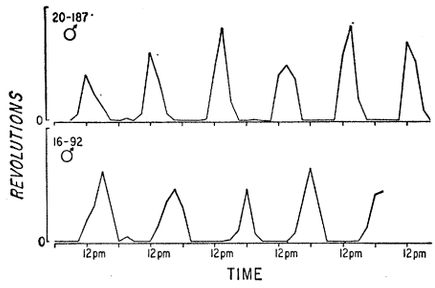

Fig. 1. Examples of rhythmic patterns of activity obtained from animals housed in activity wheels.

to 6 hours of immobilization in flexible wire mesh as previously described (*2*). On the basis of the activity pattern of the previous 5 or 6 days, 17 animals were immobilized at the beginning of the time they would have been entering their period of activity, and 13 animals were immobilized at the beginning of the time they would have been entering a trough in their particular cycle. After the period of immobilization, the animals were killed. The stomachs were removed, opened along the greater curvature, washed in saline, and the incidence of gastric erosions was recorded.

Eight animals were found to have gastric erosions. All of these came from the group immobilized during the active phase of their particular cycle. This difference in the incidence of erosions between the "peak" and "trough" animals is significant at less than the .01 level as determined by Fisher's exact probability test.

These data demonstrate conclusively the extent to which the prior state of an organism can influence its response to "stress." However, the experiment does not delineate the relative contribution of psychological as compared to physiological factors in determining this effect. For example, it was observed that of the 13 animals immobilized during a trough in their activity cycle, eight of these had some undigested food in their stomachs. In the group immobilized during their peak of activity there were two animals with food in their stomachs and neither of these developed gastric erosions. It is an impression gained from previous research with gastric erosions that the presence of food in the stomach offers some protection against the development of such lesions. Also, the research of Halberg and his associates (*4*) clearly demonstrates the existence of circadian rhythms in a variety of physiological processes, and the effects of such rhythms on responses to pathogenic stimulation. Some of these 24-hour rhythms undoubtedly parallel the activity rhythm, although their relevance to the development of immobilization-induced gastric erosions remains to be determined. In view of such data, however, it will be necessary to determine, for example, whether the plasma pepsinogen concentration, which is known to be related to the development of gastric erosions, exhibits cyclic variations which may be synchronized with the cyclic variations in activity, or, perhaps, if adrenal steroids, which do exhibit rhythmic variations, are related to the development of such lesions. Acknowledging the potential relevance of such variables, there is, nonetheless, some face validity to the behavioral hypothesis that the "perception" of restraint would be different for animals that are behaviorally prepared to be active or inactive.

In addition to the work of Halberg and his associates there have been other recent reports concerning the role of 24-hour rhythms in influencing sensitivity to stimuli such as x-irradiation (*5*) and drugs such as Nembutal (*6*). As in the experiment described here, such studies have not been concerned with periodicity in itself as much as with decreasing the commonly observed variability in response to the stimulus under study. The studies with drugs, for example, have been prompted by a recognition of certain physiological rhythms which might be expected to influence the rate at which the drug might be detoxified. Analagously, the present experiment was the result of a psychological orientation involving the behavioral relationship between overt activity and the suppression of overt activity which was the stimulus for a pathogenic process. Both approaches lead to the general conclusion that a consideration of the existing psychophysiological state of the organism is a meaningful factor in determining the organism's response to a superimposed stimulus.

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Homograft Sensitivity Induction by Group A Streptococci

Abstract. *Streptococcal cells can induce in the guinea pig a state of altered reactivity to skin homografts similar to that resulting from sensitization with homologous tissue.*

Recent reports have suggested the presence of an antigen or antigens in certain strains of group A hemolytic streptococci which exhibits or exhibit immunologic cross reactivity with human heart and skeletal muscle, as well as with rabbit tissues (*1*). This and other observations (*2*) lend support to the suggestion that similar antigenic structures may occur in groups as widely separated phylogenetically as bacterial and mammalian cells (*3*). This report is concerned with the induction of a state of altered reactivity to skin homografts in guinea pigs after treatment with suspensions of heat-killed group A, type 12 hemolytic streptococci.

Group A, type 12 hemolytic streptococci (*4*) were grown overnight at 37°C in Wannamaker's dialysate medium (*5*). They were collected and killed by heat, being placed in a 56°C waterbath for 45 minutes (*6*). The heat-killed bacterial cells were resuspended in Medium 199 (*7*), and a portion of the suspension was removed for dry-weight determinations. The remainder was emulsified in 10 ml of Freund's incomplete adjuvant (Difco). The emulsion (0.1 ml) was injected into each footpad of the animals. The test animals received dosages of the streptococcal cells ranging from 0.14 to 13.0 mg (dry weight). Four groups of control animals were included in the

study. The first group was injected with Freund's incomplete adjuvant alone; the second group received injections of an emulsion of Medium 199 in Freund's incomplete adjuvant; the third group was injected with Wannamaker's dialysate medium either alone, or as an emulsion in Freund's incomplete adjuvant. The footpad injections in control animals were similar to those used for the test group. Eleven to 14 days after injection, the guinea pigs were challenged with a skin homograft obtained from a normal donor. A fourth group of 25 untreated recipients also received skin homografts.

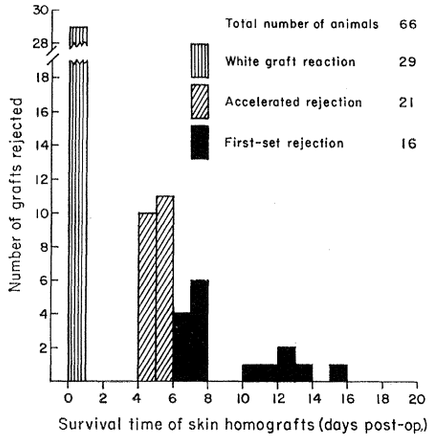

Fig. 1. Response to skin homografts in guinea pigs that had been treated with hemolytic streptococci.

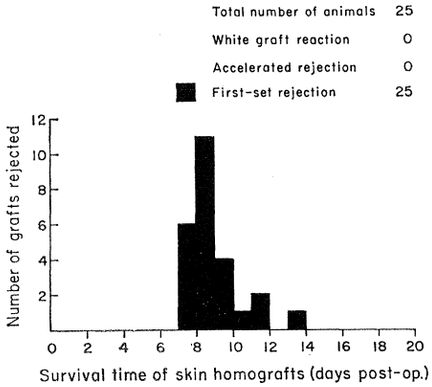

Fig. 2. Behavior of first-set skin homografts in untreated control guinea pigs.

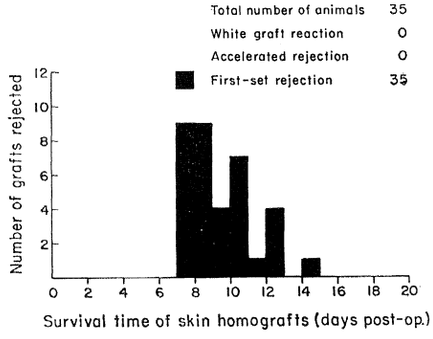

Fig. 3. Behavior of first-set skin homografts in treated control guinea pigs.

Outbred male albino guinea pigs of the Hartley strain, weighing 250 to 300 g, were used throughout this study. The skin homografts were full-thickness circular specimens measuring 11 mm in diameter. They were applied above the panniculus on the dorsal surface of the recipients (*8*). The grafts were approximated to the host skin by interrupted 5-0 silk sutures, and a pressure dressing was applied. The grafts were examined daily after the 3rd day; gross and stereomicroscopic observation of the graft surface (*9, 10, 11*) provided an accurate evaluation of graft viability in test and control animals.

As shown in Fig. 1, 50 of 66 consecutive guinea pigs sensitized with emulsions of heat-killed streptococci rejected first-set skin homografts either as white grafts or in an accelerated fashion (*9, 10*). There were 29 white graft reactions, characterized by complete absence of superficial vascularization, a parchment-white color, and gradual change of the graft into a tan-colored eschar. In some instances, these grafts also exhibited deep hemorrhages before change into an eschar. Of the 21 grafts undergoing accelerated rejection, 10 were rejected at 4 to 5 days, and 11 grafts were rejected at 5 to 6 days. The remaining 16 homografts had survival times of 6 to 14 days (mean survival time: 9.2 days); such first-set survival times were generally observed in those animals which had received low dosages of streptococcal cells (0.14 mg).

Figure 2 illustrates the behavior of first-set skin homografts applied to 25 consecutive untreated control animals. No white graft reaction was observed. Survival time of skin homografts in this group ranged from 7 to 13 days (mean survival time: 8.4 days). Figure 3 summarizes the results of prior treatment of guinea pigs with Freund's incomplete adjuvant, or with an emulsion of Medium 199 in incomplete adjuvant, or with Wannamaker's dialysate medium. Of the skin homografts applied to 35 control animals treated in this fashion, none was rejected prior to the seventh postoperative day, and no graft exhibited the white graft reaction. Survival times of skin homografts in this group ranged from 7 to 14 days (mean survival time, 8.9 days).

The survival times of skin homografts applied to control animals in the course of this study are in close agreement with those noted in previous reports of the behavior of first-set skin homografts in the guinea pig (*8*). They are in sharp contrast with the survival time of skin homografts in guinea pigs pretreated with emulsions of heat-killed streptococcal cells. In this group of animals, the response to skin homografts closely resembles that observed in recipients previously sensitized with skin or another source of tissue transplantation antigens (*10*). This study thus suggests that, under the experimental conditions described, group A, type 12, hemolytic streptococcal cells induce in the guinea pig a state of altered reactivity to skin homografts similar to that resulting from sensitization with homologous tissues. The intracellular localization of the streptococcal component or components responsible for this observation, as well as the induction of accelerated homograft rejection in other animal species by this technique, are currently under investigation.

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