

Social Factors Affecting Emotionality and Resistance to Disease in Animals

V. Early Separation from the Mother and Response to a Transplanted Tumor in the Rat

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Seven litters of Sprague-Dawley rats were permanently separated from their mothers at 15 days of age. Five control litters were weaned at the usual time of 21 days. At 45 days animals were inoculated with Walker 256 carcinosarcoma cells. Mortality rate in the early-separated animals was significantly greater than that in the controls. These data increased the generality of the previously reported findings of an increased susceptibility to disease (gastric ulcers) in prematurely weaned rats.

BEHAVIORAL CHARACTERISTICS and physiologic processes, including ultimate longevity, have been shown to be affected by premature weaning in animal subjects.^{5, 8} Also a recent series of experiments showed that, when tested as adults, rats separated from their mothers at 15 days of age were more susceptible to conflict-induced rumenal ulcers than animals weaned at the usual time of 21 days.^{1, 3} It does not necessarily follow,

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however, that rats weaned earlier in life than is usual would be more susceptible to other forms of pathogenic stimulation. It has been hypothesized² that early life experiences have the capacity to alter chronically the psychophysiologic functioning of animals and that such alterations (interacting with the psychophysiologic responses unconditionally elicited by the action of specific pathogenic stimuli) influence disease susceptibility. One method of gathering evidence on this point is to hold the independent variable constant (in this case, early separation from the mother) and subject experimental and control animals to a variety of pathogens. The present study, then, was designed to determine the effects of early weaning on susceptibility to the Walker 256 carcinosarcoma. Previous research by Newton, Bly, and Mc-

Crary⁷ had shown that responses to this transplantable tumor may be influenced by early experiences.

A preliminary experiment suggested that rats separated from their mothers at 15 days of age were more susceptible to the transplanted tumor than control animals weaned at the usual time of 21 days. The early-separated animals also appeared to be more susceptible than animals that remained with their mothers until 21 days but were prohibited from suckling after 15 days. This suggested that the observed differences were not a function of some caloric or nutritional deficiency in the prematurely weaned group. Apparently, a relatively nonvirulent strain of the tumor had been used, since fewer than 50% of the animals developed tumors and not all of these died. Although not statistically significant, these data stimulated this further study.

Method

Twelve litters of Sprague-Dawley rats were culled to 8-9 pups each within 48 hr. of birth. Seven randomly chosen litters (Group S) were separated from their mothers at 15 days of age. These animals remained in the nesting cages with shallow trays containing water and powdered food. The remaining 5 litters (Group C) were weaned at 21 days, at which time all animals were weighed, marked, and housed with same-sex littermates in groups of 4-6. Food and water were available ad libitum.

At 45 days the animals were again weighed and inoculated with a suspension of the Walker 256. At this time there were 62 S and 41 C animals. A donor* animal bearing the Walker 256 carcinosarcoma was sacrificed, and the subcutaneous tumor was exposed by relatively aseptic techniques. With sterile instruments, portions of the tu-

mor were removed; particularly fibrous or necrotic areas were avoided. The portions of tumor to be inoculated were gently ground in a 40-mesh tea strainer and diluted with an equal amount of Hank's solution containing 100 μ g./ml. of penicillin. The resulting suspension contained approximately 2.5×10^8 viable tumor cells per milliliter. Using an 18-gauge needle, 0.1 ml. of the suspension was injected subcutaneously into the back of each recipient animal. Daily observations were made to determine when a tumor reached 2 cm. in size, as measured by calipers, and when an animal died.

Results and Discussion

Analysis of body weight at 21 days showed that early separated animals, with a mean of 49.4 gm., weighed significantly less than controls, with a mean of 52.7 gm. ($t = 2.74$, $p < .01$). By 45 days, Group S males weighed 194.8 gm. and Group C males weighed 219.6 gm. ($t = 3.32$, $p < .01$). The females in Groups S and C had mean body weights of 158.3 and 163.4 gm., respectively ($t = 1.02$, $p > .10$).

Of the 103 inoculated animals, 85 developed characteristic subcutaneous tumors greater than 2 cm. in size. Fifteen other animals died without localized subcutaneous tumors, but appeared to have peritoneal involvement, with production of ascites. Two of these latter animals were autopsied, and microscopic examination revealed multifocal malignant involvement with neoplastic cells noted in the ascites. In retrospect, it would appear that these young animals were accidentally inoculated intraperitoneally rather than subcutaneously. Of the 100 tumor-bearing animals, 94 died within 30 days of the inoculation. There were no sex differences, so males and females within each group were combined in the subsequent analyses.

The number of animals that developed tumors did not differentiate between

*Our donor animals were originally supplied by Dr. Kanematsu Sugiura, Walker Laboratory, Sloan-Kettering Institute for Cancer Research, Rye, N. Y.

early-separated and control animals, and S and C groups developed 2-cm. tumors at the same rate. There was, however, a significant difference in the rate at which animals died (Fig. 1). Group S animals died a median of 21 days after inoculation, and Group C animals died in a median of 25 days. The Mann-Whitney *U* test yielded a *Z* of 2.41 ($p = .016$, two tails). Considering only those animals that developed subcutaneous tumors (i.e., eliminating those with primary peritoneal involvement), a more conservative analysis of mortality rate, beginning from the day at which a given animal was found to have a 2-cm. tumor, also indicated that the early-separated animals died at a faster rate than the controls ($Z = 2.51$, $p = .012$).

Spearman rank correlation coefficients computed between the 45-day body weights and the time to death yielded insignificant correlations of .02 and $-.15$ for the males and females of Group S and correlations of .18 $-.06$ for males and females in the control group. This lack of relation between body weight and survival time is consistent with other data on the Walker tumor⁹ and argues against explanation of these data based on the difference in body weight between the two groups. Moreover, since

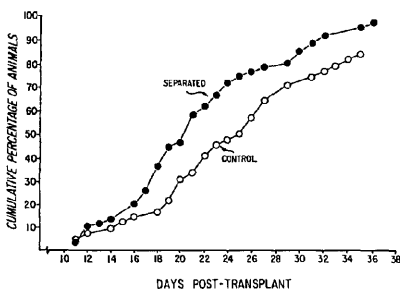


FIG. 1. Cumulative mortality rate in response to Walker 256 carcinosarcoma in rats separated from their mothers at 15 days of age (separated) and in animals weaned at usual time of 21 days (control).

a caloric deficiency has been found to retard the development of the Walker tumor,^{4, 6} the effect, if any, of the observed differences in body weight, interpreted as representing a nutritional deficit in the early-separated animals, would probably have acted to produce differences in a direction opposite to those actually observed.

The present findings serve to document further the extent to which early life experiences may influence the response to pathogenic stimuli. More specifically, these data increase the generality of the previously reported phenomenon relating early separation from the mother to subsequent resistance to disease (gastric ulcers). It is our general orientation that susceptibility to disease involves an interaction between the psychophysiologic effects of early experience on the one hand, and the psychophysiologic effects elicited by specific pathogenic stimulation on the other. Because similar effects were obtained with the transplanted tumor as had been obtained with gastric ulcers, however, these data neither support nor contradict this hypothesis. Except to eliminate the factor of body weight, it is not now possible to offer an explanation for the increased mortality rate from the transplanted tumor cells in animals separated from their mothers earlier in life than is usual. While there are data relating tumor growth to adrenal cortical function, mortality rate is undoubtedly a composite of many factors, the growth rate of the tumor being only one. Whatever the mechanisms mediating tumor growth or eventual mortality, it is assumed that the differences obtained are, in part, a result of an as yet unspecified chronic alteration in the psychophysiologic function effected by the early separation experience.

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