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Hypoxia Induced Alteration of the Skin Homograft Reaction.* (29758)

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Among the adaptive responses reported in animals exposed to altitude hypoxia are myeloid hyperplasia(1), increased hemopoiesis (2), increased adrenocortical activity associated with thymic involution(3), and a proliferation of the reticulo-endothelial system (4). Since the immunological potential of an animal is known to depend upon the functional state of both myeloid and lymphatic tissue, it was desirable to determine whether mice exposed to reduced barometric pressure exhibit any change in tolerance to skin transplants. The present study deals with the effects of altitude hypoxia on the median survival time of skin homotransplants between 2 inbred strains of mice.

Materials and methods. Seventy-eight adult male mice of the A/J and the C_{57}BL/6J strains were used in the skin transplant studies. Prior to transplantation half of the mice were maintained for 14 days in a ventilated decompression chamber at 380 mm Hg, which corresponds to simulated altitude of approximately 18,000 ft. Hematocrit measurements showed that after 14 days of continuous exposure at this pressure the mice could be considered as acclimated. The mice maintained at the ambient pressure of University Park, Pa. (1200 ft) are referred to as unacclimated.

Homotransplants were made in the following combinations: (a) unacclimated donor to unacclimated host; (b) acclimated donor to acclimated host; (c) acclimated donor to unacclimated host; (d) unacclimated donor to acclimated host. All the transplants were made using a modification of the technique described by Billingham and Silvers(5), and were of the type commonly referred to as "pinch" grafts. The grafted skin was approximately 1.5 cm in diameter, and was removed from the antero-dorsal surface of the animal. Postoperatively, all mice were maintained in individual cages at ambient pressure. The protective bandages were removed after 5 days and the animals were observed daily to follow the fate of the graft.

Data were obtained on the survival time of the grafts, and the nature and duration of the 3 stages of graft rejection—granulation, retraction, and sloughing (Table I). A separate group of 48 C_{57}BL/6J male mice was used for histological and weight analyses of the spleen and thymus (Table II).

Results. When both the host and donor were unacclimated, transplants had a median survival time of approximately 9 days, while transplants between acclimated mice were retained approximately 15 days. When skin from an acclimated donor was transplanted to an unacclimated host the median survival time was 12 days, and when the donor was unacclimated and the host was acclimated the transplant was retained 13 days. Thus, it was found that the prior acclimation of either or both the donor and host to altitude hypoxia results in an increased tolerance of mice to skin homotransplants.

Graft rejection can be grossly divided into

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TABLE I. Median Survival Time of Skin Homotransplants.

Donor	Host	n	Median survival time of graft (days + S.E.)	Granulation period‡ (days)
I. Donor and Host Maintained at Ambient Pressure (unacclimated)*				
A/J	C_{57}BL/6J	14	9.0 ± .1	6
C_{57}BL/6J	A/J	17	9.4 ± .1	6
II. Donor and Host Maintained at Reduced Pressure (acclimated)†				
A/J	C_{57}BL/6J	15	13.9 ± .1	11
C_{57}BL/6J	A/J	16	15.6 ± .3	12
III. Grafts From Acclimated Donor to Unacclimated Host				
C_{57}BL/6J	A/J	8	12.1 ± .1	9
IV. Grafts From Unacclimated Donor to Acclimated Host				
A/J	C_{57}BL/6J	8	13.0 ± .3	10

* Maintained at 730 mm Hg.
† Maintained for 16-18 days at 380 mm Hg.
‡ Retraction took 2 days and sloughing one day in all groups.

3 stages: (a) period of granulation; (b) period of retraction; (c) sloughing. Graft rejection was identical in appearance in both acclimated and unacclimated mice. However, altitude exposure of either the donor or host increased the duration of the period of granulation. If both mice were unacclimated this period lasted 6 days, but if either the donor or host was acclimated the granulation period lasted 9 to 10 days. If both donor and recipient were acclimated the duration of granulation was 12 days. Once the period of granulation terminated and graft rejection started, transplants between both acclimated and unacclimated mice were broken down and sloughed in 3 days.

Since the homotransplant response is an immunological process and the spleen and thymus are intimately associated with antibody production, it was decided to investigate the effect of acclimation to reduced barometric pressure on these organs. The data obtained on organ weight changes during acclimation indicate that the thymus involutes markedly during the first week. After 2 weeks the average thymus weight of altitude exposed mice is one-fourth that of mice kept at ambient pressure. No further involution occurs after 3 weeks. In fact, the average weight of the thymus of mice exposed for 21 days is slightly higher than that of the group exposed for 14 days. In contrast to the thymic changes, the spleen hypertrophies during exposure to reduced pressure. After 7 days exposure there is a three-fold increase in the spleen weight.

Histological analysis confirmed that thymic involution occurs after exposure to reduced barometric pressure for 7 days since thymic lobulation is no longer evident and the typical cortical-medullary pattern is absent. These changes were attributed to thymolysis, or depletion of lymphocytes from the thymus. The major histological changes in the spleen following altitude exposure are a hyperplasia of the lymphatic tissue and an increase in size of the splenic nodules. There is also a progressive increase in the amount of extranodular lymphatic tissue between the splenic sinuses of mice exposed for 14 and 21 days.

Discussion. The above findings suggest that an alteration in the animal's immune response may be one of the factors involved in the increased tolerance of acclimated mice to skin homotransplants. That additional factors play a role in homotransplant retention is indicated by the increased tolerance of unacclimated recipients to transplants from acclimated mice. Why an unacclimated host animal should retain grafts from ac-

TABLE II. Average Thymus and Spleen Weights of C_{57}BL/6J Mice Exposed to Reduced Pressure (380 mm Hg).

Days of exposure	Thymus wt* mg ± S.E.	Thymus wt mg/100 g body wt ± S.E.	Spleen wt* mg ± S.E.	Spleen wt mg/100 g body wt ±S.E.
0	63.9 ± 1.9	250.6 ± 7.5	67.0 ± 2.7	260.0 ± 8.4
7	18.1 ± 1.1	86.6 ± 4.2	166.0 ± 11.5	793.1 ± 43.3
14	14.6 ± .7	65.0 ± 3.4	86.6 ± 2.4	385.0 ± 11.7
21	20.0 ± 2.2	86.0 ± 8.6	158.0 ± 12.7	689.0 ± 45.5

* 12 mice were killed at each time indicated.

climated mice longer than from unacclimated mice is not understood. Perhaps the decreased number of lymphocytes in donor skin (and therefore in the registration of antigenicity) is responsible for the increased transplant survival observed in Group III.

Since is is known that hypoxia raises the level of circulating adrenal corticoids, the involution of the thymus in acclimated mice is probably mediated through the increased activity of the pituitary-adrenal axis. Hamer and Krohn(6) showed that ACTH injections lead to a prolonged retention of skin homotransplants in rats, and Finkel(7) demonstrated a close correspondence between spleen size and antibody production. Both of these studies suggest a relationship between the functional state of the thymus and spleen and the immunological potential of the animal.

On the basis of the above results it is postulated that the stress associated with exposure to reduced barometric pressure depletes the lymphoid elements of the animal to such an extent that a longer time is required for initiation of transplant rejection. Studies are under way to determine more precisely the manner in which altitude acclimation affects the immune response of mice to foreign antigens and skin homotransplants.

Summary. Prior acclimation of either or both the donor and host to reduced barometric pressure prolongs the retention of skin homotransplants in mice. Spleen and thymus studies suggest that the increased tolerance of acclimated mice to skin homotransplants may be due to an alteration of the animal's immunological state.

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Sero-protection Against Intracerebral *Salmonella typhosa* Infection in Chicks.* (29759)

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In attempts to assay experimentally the immunizing potency of typhoid vaccines, one of the methods commonly used is to vaccinate humans or laboratory animals and to measure the capacity of their sera passively to protect mice against fatal infection (or development of bacteremia and tissue invasion) with *S. typhosa*. Among institutions concerned with this problem, the details of testing procedure have differed. Thus, antiserum has been given intraperitoneally, intravenously, intramuscularly or subcutaneously; the time interval between injection of serum and the subsequent bacterial inoculum has been varied, from 30 minutes to 48 hours; most investigators have administered the challenge dose intraperitoneally, using large numbers of living bacilli suspended in saline or fewer organisms incorporated in suitable preparations of hog gastric mucin. The disadvantages of the respective methods have been extensively discussed in the literature (1-4) but there is still no general agreement as to which procedure is preferable; this is particularly true in regard to the mode of infection.

The intracerebral route of inoculation with

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