Chronic Allograft Rejection in the Iguana *Ctenosaura pectinata** (32966)

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Among lower vertebrates, as in homothermic species, rejection of skin allografts may be of two general types. In anuran amphibians during larval and adult stages, grafts are usually destroyed in a rather rapid or acute fashion in less than 15 days. In apodan (1) and urodele amphibians (2) the reaction is chronic, i.e., the period of rejection is slow with final median survival times of greater than 20 days. For comparative purposes we studied the rejection and fate of second-set allografts in a reptile, the iguana.

Materials and Methods. Maintenance of animals. Adult male and female iguanas, *Ctenosaura pectinata*, from the vicinity of Taxco, Guerrero, Mexico, were used. They were maintained at a temperature of 25 ± 1.0°C and fed a diet of mixed vegetables and fruits; they showed a predilection for papaya. Water was administered to each animal directly into the mouth from a small plastic bottle. Maintenance was considered successful since several females deposited as many as 30 eggs after being in the laboratory several months.

Skin grafting. The animals were anesthetized with ether and pieces of skin approximately 1 cm^2 were cut on either side of the dorsal midline and exchanged between pairs of iguanas. A control autograft was always made on the graft bed anterior to the allograft. Grafts were held firmly in place by applying collodion (J. T. Baker Chemical Co., Phillipsburg, New Jersey) to the edges instead of using sutures which had been employed previously (3). All second-set allografts were exchanged between the same hosts and donors used in first-set combinations.

Graft inspection. Grafts were examined daily by means of a dissecting microscope; this facilitated the early recognition of technical or accidental losses. Two criteria were employed for determining allograft rejection: (a) disappearance of melanophores from the scales leaving them translucent, and (b) palpation of the grafts until final rejection. Rejected grafts became detached when palpated; well-healed grafts were soft and pliable and showed no signs of pigment cell destruction.

Histology. For histologic analysis four allografts were removed after the onset of rejection but before complete destruction. As controls, pieces of normal skin were removed. Because of the toughness of the skin it was necessary to leave the tissues in 4% phenol for 3–5 days after fixation in 10% neutral buffered formalin. They were embedded in paraffin, sectioned at 6μ and stained with hematoxylin and eosin according to methods outlined by Culling (4).

Results. Gross features of graft rejection. Autografts. Generally, most autografts were cosmetically intact and showed no signs of destruction. Well-healed autografts always showed intact melanophores that were visible beneath the keratin. Occasionally a few scales in a graft showed no pigment cells, an absence presumably caused by operative trauma (Fig. 1). Autografts in 13 animals were intact at the last scoring after 28 days postgrafting and up to 7 months. Five lost their autografts either by accident or faulty techniques 4–45 days after grafting.

Allografts. Table I summarizes the survival times of allografts exchanged between adult iguanas. First-set survival times range from 48–87 days and second-sets 30–86 days. Animals 3 and 7 were male recipients of female donor skin that were grafted 14 and 16 days before the rejection of first-set grafts. These were the only two animals with accelerated

* Supported in part by funds from USPHS Research Grant AM 09030-02 from the National Institute of Allergy and Metabolic Diseases and AID Contract 1a–194.

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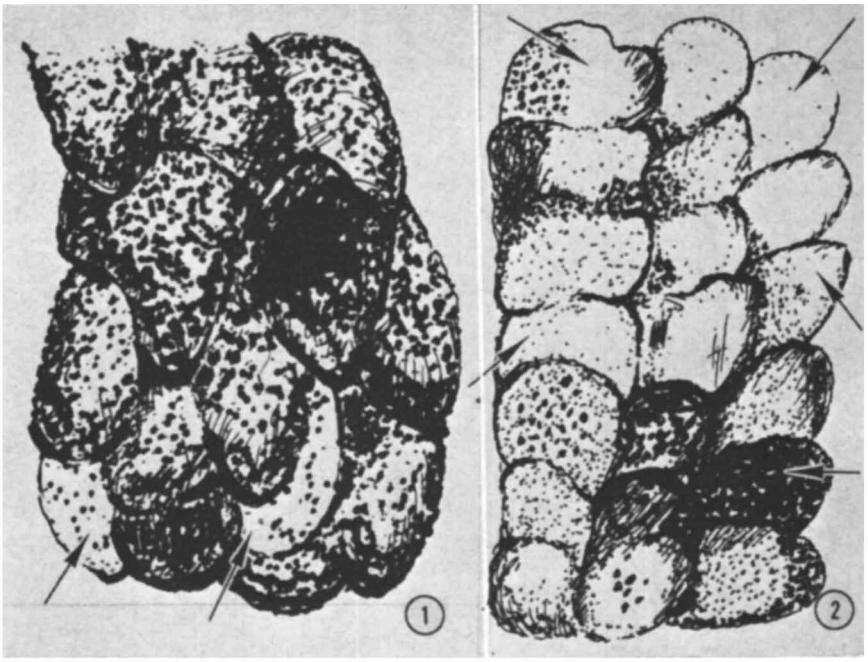

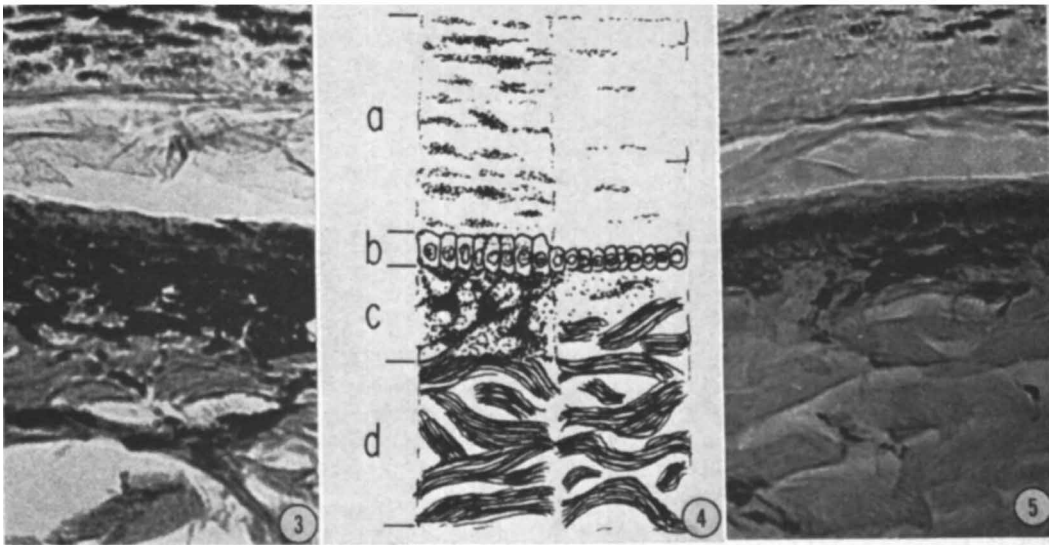

FIG. 1. Drawing of an intact skin autograft of the iguana. Arrows indicate two scales devoid of melanophores which probably resulted from trauma. Approximately × 20.

FIG. 2. Drawing of a rejected allograft in the same iguana in Fig. 1. Approximately 85% of the graft is rejected as indicated by many scales essentially devoid of melanocytes (top arrows). Bottom arrow indicates an intact scale. Approximately × 20.

FIG. 3. Section of normal skin of a dark iguana. Notice the melanin in the keratinized layer. Immediately below the epithelial cells are heavy concentrations of pigment cells. The dermal layer consists of dense connective tissue. The space between keratin and basal epithelial layer is artifactual, a line of separation when the keratin is shed. Approximately × 650.

FIG. 4. A composite drawing which represents figs. 3 and 5; (a) keratin, (b) epidermis, (c) melanophores, and (d) dermis.

FIG. 5. Section of skin of a rejected graft prior to sloughing of keratin. Notice the pronounced reduction of melanin in the keratinized layer and of pigment cells in the subepithelial region. Approximately × 650.

TABLE I. Survival Time of First- and Second-Set Allografts in the Iguana.[a]

Host no. and sex	Days since first graft to: Onset of rejection	Days since first graft to: Completion of rejection	Days between first-set rejection and second-set grafting	Days since second graft to: Onset of rejection	Days since second graft to: Completion of rejection	Sex of donor
1[f] F	45	48	0	41	58	M
2 F	43	48	0	54	64	F
3[b] M	45	55	—14	24	30	F
4 F	48	64	5	43	>43	F
5 F	51	64	5	56	82	F
6[c] F	45	66	—15	56	86	M
7[b,f] M	45	67	—16	42	58	F
8[d] F	55	87	—	—	—	F
9[e] F	38	50	0	—	—	F
10 F	48	>48	All grafts showed chronic	—	—	M
11 M	37	>37	rejection 8–15 days	—	—	M
12 M	37	>37	after onset of rejection	—	—	M
13 F	49	>49		—	—	M
14–24 (4 females, 7 males)	Death, technical or mechanical losses 7–42 days postgrafting		—	—	—	(6 females, 5 males)

[a] Arranged according to increasing survival time of first-set allografts.

[b] Second-set grafts performed before rejection of first-set graft.

[c] Second-set performed 6 days after onset of rejection of first-set.

[d] No second-set; original donor died.

[e] No second-set; mechanical loss 2 days post-grafting.

[f] Animals 1 and 7 had additional third-set grafts performed on the day of rejection of second-set. Days of initiation of rejection were 18 and 46 days, respectively. Grafts were still in chronic rejection 10 days later.

rejection of second-set grafts; the other hosts were females whose repeat grafts were enhanced with respect to first-set survival times.

Two iguanas showed first-set survival times of 50 and 87 days; no second-sets were exchanged because of death of the original donor or mechanical loss of grafts. Four had allografts that had begun rejection 8 and 15 days prior to the last observation. Third-set allografts were exchanged between animals 1 and 7 on the day of onset of rejection of the second-set graft. Rejection began approximately 18 and 46 days postgrafting and was still incomplete 10 days later.

A rejected allograft showed scales devoid of pigment cells, hence translucent. Destruction of melanocytes did not occur simultaneously in all scales of a single graft. In dark iguanas the keratin layer contains melanin; similarly, these cells are found just below the epithelium. In light skin the keratin is translucent, facilitating the observation of subepithelial pigment cells. When dark grafts began to undergo rejection they appeared as normal light colored grafts with a translucent keratinized layer. After complete rejection, subepithelial pigment cells were broken down and the pigment granules were scattered randomly; these were visible through the transparent keratin (Fig. 2). The final stages of rejection were characterized by sloughing of the keratin leaving a grey, thickened area, presumably the connective tissue which is similar to the collagen pad that remains after graft destruction in mammalian vertebrates. Because this area is translucent in iguanas vascular patterns were observed more easily at this period than before sloughage; vasodilation was generally pronounced.

Histology of graft rejection. The condition of the epithelium and melanocytes at the microscopic level provided the most reliable

indicator of graft viability. The epidermis has an outer keratin layer and a basal layer of live epithelial cells. The dermis is dense connective tissue with relatively few cells. Melanophores are present in the keratin and in the subepidermal area (Fig. 3). Epithelial cells of rejected grafts were lower than in normal skin. Pigment cells of rejected grafts were absent especially in the keratin layer (Fig. 4); melanophores were sparse beneath the epithelium. Subepithelial tissue in normal skin and rejected grafts showed only slight differences.

Discussion. The iguana, *Ctenosaura pectinata,* is capable of rejecting allografts. After an initial healing in period for autografts and allografts, subsequent gross signs of destruction of allografts were always observed. Initial features of allograft rejection, although gradual and chronic, were characterized by breakdown of melanophores in the scale similar to the condition observed in scale allografts of fishes (5). Because of the nature of the iguana integument, vasodilation and hemostasis could not be used as criteria for determining rejection as has been employed in studies of graft destruction in diverse amphibian and fish species (6). At the microscopic level some rejected grafts did not reveal much leukocytic infiltration, while others showed substantial numbers of leukocytes as well as adequate vascular connections. The intensity of the cell response may be a function of the time of biopsy; our specimens were taken when grossly identified as 75% rejection.

Although few in number, it is interesting that two male iguanas showed accelerated destruction of second-set grafts; repeat grafts were made at periods before complete rejection of the first graft. All female recipients showed enhanced survival of second-sets. This heightened reactivity by male hosts is probably not a function of sex, but it might be an additive allo-antigenic effect produced by repeat challenge prior to complete rejection of first-sets. Cohen (7) found no sexual dimorphic response in salamanders when allografts from donors of different sexes were transplanted to hosts of both sexes. Similarly, Hildemann and Cohen (8) cite much convincing evidence from mammalian studies to support one rule of their chronic rejection scheme that "allograft survival times on females are usually shorter than on males of the same strain."

The importance of temperature in immunologic studies of poikilotherms cannot be overemphasized (9). Our iguanas were maintained at a temperature of 25°C, which has been employed commonly in amphibian and fish studies. Yet, this temperature may not have been optimal for acute reactions to tissue allo-antigens. Evans (10) found that if the ambient temperature was 35°C or 40°C, desert iguanas, *Dipsosaurus dorsalis,* produce antibodies to typhoid vaccine. However, at 25°C the antibody response was poor or nonexistent. Thus, it might be argued quite strongly that the chronic response in the Mexican iguana was an effect of a low temperature and that higher temperatures might well have produced acute allograft responses. The early work of May (11) suggests that chronic rejection may be the pattern in reptiles but his lizards were also maintained at a relatively low temperature of 23.5°C.

May performed auto- and allotransplants in the chameleon, *Anolis carolinensis,* in an attempt to understand the specificity of pigment cell migration after transplantation. In this connection, but independent of pigment cell studies, he regularly observed rejection of allografts at periods ranging from 60 to 90 days; one exception, however, was a graft that was still intact after 4 months. His criterion of rejection was the disappearance of pigment after grafts were well healed; subsequently the scales were sloughed, leaving an area covered by a thin scaleless integument. Although first-set survival times of iguana allografts seem equivalent to May's we have no information pertaining to anamnesis in that species since he performed no second-set grafts. The iguana may be an advantageous reptile for studies of graft survival since transplants are relatively easy to perform. Besides, ecdysis apparently does not occur in the iguana as is routinely the case in *Anolis,* a condition which could possibly render assessment of graft survival difficult in lizards.

Summary. Adult male and female iguanas, *Ctenosaura pectinata*, were auto- and allografted. Skin allografts and autografts behaved like those of other vertebrates; they healed in initially but allografts later showed signs of rejection recognized mainly by pigment cell destruction. At 25°C chronic rejection of first-set allografts in the iguana, as indicated by the survival times, is more like the rejection pattern of urodeles and apodans than that of anuran amphibians or fishes. The data support a view that allograft rejection is by an immune process since accelerated rejection of some second-set grafts occurred while the rest showed enhanced survival. In addition, an inflammatory response, characterized by lymphocytic infiltration, was always associated with destroyed grafts.

The authors wish to extend appreciation to Miss Maria de la Paz Rodriguez, Mrs. Gloria Aponte, Mrs. Margarita Moncada and Mrs. Alicia Bifano for technical assistance. We also wish to thank Dr. Francisco Garcia-Herrera who provided the laboratory facilities.

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Received Dec. 22, 1967. P.S.E.B.M., 1968, Vol. 128.

Studies on the Nature of the Synalbumin Insulin Antagonist* (32967)

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Excess plasma synalbumin insulin antagonism has been proposed to explain coexistence of normal or above normal plasma insulin levels and abnormal glucose tolerance in some maturity-onset diabetics (1). Although it is generally agreed that something associated with plasma albumin antagonizes the effect of insulin on glucose uptake by the rat hemidiaphragm *in vitro* (1–4) and in other *in vitro* systems (5–7), there are conflicting reports regarding the ability of synalbumin to function in whole animals (8,9). Consequently, the actual role of this antagonist in the pathogenesis of diabetes is not clear.

Recently published data indicates that at least part of the antagonism exhibited by Debro "albumin" is due to an artifactual antagonist derived from the Visking tubing used to dialyze the "albumin" (10). The present studies were carried out to gain information on the nature of synalbumin antagonist extracted by the Debro procedure.

Materials and Methods. The rat hemidiaphragm assay of Vallance-Owen (1) was used to measure insulin antagonism. Rats used as donors of hemidiaphragms were Upjohn Wistars or Upjohn pathogen free rats. Insulin concentration was 1000 μU/ml with hemidiaphragms from Wistars and 500 μU/ml with tissue from pathogen free animals. Boiled dialysis membranes were prepared by boiling 50-cm lengths of Visking tubing, size 27/32, (Union Carbide Company) in 4 liters of distilled water for 4 hours with the water changed at 1-hour intervals. Antagonistic "albumin" was extracted from outdated human

* This investigation was supported in part by a U. S. Public Health Service Research Grant (AM-10997-01) from the National Institute of Arthritis and Metabolic Diseases.