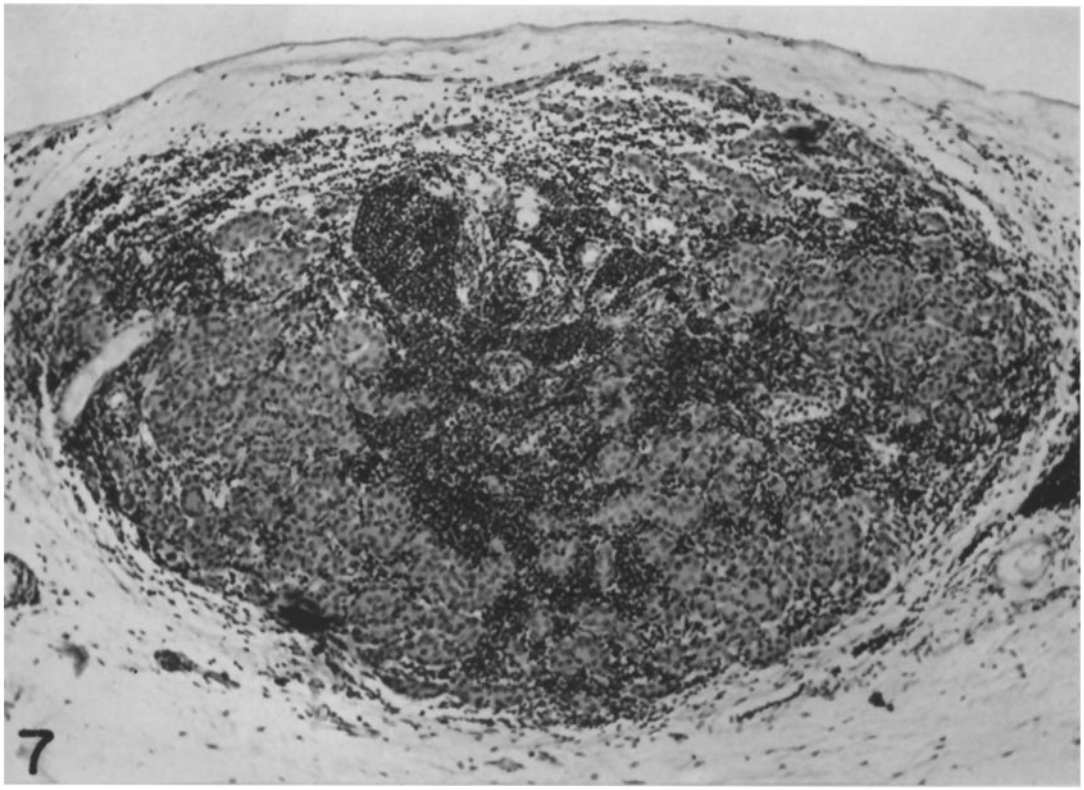
PLATE 68

FIG. 7. Functioning Wistar parathyroid homograft removed 18 days after completion of second set skin rejection from Wistar recipient. Intact islands of glandular acini lie between dense collections of inflammatory cells concentrated along the connective tissue septa and about the periphery of the graft. (Animal 2-B-II). Hematoxylin and eosin. × 127.

FIG. 8. Biopsy of second set skin graft taken 18 days before parathyroid, shown in Fig. 7, was removed. The vigorous inflammatory cell response and complete loss of epithelium 16 days after grafting are characteristic of an accelerated reaction and indicate an appreciable degree of sensitivity to skin. (Animal 2-B-II). Hematoxylin and eosin. × 122.



(Gittes and Russell: Parathyroid transplants)