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Differential Survival to Leukemia as a Function of Infantile Stimulation in DBA/2 Mice.* (25140)

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The influence of environmental factors on physiological activity has been well documented. The effects of emotional stress(1) and periodicity(2) are examples of environmental influences on physiological functioning. Recently there has appeared evidence to indicate that the nature of environment during organism's infancy exerts profound influence on physiological activity later in life. For example, rats which received extra stimulation during infancy show less mortality(3) following 120 hours of food and water deprivation and less adrenal hypertrophy 24 hours following an injection of hypertonic glucose(4) than do nonstimulated controls. This communication presents findings concerning the effects of infantile stimulation on length of survival following implantation of leukemia 1210 in DBA/2 mice.

Materials and methods. Ninety mice were used. Litters were assigned at birth alternately to either the stimulated or non-stimulated condition. Experimental treatment consisted of removing the pup from the nest, placing it in a 2 x 3 x 6″ compartment for 3 minutes, then returning the infant to the nest. This procedure was initiated on day following birth and was continued once daily until weaning on day 24. There were 42 mice in this treatment group. The non-treated subjects (N = 48) remained in the nest and were not treated in any manner until weaning. On day 24 all mice were weaned and placed in group cages. No animals were manipulated from weaning until time of implantation. All animals were implanted interperitoneally with 2 x 10^6 leukemic cells in 0.1 ml volume of Locke's solution when they reached a weight of at least 18 g, *i.e.,* between ages 45 to 55 days. The tumor used, L-1210, is a transplantable lymphoid leukemia induced in DBA/2 mice(5), maintained in the tumor bank at Battelle Memorial Inst. by weekly implantation of 2 x 10^6 tumor cells into the peritoneal cavity. This tumor has been very consistent in causing death in 100% of implanted animals with mean survival time over the past year between 8 to 9 days, and with a latent period of 2 to 3 days after implantation.

Results. (Table I) shows that animals stimulated by manipulation during postnatal period have lower survival times than nonstimulated mice. In the first and second replications there was no overlap between groups, thus obviating the need for statistical analysis. A median test was used for the third replication and yielded an X^2 of 20.16 significant beyond the .001 level of probability. Although there were some differences between the replications, these may in part be attributable to the fact that a transplantable tumor shows changes during the course of repeated transplantation. These changes may manifest themselves as slight variations in mean survival time of animals implanted. Furthermore, the experimental subjects, although highly inbred, may show fluctuation in re-

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TABLE I. Survival Time in Days after Tumor Implantation.

		N	5	6	7	8	9	10
Rep 1	S	10			10			
	NS	12					12	
2	S	7	7					
	NS	8					8	
3	S	25		18	6	1		
	NS	28		3	23		1	1

S = Stimulated during infancy.
NS = Nonstimulated controls.

sponse to implanted tumors because of seasonal variation, or the state of the colony in relation to endemic disease. However, the direction of differences is consistent and significant between replications.

In every test the early stimulated mice have shown significantly earlier death to the implanted leukemia.†

Discussion. These data are contrary to what might have been expected on the basis of previous research. Whereas in the past, viability has been increased by infantile stimulation, the opposite is found in these experiments.

It has been proposed that stimulation as used in this study, constitutes a stress for the infant organim. The effects of experience to chronic stress in infancy is hypothesized to modify the "neural stats"(6) resulting in a modified pattern of response to later stressful stimuli. More specifically this hypothesis states that the nature of the modification is a reduction of neurohumoral response to physiological insult. In view of certain constitutional factors such as size, developmental rate and genetic hyperemotionality of the DBA/2 mouse it appears that handling may be even more stressful to the mouse, thus reducing still further the neurohumoral response to later stress.

It is possible, therefore, that survival to the implanted leukemia may depend upon the organism's ability to produce a maximal neurohumoral response and when this response is effectively reduced less viability results.

These hypotheses are at best tentative. Any attempt to elucidate mechanisms must await further research. It has been suggested, however, that our results could be explained on the basis of a mild, inapparent infection caused by the handling procedure. This suggestion does not appear tenable for 2 reasons: (1) The background data for this study was the response of more than 3,000 mice implanted with L-1210. Many of these mice were also used in a study of prevalence of salmonella organisms in experimental mouse stocks. Survival time from leukemia was quite consistent and was not affected by presence of a low grade infection; and (2) the effects of infantile stimulation have been shown when the stimulating procedure did not involve handling(7).

Summary. DBA/2 mice were stimulated by being removed from the nest for 3 min. daily from birth to day 24. Control mice received no stimulation. Between days 45 and 55 all animals were implanted with leukemia 1210. The stimulated mice showed significantly shorter survival time than their nonstimulated controls. This effect is assumed to be a result of early chronic stress leading to a reduced neurohumoral response to later challenge.

† Since writing this report, 2 subsequent experiments have been done. One experiment using hybrid BDF/1 showed the same results as those presented here, and an additional replication using DBA/2 mice yielded the same significant results.

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