experimental data and the previous experimental data on wetting-phase electrical conductivity can be interpreted in terms of the network model, and both suggest about the same degree of pore interconnection (*6*).

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8 August 1959

Effect of Stress on Skin Transplantation Immunity in Mice

Abstract. Chronic avoidance-learning stress was found to depress the immune reaction responsible for skin homograft rejection to a modest but significant degree. This effect was observed in a genetically uniform as well as a heterogeneous line of mice.

Although diverse types of systemic stress have long been known to modify the immunological responses of mammals (*1*), precise experimental investigations have recently elucidated the effect of such stress on particular immune reactions. Thus, mice subjected to a standardized avoidance-learning type of stress show an increased susceptibility to *Herpes simplex* virus infection (*2*) as well as a decreased susceptibility to passive anaphylaxis (*3*) and a depressed colloid-clearing capacity of the reticuloendothelial system.

The study discussed here was undertaken to determine the effect of controlled stress on skin-homograft rejection. The immunologic basis of the homograft reaction has been well established and shows the characteristics of a typical hypersensitivity of the delayed type (*4*).

The stressing procedure employed has been described in detail by Rasmussen *et al.* (*2*). The apparatus makes use of a shuttle box with wired floor each half of which is alternately electrified with a 20- to 30-volt current painful to the mouse. Alteration of current from one side to the other is preceded by signals from a light and buzzer. Mice soon learn to avoid the shocking current, which occurs at about 5-minute intervals. The animals are subjected to this stress 6 hours per day, 6 days per week. Such stress regularly engenders significant changes in the weights of organs: the weights of the thymus and spleen decrease, whereas those of the liver and adrenal increase. Moreover, a progressive leukopenia occurs as the stress is continued over several weeks. Randomly bred Swiss-Webster BRVS mice for which the stress parameters have been determined (*2*) were employed, along with highly inbred C57B1 and A-line mice. Single, orthotopic skin homografts were made by the technique of Billingham and Medawar (*5*) in two donor-recipient combinations—A→C57B1 and C57B1→Swiss. Thus, both inbred and genetically diverse recipients were tested. The Swiss recipients were all virgin females, whereas both sexes were represented in the C57B1's.

Mice about 5 weeks old were exposed to the standardized stress experience for 2 weeks before grafting. On the day after grafting, the mice were again subjected to stress until homograft rejection was complete. The control mice received grafts in the same manner but were not exposed to experimental stress. Protective bandages were removed for the initial inspection on the 8th day, and graft survival was scored daily thereafter. Intermediate stages of breakdown were estimated by gross inspection and confirmed in several instances by histologic examination of biopsy sections stained with hematoxylin and eosin. Zero survival end points were assessed on the basis of no surviving graft epithelium. Median survival times as well as tests for parallelism and reaction-time ratios, with their 95-percent confidence limits, were computed by the method of Litchfield (*6*).

The cumulative percentage of homografts destroyed in each experiment is plotted against days after grafting in Fig. 1. While it is apparent that the time-mortality distributions of grafts in the comparable groups of control mice and stressed mice are distinctive, the prolongation of skin homograft survival in the stressed mice was not extensive. Also, the figure reveals that the uniform C57B1 recipients showed a narrow range of graft-survival times, whereas the Swiss mice showed the broad distribution characteristic of genetically diverse recipients. The results are summarized in Table 1. When the data were subjected to the parallelism and reaction-time ratio tests of significance, the difference between stressed and control mice in both combinations is significant at the 95-percent level of probability.

Although the stress applied is known to induce profound physiological changes in mice, it appears probable that the observed inhibition of transplantation immunity in stressed mice is affected primarily by hypersecretion of adrenal corticosteroids. Indeed, the decrease in weight of the spleen and the progressive leukopenia in stressed mice can be duplicated by administration of cortisone. Since homograft immunity, like other delayed types of hypersensitivity, is clearly mediated by lymphoid cells, a substantial depression of such cells by corticosteroids would be expected to allow a prolongation of skin homograft survival. Nevertheless, the endocrine situation is complex. While the normal mouse secretes principally corticosterone and little if any cortisone and hydrocortisone (*7*), Medawar and Sparrow (*8*) have shown that injection of the latter compounds but not of corticosterone will prolong homograft survival time in mice. An analysis of the endogenous corticosteroid levels in stressed mice now under way in this laboratory should indicate whether such mice preferentially secrete the hormones known to prolong homograft survival. The possibility remains, of course, that the stress-induced inhibition of the homograft reaction is mediated mainly through channels other than the adrenal corticoids. In this connection, studies with rats (*9*) have revealed that adrenal corticoid output may actually decrease below normal levels during prolonged stress.

It should be noted that our control mice were unfortunately exposed to the periodic noise of building reconstruc-

Table 1. Summary of results.

Group	Donor-recipient combination	No. of mice	Median survival times (days) with 95% confidence limits
Control	A→C57B1	7	8.2 (7.2–9.3)
Stressed	A→C57B1	11	9.6 (9.3–9.9)
Control	C57B1→Swiss	23	8.5 (8.3–8.7)
Stressed	C57B1→Swiss	21	9.2 (8.6–9.9)

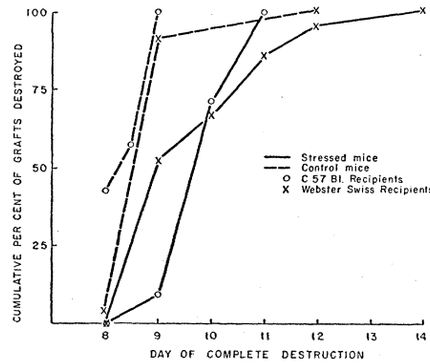

Fig. 1. Cumulative time-mortality curves for skin homografts in stressed and non-stressed mice.

tion and thus were moderately stressed. Some additional stress may also have resulted from caging the animals singly. Hence, the difference between experimental and control groups might be more striking under ideal conditions.

Even though the physiological pathways of action are still poorly defined, it is clear that chronic, avoidance-learning stress induces a prolongation of homograft survival times to a small but significant degree in genetically homogeneous as well as in heterogeneous lines of mice (*10*).

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31 August 1959

Growth Pattern in the Green Hydra, Chlorohydra viridissima

Abstract. A method was employed whereby it was possible to determine the growth pattern of hydra without using the traditional techniques of grafting and vital staining. It has been shown that hydra possess a growth region just below the hypostome where constant cell proliferation renews the tissues of the animal completely every few weeks.

In 1949 Brien and Reniers-Decoen (*1*) published an account of the growth pattern exhibited by the common brown hydra, *Pelmatohydra oligactis* (Pallas). These investigators grafted upper portions (mouth, tentacles, hypostome, and the region just below the hypostome) of animals which had been stained vitally with methylene blue, neutral red, and Nile blue sulfate to the stalk or peduncular region of unstained animals. During the next few weeks the stained material migrated towards the basal disk, where, after a period of from 3 to 5 weeks, the stained material disappeared entirely. These workers concluded from the experiments that hydra possess a growth region just under the hypostome where rapid cell division occurs in both epidermis and gastrodermis. As cells divide they force nondividing cells proximally to the basal disk and distally to the tentacle tips, where these migrating cells die and are sloughed off. Thus, it appears that the hydra is constantly renewing its tissues every 3 to 5 weeks.

This discovery of Brien and Reniers-Decoen, if correct, will influence to a great extent future interpretations of experiments dealing with growth and cell differentiation in hydra, because it was formerly believed that the hydra's "immortality" was dependent only upon a constant tissue replacement by interstitial cells in all areas of the body.

The present study was an attempt to confirm the existence of a growth region in hydra and to determine whether a growth region exists in species of hydra other than *Pelmatohydra oligactis*. Also, it was necessary to devise a method of staining which did not involve vital dyes, since there is a possibility that these dyes are capable of diffusing from cell to cell, thus mimicking cell movement. Furthermore, it was desirable to eliminate the grafting techniques applied to experimental animals, because it appeared that these procedures were not altogther satisfactory for studying the normal growth process of an animal. In any grafting precedure applied to hydra a wound must be made in each of the pieces to be joined. Once the pieces are united, a certain amount of regeneration is necessary to repair the wounded tissue. New nervous and muscular tissue must be made, for example. It is never possible to cut sections which exactly complement each other from two different animals. In a given hydra there is a certain distance which separates the hypostome from the budding region, the distance depending upon the dominance exerted by the mouth in the immediate vicinity. This distance is further dependent upon the size and physiological state of the animal. Thus, when a proximal portion of one animal is grafted to the distal portion of another, a process of regulation must occur while the body regions of the newly formed animal are being adjusted. During this period of regeneration and regulation it is possible that cells are called to service from distant body areas to aid in repair processes and so on. Also it appeared possible that active cell proliferation might have begun at the site of the wound, thus establishing a temporary growth region.

A relatively simple procedure was found for marking the cells of the green hydra, *Chlorohydra viridissima*. Whitney (*2*) found that a green hydra when placed in a 0.5-percent glycerin solution for a few days voided its algal bodies. Several "white" animals resulting from this treatment were cut through the center of their budding zones into two pieces. The piece containing the head and tentacles was then grafted to the base, stalk, and lower half of the budding region of a normal green animal. This results in an animal whose budding region is half green and half white. The buds produced from such animals are also sometimes half green and half white, but usually the buds are white with irregular green blotches. These irregularly colored forms were then allowed to reproduce, and after several hundred of their offspring and of the offspring of succeeding generations had been examined, it was possible to find about two dozen animals with only a single patch or two of algal-laden digestive cells in their bodies. Thus, we had essentially a stained animal which was not directly involved in grafting procedures. Also, it may be assumed that no diffusion of algal cells from one cell to another in hydra will occur, since an individual algal cell is as large as the digestive cell nucleus. All that was necessary at this point was to observe the fate of algal-laden digestive cells in the growth region over a period of several days.

In 15 animals examined there was a distal and proximal migration of colored cells from the growth region. In many cases the colored material moved down only one side of the body column, showing that diffusion was not taking place. It usually took from 2 to 3 weeks for cells to be forced from the growth region to the basal disk and less than a week (average, 5 days) for cells to be forced from the growth region to the tips of the tentacles.

It may be concluded that a growth region does exist in *Chlorohydra viridissima* similar to that found in *Pelmatohydra oligactis*. Furthermore, these experiments indicate that the cell migration witnessed after staining *P. oligactis* with vital dyes was not due to regenerative and regulative processes involved in grafting procedures.

It is hoped that this simple marking procedure will be employed more in the future for the study of such processes as budding, wound healing, and reorganization of animals from bits of tissue.

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