ROLE OF THE THYMUS IN IMMUNE REACTIONS IN RATS

II. Suppressive Effect of Thymectomy at Birth on Reactions of Delayed (Cellular) Hypersensitivity and the Circulating Small Lymphocyte*

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Plates 13 and 14

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In the first paper of the present series (1) we have shown that, of rats thymectomized within the 1st weeks after birth, many fail to produce antibody of the precipitating or hemagglutinating type or to develop delayed sensitivity against the specific antigen following foot-pad injection of bovine serum albumin in adjuvant, though sham-operated and other control animals do so with great regularity. In the remaining thymectomized animals, these responses occur but are delayed in appearance and somewhat diminished in degree. In the present paper, we wish to present data showing that there is a similar suppression or retardation of other immune responses of the delayed type and a striking depression of the level of circulating small lymphocytes in these animals (2, 3).

Materials and Methods

Thymectomy.—The details of anesthetic and surgical technique are given in the previous paper (1).

Tuberculin Sensitization.—Tuberculin sensitization in the rat has been described in detail by Flax and Waksman (4). In the present experiments, rats received a single intradermal injection (0.05 to 0.1 ml) of killed tubercle bacilli[1] in oil[2] (3 mg per ml) in the left hind foot-pad.

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[1] Obtained through the courtesy of Dr. I. S. Danielson of the Lederle Laboratories, Pearl River, New York.

[2] Bayol F, obtained from Esso Standard Oil Company, Linden, New Jersey.

These animals were skin-tested at approximately 10 days and again at 20 days by intradermal injection of old tuberculin 1:10 (OT) in the shaved flank. The reactions were read at 24 and 48 hours for the diameter and degree of subcutaneous and intracutaneous induration. The tuberculin reaction characteristically shows little erythema in the rat (4).

Passive Transfer of the Tuberculin Reaction.—Normal, adult Sprague-Dawley rats, injected in all four foot-pads with tubercle bacilli in oil, were sacrificed at 7 days. Their lymph nodes were removed and a suspension of lymph node cells prepared, washed twice, and finally resuspended in Hanks' solution. 5×10^7 viable cells, in a volume of 0.05 ml, were injected intracutaneously in each ear of test animals, on the left without added antigen and on the right with OT 1:10 included in the medium. Reactions (4) were read at 24 and 48 hours.

Adjuvant Arthritis.—This disease process has been described in detail by Pearson, Waksman, and Sharp (5). All animals injected with mycobacteria in oil and skin-tested for tuberculin sensitivity were also observed daily for the development and intensity of arthritic manifestations in various peripheral joints and of nodular lesions on exposed skin surfaces. An over-all grade of severity between 0 and +++ was assigned to each animal. No histologic observations were made.

Experimental Allergic Encephalomyelitis.—For a description of this disease process, see reference (6). Antigenic mixtures were prepared from fresh normal rat spinal cord by grinding in a mortar to give a fine suspension in oil containing tubercle bacilli (3 mg per ml). The proportions used were approximately 1 part of cord to 2 or 3 parts of oil with bacilli. Sensitization consisted of a single intradermal inoculation of 0.05 to 0.1 ml of this mixture in the left hind foot-pad. Animals were observed daily for the onset of neurological disease, *i.e.* paralysis, ataxia, tremulousness, and were autopsied shortly after the onset. Those animals which appeared to remain free of disease were autopsied at a constant time, approximately 4 weeks. Paraffin sections of cortex, cerebellum, and several levels of spinal cord, stained with hematoxylin and eosin, were studied for the presence, number, and intensity of the characteristic lesions. Each animal was assigned a grade of 0 to +++ based on the histologic observations. There was a close correlation between the intensity of disease as judged clinically and the number and extent of histologic lesions.

Skin Homografts.—Circular pieces of flank skin, 6 mm in diameter, were removed with a biopsy punch, cleaned of adventitious fat and muscle, and fitted into holes of the same size on the upper anterior flank of adult Sprague-Dawley recipients. Each rat received two autografts and two homografts (from Sherman strain rats). The grafts were maintained in position with vaseline gauze and a light plaster cast. The cast was removed at 10 days, and the gross appearance of the grafts noted at 10 and 20 days. One autograft and one homograft were removed for histologic study at each of these times. Graft rejection was evaluated histologically by noting the degree of cell infiltration in the base and upper portion of the dermis as well as the amount of epidermal destruction and destruction of follicles. There was a very precise correlation between the degree of rejection estimated in the gross and the degree of these changes observed histologically. In a second experiment, grafts were allowed to remain in place, and the time of graft destruction estimated visually.

Total White Counts and Differential Counts.—Counts were performed on tail blood of 6 to 8 week old rats before experimentation and, in some animals, 3 to 4 weeks after they had received Freund's adjuvant in connection with the immunologic studies. All smears used for differential counts were stained with Wright's stain. A frequency distribution of lymphocyte and monocyte diameters was obtained by micrometer measurement of individual cell diameters (at least 100 cells counted) in selected smears.

RESULTS

Tuberculin Sensitization.—The pooled data of several experiments are shown in Table I. There is a clear difference between thymectomized and control ani-

mals, the 48 hour reactions being markedly reduced in the former. By this criterion, half the thymectomized animals failed to develop sensitivity. However, some reactivity was present even in these rats, as judged by 24 hour readings. In animals injected with BSA and adjuvant or rat spinal cord and adjuvant (not shown in table), tuberculin testing was also carried out and led to exactly similar findings; *i.e.*, a partial inhibition of sensitization most noticeable in readings at 48 hours, in animals thymectomized at birth.

TABLE I

Decrease of Tuberculin Sensitization in Rats Thymectomized at Birth

Group	No. of rats	Day of test*	Reading at	No. of rats with reactions		
				0–4 mm	5–9 mm	10–14 mm
			hrs.			
Thymectomized	13	9–11	24	2	8	3
			48	6	4	3
		20–21	24	1	11	1
			48	6	7	0
Control	15	9–11	24	0	8	7
			48	1	4	10
		20–21	24	1	9	5
			48	2	9	4

* With OT 1:10, 0.1 ml intradermally.

TABLE II

Failure of Thymectomy at Birth to Affect Adjuvant Arthritis

Group	No. of rats with arthritis				Average day of onset
	0	+	++	+++	
Thymectomized	2	7	2	2	14
Control	3	4	5	3	12

In the passive transfer experiment, seven thymectomized and seven sham-operated rats served as unsensitized recipients. Injection of sensitized donor cells mixed with tuberculin into the ear skin produced typical local tuberculin reactions (erythema and induration, maximal at 24 to 48 hours), which exceeded markedly the reactions produced by the same cells injected without antigen into the contralateral ear. These local passive reactions were produced with equal success in thymectomized and control rats. There was therefore no reason to suspect a local abnormality in the skin or some unknown general alteration which interfered with the expression of tuberculin sensitivity in thymectomized rats.

Adjuvant Arthritis.—Arthritis and the characteristic nodular skin lesions on the ears, tail, and feet were observed in a high proportion of rats injected with tubercle bacilli in oil. There was little difference between thymectomized and control animals (Table II), though disease tended to be slightly milder and slightly later in onset in the experimental group.

Allergic Encephalomyelitis.—As indicated in Table III there was a striking loss in the thymectomized rats of ability to develop allergic encephalomyelitis following a single food-pad injection of homologous spinal cord in adjuvant. However, animals with residual thymus developed this disease as readily as the controls. As will be noted in the last paper of the present series, the two thymectomized rats which did react showed incomplete depletion of small lymphocytes in the spleen and lymph nodes.

TABLE III

Loss of Ability to Develop Allergic Encephalomyelitis in Thymectomized Rats

Rats	Degree of disease*		
	0	+	++ and +++
Thymectomized	8	0	2
Thymectomized, residual thymus	0	1	3
Sham-operated‡	1	1	5
Non-operated	0	0	4

* Based on histologic evaluation.

‡ Single rat which failed to develop disease had only small amount of residual thymus and showed depletion of all lymphoid tissues.

Skin Homograft Rejection.—Tables IV and V present data obtained in our homografting experiments. Control and sham-operated rats rejected homografts with great uniformity, rejection being nearly complete by the 10th day and the replacement of the graft by host tissue well advanced by the 20th day. Many of the thymectomized rats showed little or no evidence, in the gross or histologically, of rejection even at 20 days. However, grafts observed for a longer period were rejected by 30 days. The characteristic findings are illustrated in Figs. 1 and 2. Grafts in five rats, thymectomized at 2 weeks of age and grafted 4 weeks later, were rejected rapidly, in a manner indistinguishable from rejection in sham-operated controls.

Blood Counts.—Total and differential white blood cell counts in 6 to 8 week old, unstimulated rats revealed a striking diminution of lymphocytes in the peripheral blood of thymectomized animals as compared with sham-operated litter mates (Table VI). The lowered lymphocyte level in these animals, approximately 40 per cent of that in controls, was associated with a depletion of small lymphocytes in spleen and lymph nodes (described in the following paper);

TABLE IV

Suppression of Skin Homograft Rejection in Thymectomized Rats

Rats	Residual thymus	Gross rejection		Rejection estimated histologically	
		10 days	20 days	10 days	20 days
	mg				
Thymectomized					
1	0	0	0	±	0
2	0	0	0	+	0
3	0	±	±	+	+
4	0	0	0	+-++	+
5	30	0	+-++	+	+-++
6	40	+	++	++	+++
7	50	±	±	+-++	0
Sham-operated					
8	130	++	+++	+++	+++
9	220	++	+++	+-++	+++
10	340	++	+++	+++	+++
11	390	++	+++	+++	+++
12	440	+++	+++	+++	+++
13	470	++	+++	+++	+++
Non-operated					
14	330	+++	+++	++	+++
15	450	++	+++	+++	+++
16	450	++	+++	+++	+++
17	510	++	+++	+++	+++
18	540	++	+++	++	+++
19	—	++	+++	+-++	+++
20	—	++	+++	+	+++
21	—	+++	+++	++	+++

TABLE V

Suppression of Skin Homograft Rejection in Thymectomized Rats

Rats	Residual thymus	Gross rejection at			
		10 days	20 days	25 days	30 days
Thymectomized					
22	0	0	0	+	Scab
23	0	0	±	++	—
24	0	0	0	+	Scab
25	0	0	0	++	—
26	0	0	+	++	Scab
27	60 mg	±	++	+++	Scab
28	90 mg	0	+	++	Scab
Sham-operated					
29	Normal	++	+++	Scab	Scab
30	Normal	+++	Scab	Scab	Scab
31	Normal	++	Scab	Scab	Scab
32	Small abscess	++	Scab	Scab	Scab

TABLE VI

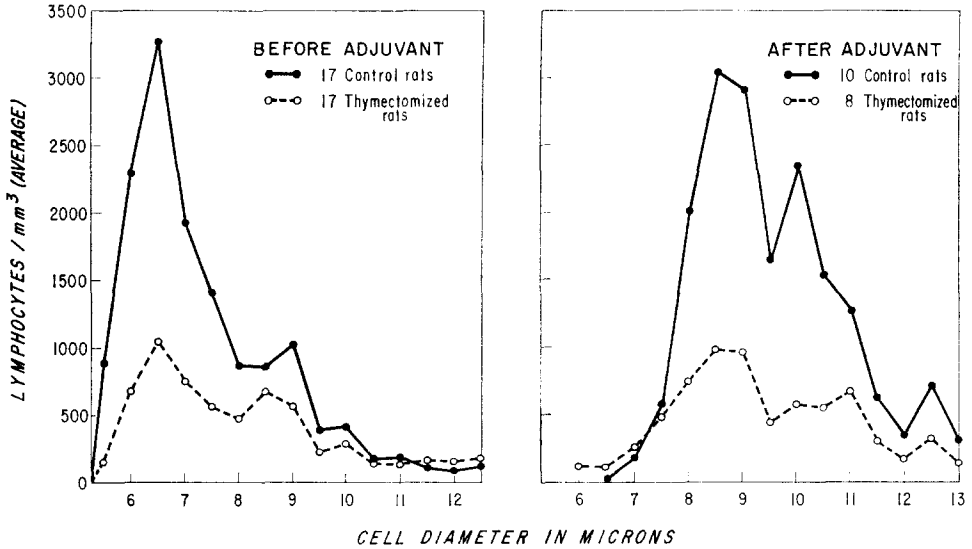
*Lymphocyte Counts in Adult Rats, Unsensitized or Sensitized by Injection of Adjuvant Mixtures**

Group	No. of rats	No. of rats with lymphocyte count, *cells/mm³*				Average count
		2000–6000	6000–10,000	10,000–14,000	>14,000	
Unsensitized						
Thymectomized	24‡	15	6	3	0	5,900
Residual thymus	8	3	2	2	1	8,300
Sham-operated	19	2	4	5	8	14,500
Non-operated	14	0	3	6	5	14,350
Sensitized						
Thymectomized	20§	6	10	3	1	8,200
Residual thymus	9	0	4	1	4	13,500
Sham-operated	18	0	5	6	8	15,100
Non-operated	8	0	1	2	5	15,400

* Adjuvant alone or adjuvant mixtures containing BSA or rat spinal cord.

‡ Including two rats which, while showing no residual thymus, did respond to subsequent antigenic stimulation.

§ Including five rats which showed no residual thymus at postmortem but did respond to antigenic stimulation.



TEXT-FIG. 1. Frequency distribution of lymphocyte diameters (monocytes not included) in blood smears from thymectomized and control rats before and after stimulation with foot-pad injection of adjuvant mixtures.

those animals which showed the lowest blood levels had the greatest tissue depletion of these cells. Rats thymectomized as late as 3 weeks after birth (not shown in table) showed a comparable lowering of blood lymphocytes at 8 weeks. Rats found at postmortem to have had incomplete extirpation showed lymphocyte counts intermediate between those of thymectomized and control animals. 2 to 3 weeks after an injection of Freund's adjuvant, the absolute lymphocyte counts in completely thymectomized animals were only slightly elevated above baseline levels; animals with residual thymus showed a more striking elevation to levels approaching those in control rats.

Micrometer counts revealed the depression of lymphocytes in thymectomized rats to involve small lymphocytes (6 to 8 μ) preferentially; there was also some depression of medium lymphocytes (9 to 10 μ) but none of large lymphocytes (11 to 12 μ) (Text-fig. 1). After adjuvant injection, there was a shift of the entire lymphocyte series toward larger cells. Small lymphocytes were no longer found, either in control or in thymectomized animals. Other formed blood elements, polymorphonuclears, eosinophils, basophils, and monocytes, were counted as routine but showed no differences between thymectomized and control animals. The hematocrit reading, in a limited number of thymectomized animals, was also normal.

DISCUSSION

The data presented here show a suppressive effect of thymectomy at birth on several well standardized immunologic reactions which, on the basis of available evidence, are regarded as having a similar mechanism, so called delayed hypersensitivity. In the case of allergic encephalomyelitis, the suppression appeared to be nearly complete. In the other cases studied, the effect was partial and in a single instance, the adjuvant arthritis, was insignificant. Our observations with regard to prolonged survival of skin homografts in thymectomized rats agree well with the findings of Miller (7) and Martinez *et al.* (8) who showed that, in mice thymectomized at birth, homograft survival was prolonged to a varying degree. Nevertheless, in their experiments as well, rejection occurred and was more rapid the more efficient the antigenic stimulus (8). A slightly prolonged survival of grafts has also been reported in chickens thymectomized by early treatment with testosterone (9).

The present findings shed an interesting light on the question of the genesis of delayed hypersensitivity. The evidence that delayed reactions form a coherent group of immunologic phenomena, having a common morphology and probably a common mechanism, has been presented in many recent publications. Their principal histologic feature is perivenous infiltration of mononuclear cells resembling histiocytes, Gell's "perivascular island" (10). There is evidence that the infiltrating cells may be derived from specifically sensitized cells in the blood

stream (11); and this contention has received strong support, for certain of the lesions under consideration, from recent experiments in which tracer methods were employed (12, 13). The identity of the sensitized cells, however, remains uncertain. They may be small, medium, or large lymphocytes, since these cells are constantly present in the blood, as well as in suspensions such as thoracic duct lymph, which have been used to transfer delayed reactivity passively. The effectiveness of specific lymphocyte antiserum, which depresses the level of circulating lymphocytes, in abolishing various delayed reactions (14) has particularly focussed attention on lymphocytes as possible precursors of the histiocyte-like cells in the lesions. Following Maximow (15), a considerable body of experimental data has developed to suggest that lymphocytes may become "histiocytes" (reviewed in reference 16); but the direct transformation of one cell type into the other has not been satisfactorily demonstrated.

Thymectomized rats show a striking deficiency of peripheral blood lymphocytes. This defect was first noted in 1904 and has been many times corroborated (17). We have shown that the loss of lymphocytes is relatively selective, small lymphocytes showing the greatest decrease, medium lymphocytes considerably less, and large lymphocytes little change. The depletion of circulating small lymphocytes, in turn, was closely correlated with a great depletion of these cells in the spleen and lymph nodes (18). After an injection of adjuvant, these cells were no longer found in the circulation, either in control or in thymectomized animals. This would suggest that small lymphocytes can become larger cells and that those present in thymectomized rats are as capable of response to adjuvant as are those of normal animals. Since the capacity of thymectomized animals to respond to non-specific irritation with an inflammatory lesion (1) or to support the development of a local passive tuberculin reaction was normal, they appear to have no local impediment to the expression of hypersensitivity. The marked depression of various types of delayed reactivity in thymectomized rats would seem then to depend specifically on their deficiency of small lymphocytes. In individual animals of the BSA and allergic encephalomyelitis experiments, the occurrence of a reaction was, in fact, found to be closely correlated with the presence of residual small lymphocytes in the spleen and nodes.

We cannot distinguish at present between two major possibilities: (*a*) that the small lymphocytes are themselves the sensitized cells, and (*b*) that they are precursors of other mononuclear cells, formed in the lymphoid organs in response to the sensitizing stimulus and subsequently released into the circulation. We favor the second possibility, since the evidence of other workers (17) as well as our own (18) strongly implies that small lymphocytes are formed in the thymus and proceed thence to the peripheral lymphatic organs and, further, that thymus cells may not themselves be immunologically competent.

We must account for the fact that many animals, even after complete thymectomy, showed some degree of residual delayed reactivity or expressed re-

activity more slowly than the controls. Since rats which gave one or more immune responses in intense form were animals in whom the lymphoid tissues showed only partial depletion of lymphocytes (18), it may be presumed that some functional immunologically competent cells were present in all our thymectomized animals. They could then respond to some degree depending on the intensity or duration of the antigenic stimulus. In the study of Martinez *et al.* there was almost no evidence of suppression of the homograft response in thymectomized mice when there was a sufficient antigenic difference (at the H-2 locus) between donor and recipient (8). Alternatively our test reactions, for example the tuberculin reaction or the adjuvant arthritis, may have included elements of other immunologic mechanisms not influenced by thymectomy.

SUMMARY

In rats thymectomized at birth and tested in adult life, ability to develop autoallergic encephalomyelitis was completely suppressed, there was a marked diminution in the degree of tuberculin sensitization appearing after a single injection of mycobacteria in oil, rejection of skin homografts was markedly delayed, and adjuvant arthritis was not appreciably affected. At the same time there was a striking decrease in the circulating level of small lymphocytes.

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EXPLANATION OF PLATES

PLATE 13

FIG. 1. Comparison of graft rejection in thymectomized rat (34-4) and sham-operated rat (34-3) at 10 days. Each rat has duplicate homografts from Sherman strain donors on left and duplicate autografts on right. Rejection of the homografts is well advanced in the control animal.

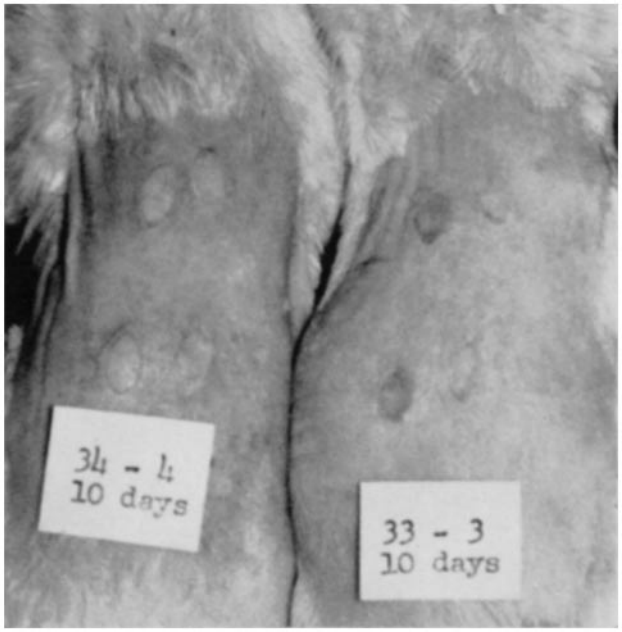
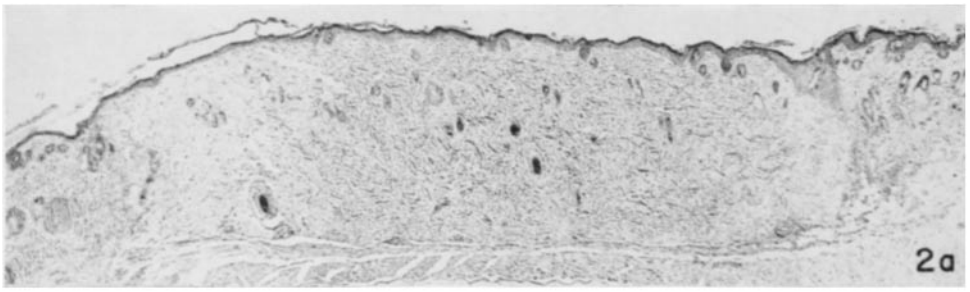
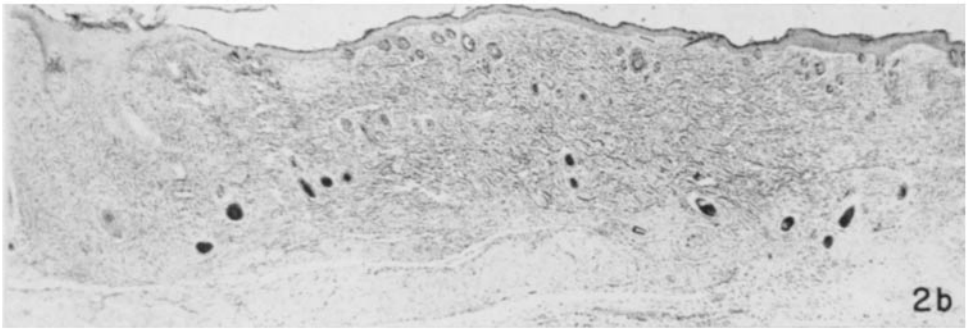


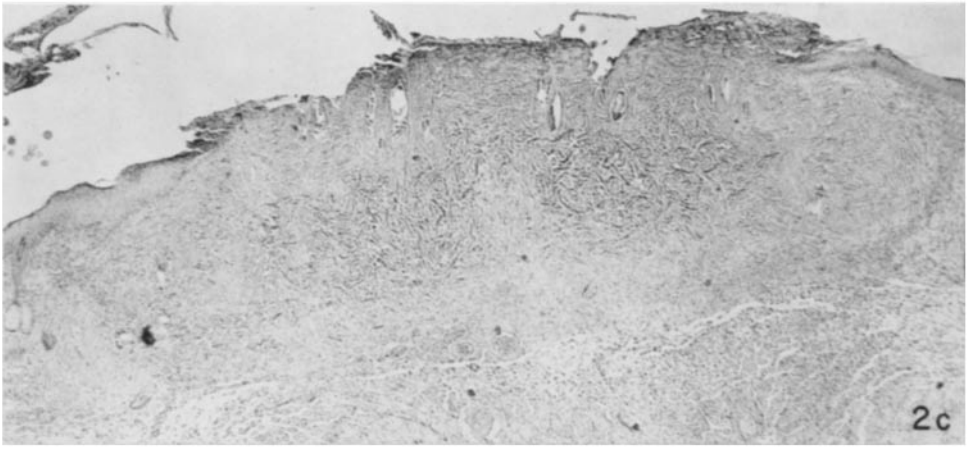
FIG. 1

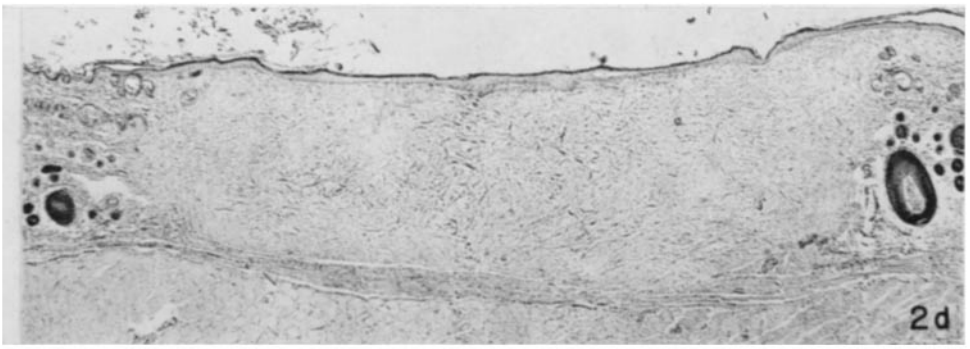
PLATE 14

FIGS. 2 *a* to 2 *d*. Homografts in thymectomized rat (2 *a* and 2 *b*), and sham-operated control (2 *c* and 2 *d*) at 10 days (2 *a* and 2 *c*) and 20 days (2 *b* and 2 *d*). Control at 10 days (2 *c*) shows massive cellular infiltration of graft bed, destruction of follicles and of surface epidermis, and ingrowth of host epidermis. 20 day control (2 *d*) shows total absence of follicles and complete replacement of graft epidermis by host cells. In thymectomized host, at 10 and 20 days, there is minimal infiltration and both follicles and surface epidermis of graft remain intact. All hematoxylin and eosin. × 20.









(Arnason *et al.*: Thymus role in immune reactions in rats. II)