References and Notes

1. F. C. Fuglister, in *Studies in Physical Oceanography, A Tribute to Georg Wüst on His 80th Birthday*. A. L. Gordon, Ed. (Gordon & Breach, New York, 1972), vol. 1, p. 137.
2. C. E. Parker, *Deep-Sea Res.* **18**, 981 (1971).
3. D. Y. Lai and P. L. Richardson, *J. Phys. Oceanogr.* **7**, 670 (1977).
4. P. L. Richardson, R. E. Cheney, L. V. Worthington, *J. Geophys. Res.* **83**, 6136 (1978).
5. H. Kawai, in *Proceedings of the Fourth Cooperative Study, Kuroshio (CSK), Tokyo, February 1979* (Saikon, Tokyo, 1979), p. 250.
6. C. S. Nilsson, J. C. Andrews, P. Scully-Power, *J. Phys. Oceanogr.* **7**, 659 (1977).
7. R. E. Cheney, W. H. Gemmill, M. K. Shank, P. L. Richardson, D. Webb, *ibid.* **6**, 741 (1976).
8. P. H. Wiebe, K. H. Burt, S. H. Boyd, A. W. Morton, *J. Mar. Res.* **34**, 313 (1976).
9. R. A. Doblar and R. E. Cheney, *J. Phys. Oceanogr.* **7**, 944 (1977).
10. A. Leetmaa, *Science* **198**, 188 (1977).
11. A convenient way of expressing the magnitude of the cold-core ring anomaly is to indicate the minimum depth to 15°C at the ring center. The Slope Water–Gulf Stream boundary is commonly determined by the 15°C isotherm at 200 m, with this isotherm being shallower to the north and deeper to the south; thus, the shallower the 15°C isotherm in a cold-core ring the greater the anomaly.
12. N. E. Huang, C. D. Leitao, C. G. Parra, *J. Geophys. Res.* **83**, 4673 (1978).
13. R. E. Cheney and J. G. Marsh, in preparation.
14. D. E. Hagan, D. B. Olson, J. E. Schmitz, A. C. Vastano, *J. Phys. Oceanogr.* **8**, 997 (1978).
15. P. Mukherji and D. R. Kester, *Science* **204**, 64 (1979).
16. F. A. Richards and A. C. Redfield, *Deep-Sea Res.* **2**, 182 (1955).
17. We use here the same terminology as in Backus *et al.* (*43*).
18. P. L. Richardson, *J. Phys. Oceanogr.* **10**, 90 (1980).
19. ———, C. Maillard, T. B. Sanford, *J. Geophys. Res.* **84**, 7727 (1979).
20. F. C. Fuglister, in *A Voyage of Discovery, George Deacon 70th Anniversary Volume*, M. V. Angel, Ed. (Pergamon, New York, 1977), p. 177.
21. A. C. Vastano, J. E. Schmitz, D. E. Hagan, *J. Phys. Oceanogr.* **10**, 493 (1980).
22. C.-G. Rossby, *J. Mar. Res.* **2**, 38 (1939).
23. B. A. Warren, *Deep-Sea Res.* **14**, 505 (1967).
24. J. McWilliams and G. R. Flierl, *J. Phys. Oceanogr.* **9**, 1155 (1979).
25. R. Mied and G. J. Lindemann, *ibid.*, p. 1183.
26. A. C. Vastano, D. E. Hagan, J. E. Schmitz, in preparation.
27. J. R. Barrett, *Deep-Sea Res.* **18**, 1221 (1971).
28. R. E. Cheney and P. L. Richardson, *ibid.* **23**, 143 (1976).
29. G. R. Flierl, *J. Phys. Oceanogr.* **7**, 365 (1977).
30. D. B. Olson, *ibid.* **10**, 514 (1980).
31. D. C. Smith, thesis, Texas A & M University (1980).
32. R. B. Lambert, Jr., *Deep-Sea Res.* **21**, 529 (1974).
33. R. L. Molinari, thesis, Texas A & M University (1970).
34. J. E. Schmitz and A. C. Vastano, *J. Phys. Oceanogr.* **5**, 93 (1975).
35. A. C. Vastano and D. E. Hagan, *ibid.* **7**, 938 (1977).
36. P. H. Wiebe and S. H. Boyd, *J. Mar. Res.* **36**, 119 (1978).
37. A. Fleminger and K. Hulsemann, *Mar. Biol.* **40**, 233 (1977).
38. P. H. Wiebe, E. M. Hulburt, E. J. Carpenter, A. E. Jahn, G. P. Knapp III, S. H. Boyd, P. B. Ortner, J. L. Cox, *Deep-Sea Res.* **23**, 695 (1976).
39. P. B. Ortner, P. H. Wiebe, L. R. Haury, S. H. Boyd, *Fish. Bull.* **76**, 323 (1978).
40. S. H. Boyd, P. H. Wiebe, J. L. Cox, *J. Mar. Res.* **36**, 143 (1978).
41. P. B. Ortner, E. M. Hulburt, P. H. Wiebe, *J. Exp. Mar. Biol. Ecol.* **39**, 101 (1979).
42. P. B. Ortner, P. H. Wiebe, J. L. Cox, *J. Mar. Res.* **38**, 507 (1980).
43. R. H. Backus, J. E. Craddock, R. L. Haedrich, B. H. Robison, C. E. Karnella, *Mem. Sears Found. Mar. Res.* **1** (part 7), 266 (1977).
44. B. G. Nafpaktitis, R. H. Backus, J. E. Craddock, R. L. Haedrich, B. H. Robison, C. Karnella, *ibid.*, p. 38.
45. C. Karnella, personal communication.
46. D. R. Watts and D. B. Olson, *Science* **202**, 971 (1978).
47. W. R. Holland, *J. Phys. Oceanogr.* **8**, 363 (1978).
48. In accordance with Lai and Richardson (*3*), we assume that the area affected by cold-core rings is that part of the northern Sargasso Sea west of 50°W, about 3×10^{12} m^2, and the volume affected is that part of the area above the permanent thermocline, or about 3×10^{15} m^3. The southern boundary of the northern Sargasso Sea is judged to lie at about 28°N, corresponding to the southern limit of the principal westward return flow of the Gulf Stream (*49*).
49. L. V. Worthington, *Johns Hopkins Oceanogr. Stud.* **6** (1976), figure 42.
50. C. Wunsch, *Rev. Geophys. Space Phys.* **16**, 583 (1978).
51. W. J. Schmitz, Jr., *J. Mar. Res.* **38**, 111 (1980).
52. G. T. Rowe and W. D. Gardner, *ibid.* **37**, 581 (1979).
53. R. G. Fairbanks, P. H. Wiebe, A. W. H. Bé, *Science* **207**, 61 (1980).
54. MODE group, *Deep-Sea Res.* **25**, 859 (1978).
55. P. H. Wiebe, S. H. Boyd, J. L. Cox, *Fish. Bull.* **73**, 777 (1975).

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Psychoneuroendocrine Influences on Immunocompetence and Neoplasia

Vernon Riley

"Stress" is a widely used term for describing emotional and biological responses to novel or threatening situations. There is, thus, an extensive variety of experimental or other circumstances in which "stress" serves as a convenient word to express complex and incompletely understood psychological and physiological phenomena (*1–3*).

In studies at this laboratory, we use the term "stress" in a more restricted experimental sense to relate specific stress-inducing stimuli, or stressors, to their physiological consequences. The latter include specific biochemical, cellular, and tissue alterations that are associated with an emotional activation of the adrenal cortex by way of the pituitary and its secretion of adrenocorticotropic hormone (*4, 5*). Within the biological systems that we have used, several key parameters characterize the physiological manifestations of stress, and relate to pathological and other changes that may be observed in stressed experimental animals.

Although emotional stress brings about many biochemical changes, in our studies with mice we have focused our attention on the adrenal cortex and have measured with precision the most conspicuous, and what appears to be the most relevant, of the biochemical substances elaborated by this organ in response to anxiety, namely, corticosterone. Immediately after an animal is subjected to an emotional stimulus, or perceives a situation that generates anxiety, the adrenal cortex in response to signals from the hypothalamus, via the pituitary, produces increased quantities of corticosterone. The rapidity of the appearance of corticosterone in the plasma can be readily measured by appropriate microassay techniques (*6–8*).

Immunological and Pathological Consequences of Stress

Secondary manifestations that result from increased corticosterone in the blood plasma that are readily observed include (i) lymphocytopenia, or decreased circulating lymphocytes, (ii) thymus involution, and (iii) related loss of tissue mass of the spleen and peripheral lymph nodes. Details of these cellular and tissue stress effects will be discussed later, but it is relevant to note here that the physiological consequences of such stress-mediated events have significant

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adverse effects on important elements of the immunological apparatus. By appropriate experiments with mice, it is possible to demonstrate the pathological effects of a stress-associated decrease in immune competence on cancer processes, virus infections, and other diseases subject to immunological control.

Summary. Emotional, psychosocial, or anxiety-stimulated stress produces increased plasma concentrations of adrenal corticoids and other hormones through well-known neuroendocrine pathways. A direct consequence of these increased corticoid concentrations is injury to elements of the immunological apparatus, which may leave the subject vulnerable to the action of latent oncogenic viruses, newly transformed cancer cells, or other incipient pathological processes that are normally held in check by an intact immunological apparatus. This article describes studies that examine the adverse effects of increased plasma concentrations of adrenal corticoids on the thymus and thymus-dependent T cells, inasmuch as these elements constitute a major defense system against various neoplastic processes and other pathologies. The studies demonstrate that anxiety-stress can be quantitatively induced and the consequences measured through specific biochemical and cellular parameters, providing that authentic quiescent baselines of these conditions are obtained in the experimental animals by the use of low-stress protective housing and handling techniques.

Although it may be hazardous to extrapolate biological findings from mice to other species, it would be equally imprudent to ignore the many physiological similarities and analogous biochemical relationships that evolutionary biologists have demonstrated in animals belonging to the same phyla. Thus fundamental biological principles that are further delineated through the study of animal models may be expected to have application to man.

Early Studies and the Emergence of Psychoneuroimmunology

Many investigators have examined the relations between various forms of stress and neoplastic processes. However, difficulties inherent in the establishment of authentic quiescent baseline conditions for experimental animals complicated the interpretation of some of the earlier studies. Furthermore, in many instances there was a lack of access to the biochemical and cellular measurements that permit an objective assessment of the basic physiological manifestations of stress. Consequently, results between laboratories tended to be inconsistent, undermining confidence in the reliability of research in this field. However, rapid developments in the past few years delineating and characterizing many new facets of immunology, endocrinology, and neurobiology provide a more effective base for reexamining both the potentialities and the limitations of the effects of stress on neoplastic and other disease processes.

Our original incentive for studying stress was the need to better understand the seemingly mysterious and uncontrollable factors that contributed to the relative accuracy and reproducibility of experimental results in vivo. The unexpected and revealing consequences of these experiments made us increasingly aware that the primary factors responsible for the pathological effects associated with stress were related to impairment of certain elements of the immunological apparatus. Thus, our present perspective in the study of behavioral and stress phenomena naturally calls for emphasis in examining the interrelations between stress and immune competence. This is the biological basis for the entry of a new discipline, psychoneuroimmunology, which is a primary ingredient of an emergent field, behavioral medicine (*9, 10*).

The Biochemical Nature of Stress

Anxiety, as well as other emotional or psychosocial stresses in experimental animals, produces a series of well-known neuroendocrine and biochemical events (*4*). At least one of these biochemical responses to stress has an easily demonstrable destructive effect on specific cells and tissues that are required for optimum immunological defense. As a consequence, the stress-compromised animal is less capable of defending itself against cancer cells, infectious agents, and other disease processes that are normally responsive to cell-mediated immunity. Thus, uncontrolled stress factors are important elements to be reckoned with in designing and carrying out most biological experiments.

There are, of course, many varieties of stressors, some of which may activate various physiological systems, either singly or as complexes. For purposes of simplification, the discussion and data presented in this article deal largely with the effects of psychosocial arousal associated with the activation of the adrenal cortex. A characteristic biochemical expression of such a response is an abrupt increase of adrenal corticoids in the blood plasma. In rodents, the most conspicuous adrenal cortical hormone is corticosterone; in primates, including man, it is largely cortisol. These adrenal hormones are only one of many types of substances that are increased in response to a variety of stressors. Such stress-induced increases in corticoid hormones produce secondary effects involving T cells, B cells (bursa equivalent), NK (natural killer) cells, and thymic components, all vital elements of the immunological apparatus. Relevant metabolic and biochemical alterations, such as protein breakdown and synthesis, also occur through the influences of increased glucocorticoid action. Within the framework of this definition of stress, no special assumptions are made concerning activation of the adrenal medulla by the stimuli involved with adrenal cortical arousal. It is known, however, that the adrenal medulla releases potent central nervous system–active catecholamines when fear or rage is a component of the inciting stimulation (*11*).

The rationale of stress-mediated disease follows logically from these physiological events that bring about immunological impairment. Although the overall biochemical phenomena associated with stress are complex and have many subtle consequences, the primary events relevant to disease processes, at least those involving the adrenal cortex, appear to be straightforward. This is demonstrated by the following experimental data carried out under new and improved experimental conditions.

Low-Stress Animal Housing

Critical experiments cannot be carried out with confidence when conventional animal facilities are used, because such housing is not suitable for the maintenance of quiescent baseline values of the stress-associated hormonal and cellular elements that influence or control immunological and thus pathological processes. We have designed animal facilities that not only serve the experimental needs of stress and immunological research but, in addition, provide safer facilities for working with infectious or allergenic agents. Prototypes of

such low-stress animal facilities are described elsewhere (*12–14*).

From the standpoint of the low-stress environment that is essential for most biological research in vivo, individually ventilated shelf units offer several beneficial features. For example, the enclosed shelves provide a substantial amount of soundproofing, which is important because it has been established that animals are stressed by a wide variety of noises. These noises stimulate neuroendocrine reactions that may have subsequent adverse effects on immunocompetence (*3, 15, 16*). Stressful cage motion and a variety of noises are prevalent in most conventional animal rooms, particularly where there are rolling metal racks, metal cages, transistor radios, shouting, frequent cage cleaning with rough handling, and other uncontrolled stress-inducing practices (*17*).

For comparison, mice housed under low-stress conditions show low baseline values of 0 to 35 nanograms of corticosterone per milliliter of plasma, whereas mice maintained in conventional, communal animal facilities usually have plasma corticosterone values in the range of 150 to 500 ng/ml. This constitutes an increase of 10 to 20 times the concentrations in mice maintained under quiescent conditions.

If one examines the inconsistent results obtained in earlier studies (*3*), it is apparent that the experimental nuances and complexities associated with the various forms of stress imposed on cancer-bearing subjects exceeded our ability to control and evaluate the variables involved and thus to provide the necessary experimental conditions and essential controls. Most of these difficulties occurred because of a variety of unappreciated and uncontrolled stress factors and their physiological consequences, including the effects of population density and male-female proximity (*18, 19*).

Effect of population density on tumor regression. Since tumor regression in a properly balanced tumor-host model is a good index of cell-mediated immune competence, and thus of stress status, we have used tumor regression as a means for determining the optimum number of mice per standard plastic cage (*18*). In C3H/He female mice housed alone (one animal per cage), the regression rate of implanted 6C3HED lymphosarcomas was 60 percent. In all the other population densities tested (2, 3, 5, 10, 15, or 20 mice per cage), the regression rate of implanted tumors ranged from 80 to 100 percent, the average being 93 percent ($P < .001$). Thus, based on the immunological ability of female mice to reject a tumor challenge, all tested densities of group housing were preferable to that of the single animal per cage. In a similar experiment, male C3H/He mice housed one or two per cage had less ability to reject the 6C3HED lymphosarcoma than did those housed 3, 5, 10, 15, or 20 per cage. Thus, unlike female mice, male mice housed two per cage exhibited no advantage over isolated mice with respect to tumor regressions. Within the range of 3 to 20 mice per cage, no optimum number per cage was detectable. The importance of using an appropriate tumor-host model for the detection of such effects is shown by the finding that population density within the range reported here had no effects on the growth of a rapidly progressing B-16 melanoma under the protected conditions of our animal facilities. For additional work done by others see (*19*).

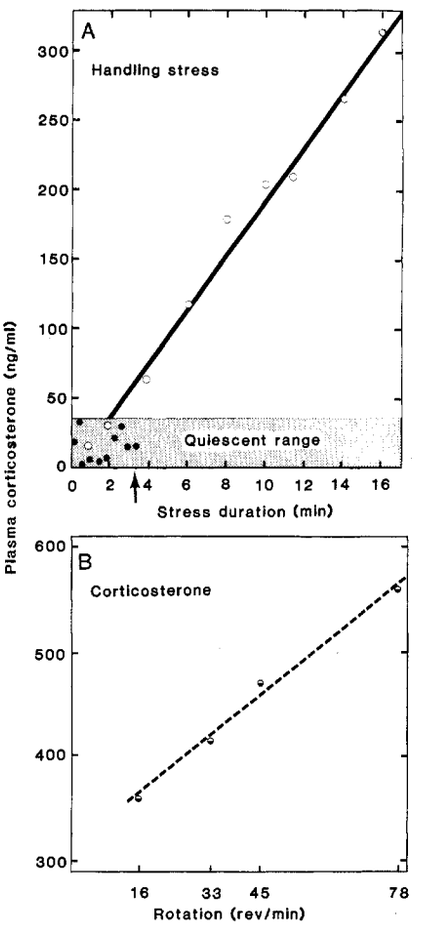

Fig. 1. Increases in corticosterone concentrations associated with anxiety stress. (A) The influence of capturing and handling "normal" mice on the concentration of corticosterone in the plasma as a function of time. The unconnected points falling within the unstressed range of corticosterone concentration represent ten animals whose blood samples were obtained within 3.5 minutes of their being removed from their protective facilities. The ascending linear corticosterone curve shows the systematic increase in plasma corticosterone with time after removal of the cage of mice from the low-stress storage shelf, and capture and bleeding of individual animals. The open circles represent individual mice that were selected randomly and bled at the times indicated. The slope of the curve indicates the rapidity of the physiological response to stress as measured by corticosterone concentrations. All the mice were bled by the orbital bleeding technique. (B) Linear relation between speed of rotation and plasma corticosterone concentrations in mice subjected to 20 minutes of rotation in their home cage placed on a specially designed stress-inducing machine. The highest speed used generates less than 1*g* at the maximum radius of the cage. This instrument produces a mild spatial disorientation with an attending anxiety and quantitative corticosterone response.

Male-female proximity and chronic stress. Mice housed in separate cages, but maintained in proximity to cages containing mice of the opposite sex, exhibited a four- to sevenfold increase in plasma corticosterone (*18*). The increases in females (80 to 120 ng/ml) remained for more than 80 days; male mice were less affected than analogous females when observed over a prolonged period of time. Mice housed in the same cage with mice of the opposite sex exhibited similar corticosterone elevations. Thus, the mixing of the sexes within the same vicinity, even if housed in separate cages, may have physiological consequences that can distort biochemical and immunological parameters, and may also affect tumor-associated parameters. However, the effects of male-female proximity may not be detectable under the stressful conditions of conventional animal rooms in which the mice usually have plasma corticosterone concentrations that are 5- to 20-fold above those of mice maintained under quiescent conditions.

Physiological Response to Animal Handling

Another troublesome aspect of earlier studies was the failure of investigators to appreciate the extreme sensitivity and rapidity of the physiological alterations occurring in animals exposed to experimentally or environmentally induced stress. In mice, critical phases of the stress syndrome are initiated immediately after the slightest disturbance. The physiological consequences of this stress may continue for hours or days, depending on the nature, severity, and duration of the stimulus.

The rapidity of the physiological response to handling-induced anxiety-stress is indicated by the measurable increase in plasma corticosterone only minutes after the animals have been agi-

tated by simple capturing procedures. For example, the curve in Fig. 1A demonstrates that the response to handling is so rapid that its manifestation in the form of increased plasma corticosterone is detectable within less than 5 minutes. Thus accurate measurements of quiescent corticosterone concentrations can be obtained only when the blood samples are removed within 3.5 minutes of the initial disturbance produced by transferring the animals from their protective shelves to the bleeding area. This imposes a rigorous time limit on the investigator for obtaining blood samples that establish true baseline levels of plasma corticosterone in control animals, as well as for quantifying the physiological effects of experimentally induced stress.

The increase in corticosterone illustrated in Fig. 1A was induced not by the bleeding procedure itself, which took only 5 seconds, but by the contagious anxiety induced in the entire cage population during the sequential capture of each mouse. Similar effects have been observed in each of four different mouse strains tested (BDF, BAF, C3H, and CBA), as well as in mice of different ages, ranging from 7 weeks to 24 months. We have demonstrated that rapid increases in plasma corticosterone can be generated by the routine practice of capturing animals for injections, cage transfer, bleeding, or other experimental procedures, or even by simply transferring the animals from their protective holding facilities to the laboratory bench. This phenomenon further demonstrates the need for both quiescent protective animal facilities and the use of appropriate animal handling techniques in biological studies, especially if optimal immunocompetence and normal physiology are of relevance.

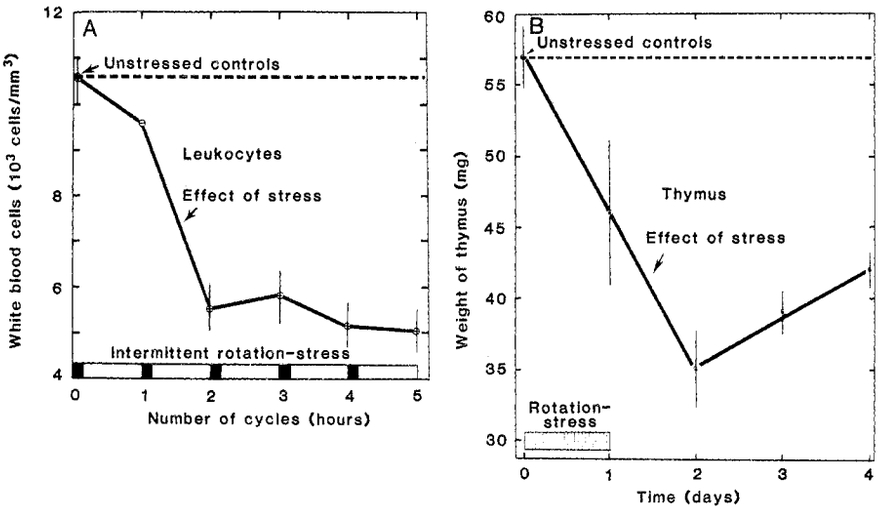

Fig. 2. Effects of anxiety stress on lymphocytes and thymocytes. (A) Leukocytopenia induced in mice by mild, nontraumatic rotational stress at 45 rev/min for five 60-minute cycles consisting of 10 minutes of rotation followed by 50 minutes of rest. A 50 percent leukocytopenia was produced by the end of the second cycle, and was maintained throughout the 5 hours of intermittent stress. Vertical lines through each point represent standard errors. (B) Thymus involution in mice subjected to mild rotational stress at 45 rev/min for 24 60-minute cycles consisting of 10 minutes of rotation followed by 50 minutes of rest. Evidence indicates that the thymocytes are lysed by the increased concentration of plasma corticosterone that accompanies stress.

A Nontraumatic Stress-Inducing Machine

The contradictory results of some previous studies demonstrated the need for a simple, reproducible, and nontraumatic means for the quantitative induction of simple anxiety stress in experimental animals. Such stress should preferably be produced without significantly activating hormonal systems other than those of the adrenal cortex, or depriving the animals of free access to food and water by restraint or other means, or subjecting them to unnecessarily harsh treatment. We have designed a simple stress-inducing device that provides a readily reproducible form of quantifiable stress and permits automatic programming of a wide variety of ratios of stress periods to rest periods (*20*). This machine is a modified turntable with the four standard speeds of 16, 33.3, 45, and 78 revolutions per minute. The instrument has been designed so that an entire cage of animals can be rotated without changing the familiar arrangement of their living quarters. Thus, with this benign stressing device, it is not necessary to submit the animals to disturbing novel environments, to restrain them, or to alter the availability of their food and water, inasmuch as the slow rotational speeds permit the animals to move about their cage and to continue eating and drinking. The maximum lateral gravitational force experienced by the mice is less than 1*g*. This instrument is not a centrifuge in the usual sense, but is essentially a mechanical means for inducing mild spatial disorientation, and possibly vertigo or dizziness, with an associated anxiety that activates the adrenal cortex and results in a prompt and predictable increase in plasma corticosterone.

In most of the experiments reported here, the intermediate speed of 45 rev/min was used to induce stress. For prolonged exposure to stress, the animals were usually rotated for 10 minutes and then allowed to rest for 50 minutes. This, or other on-off cycles, can be repeated for any desired periodicity, or for any time sequence, by using preprogrammed rotation patterns. The ability to control the magnitude of the stress stimulus, and to quantitate stress induction in terms of the biochemical and cellular response of the stressed subject, has been important in making our studies quantitative and easily reproducible.

Controlled increases in corticosterone induced by slow rotation. The ability of the stress-inducing device to produce variable intensities of stress is illustrated in Fig. 1B, where plasma corticosterone values are plotted against rotational speed. After the rotation of separate groups of mice at each of the four speeds for 20 minutes, a graded and systematic increase in plasma corticosterone occurred.

Stress-induced lysis of cells and tissues. Figure 2A shows the effect of programmed rotation on the leukocyte count of mice subjected to a rotation schedule of 10 minutes out of each hour at 45 rev/min. Stress-induced leukocytopenia is an immediate consequence of increased plasma corticosterone concentrations and is a key factor in bringing about an impairment of the rodent immunological apparatus. Most of the circulating mouse leukocytes are T cells, and their loss acquires special significance in view of the substantial damage also done to the thymus, which may prevent or delay processing of the replacement cells. Thus, in terms of the sequence of events, an increased concentration of corticosterone appears in the blood within a few minutes of exposure to anxiety stress and this, in turn, brings about circulating lymphocyte damage within

or 2 hours. Thymus involution follows; however, the disintegration of the solid organ requires more time, with a measurable weight loss occurring within less than 24 hours. This time course of thymus involution is shown in Fig. 2B.

Tumor-Host Models for Stress Studies

A proper tumor-host combination is critical for experiments in which neoplastic growth is to be used as an indicator of the effects of stress on immunocompetence. Tumors that are completely histocompatible with their hosts are not usually affected by impairments of the immune system, and thus cannot be used for such studies. In Fig. 3A, the growth of the 6C3HED lymphosarcoma is compared in two different C3H mouse substrains. The differences in tumor growth rates and tumor regressions indicate that this lymphosarcoma is more histocompatible with the C3H/Bi substrain than with the C3H/He mice. The C3H/He substrain is thus especially useful for detecting and measuring stress-induced impairments of immunocompetence. Under quiescent conditions, C3H/He mice have the capacity to partially or totally restrain growth of the 6C3HED tumor; however, growth of this tumor is enhanced in these mice when they are subjected to stressful stimuli (see Fig. 3B). Growth of the 6C3HED tumor progresses with equal rapidity in both stressed and nonstressed mice of the C3H/Bi substrain. This substrain is useful, however, for demonstrating enhanced immunological competence that may be expressed by a reduction in tumor growth and an increase in the percentage of 6C3HED regressions. In general, rapidly growing tumors that are syngeneic with their hosts are not responsive to immunological impairments, whether induced by stressful stimuli or by other means. However, more slowly growing tumors that are under partial immunological control are capable of responding to the biochemical and cellular events induced by anxiety-stress.

Enhancement of tumor growth after rotation-induced stress. In the experiment shown in Fig. 3B, half of the population of the tumor-bearing C3H/He mice were rotated at 45 rev/min for 10 minutes out of each hour during days 4, 5, and 6 after subcutaneous implantation of the 6C3HED lymphosarcoma. We assume that the stress caused by the rotation reduced the immunological competence of these mice and thus permitted rapid tumor growth, whereas the control animals retained their known capability for restraining the optimum growth of the tumor. Such a stress-induced decrease in immunological competence is consistent with the T cell and thymus damage caused by increases in plasma corticosterone. The similarity of the tumor growth curves in Fig. 3, A and B, demonstrates that anxiety-stress causes 6C3HED tumors in C3H/He mice to grow in the same way as they do naturally in the more histocompatible C3H/Bi substrain in the absence of stress.

Simulation of stress by the administration of corticoids. The biochemical effects of stress can be simulated by the exogenous administration of either natural or synthetic corticoids. Corticosterone, dexmethasone, or fluocinolone acetonide (FCA) were used in separate illustrative experiments (*18, 20*). These corticoids were either injected intraperitoneally as saline suspensions of low solubility, or implanted intramuscularly or subcutaneously into the hip in the form of solid corticoid-containing spermaceti pellets. Because of their low solubility, the corticoids were released slowly into the plasma of the recipient mice over several days. The concentrations attained approximated the high endogenous concentrations in the blood that are associated with mild anxiety stress.

Effects of stress on Moloney sarcoma virus–induced tumors. The Moloney sarcoma virus (MSV) tumor system provides a useful model for studying the rare phenomenon of spontaneous regression of autochthonous tumors (*21–23*). The following experiments demonstrate the influence of induced anxiety-stress and its endogenously produced biochemical products on this viral-neoplastic system. Enhancement of MSV-induced tumor incidence and tumor growth was observed, together with a delay in the usual prompt regression of the tumors, when programmed, rotation-induced anxiety-stress was administered immediately following MSV inoculation (see Fig. 4A). Similar tumor enhancement was obtained by a biochemical simulation of the typical physiological consequences of stress, specifically by the administration of natural or synthetic corticoids. Single, slow-release repository doses of corticosterone or dexamethasone produced effects on the Moloney virus–tumor system similar to those induced by rotational stress and the resulting endogenous corticosterone elevation. In order to examine the concomitant effect of a second, non-oncogenic virus as an additional biological stress-inducing factor, the LDH virus (lactate dehydrogenase–elevating) was also examined with this system (*24*). As shown in Fig. 4A, when the LDH virus was injected at an appropriate time in reference to the inoculation of the Moloney virus, tumor growth

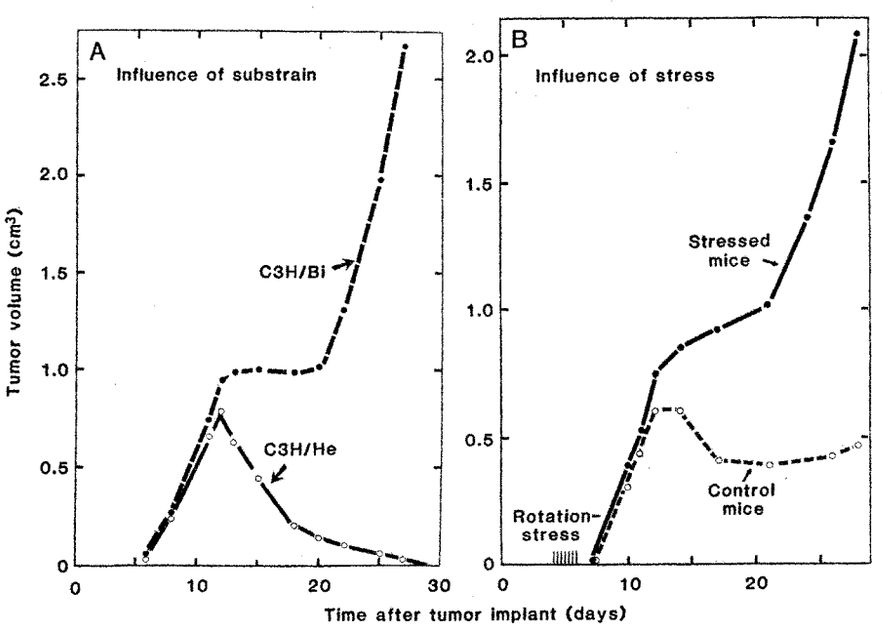

Fig. 3. Differential growth of a lymphosarcoma under various conditions. (A) Differential tumor growth and regression behavior of the 6C3HED lymphosarcoma transplanted into two substrains of C3H mice. The differences in histocompatibility provide useful tumor-host models for the detection of alterations of immunocompetence induced by stress or other factors. (B) Stress-associated influences on the growth of the 6C3HED lymphosarcoma in female C3H/He mice exposed to intermittent anxiety-stress. The stress was induced by rotation at 45 rev/min for 10 minutes out of each hour on days 4, 5, and 6 after tumor implantation. Food and water were freely available during the course of the experiments in (A) and (B).

enhancement was obtained. Thus, three extraneous factors, all having the common capability of producing or simulating elevated adrenal hormones, enhanced this neoplastic system. Figure 4A compares the effects of these three varieties of stress on the growth behavior of tumors induced by MSV. Tumor growth enhancement was expressed by significantly larger maximum tumor volumes attained in all three experimental groups as compared to the unstressed and untreated MSV-inoculated controls ($P < .001$ for all three experimental modalities). Our interpretation of these phenomena is consistent with other related data, namely, that all of the above stress stimuli cause an indirect impairment of cell-mediated immunocompetence.

Other influences of the LDH virus on experiments involving neoplasia or immunological responses. Various effects of the LDH virus on experiments involving neoplasia or immunological responses may include the following (*13, 24*): (i) increased plasma corticosterone during the acute phase of the LDH virus infection; (ii) lysis of circulating lymphocytes, largely T cells; (iii) subsequent thymus involution; (iv) splenic hyperplasia resulting from continuous immunoblast proliferation in the thymus-independent regions; (v) enhancement of spleen antibody-forming B cells during acute infection, followed by B cell inhibition during the subsequent life-long phase of the infection; (vi) increased incidence of tumors induced by various oncogenic viruses; (vii) reduced regression rates of certain virus-induced tumors; (viii) enhanced growth rates of specific nonsyngeneic transplantable tumors; (ix) a 3- to 20-fold enhancement in the production of spleen tumor foci after intravenous inoculation of Friend virus; and (x) impairment of the clearance of various enzymes from the plasma, together with a simultaneous increased influx of enzymes into the plasma, which persists for the lifespan of the infected mouse. In the presence of a growing tumor, these physiological alterations result in a synergistic increase in the concentrations of certain endogenous enzymes, notably lactate dehydrogenase, that may amount to more than 100-fold over normal values. Synergistic interactions with other infectious entities have also occurred. It would be presumptuous to assume that this is the only infectious entity capable of such physiological influences. Since the LDH virus will enhance the growth of certain immunoresponsive tumors, regardless of whether the virus is intentionally inoculated or transmitted as an inadvertent contaminant, efforts to study the effects of stress on such an unsuspectedly contaminated tumor would probably be confusing and difficult to evaluate (*24*).

Effects of chemically simulated stress on titers of latent LDH virus. Figure 4B shows the physiological consequences of a single injection of the synthetic corticoid dexamethasone on thymus weight, as well as an inverse enhancement of the equilibrated LDH virus titers in mice chronically infected with this virus. During the acute phase of the LDH virus infection, the plasma concentration of corticosterone is increased. The concentration returns to normal in 1 or 2 days, after which the virus titer decreases from approximately 10^{10} to 10^6 median infectious doses (ID_{50}) per milliliter of plasma. The dexamethasone-induced secondary increase in the titer to the high level usually seen only during the acute infectious phase suggests that stress-induced increases in plasma corticosterone may potentiate other viruses, possibly with pathological consequences. The question thus arises as to whether stress may be directly or indirectly responsible for the appearance of certain virus-induced tumors. Thus stress might promote the activation of latent carcinogenic viruses or the increase of titers of viruses present in nonpathological, chronic states. For example, the Bittner mammary tumor virus (MTV) may be present in C3H mice without causing pathological effects (see Fig. 7A). The enhancement of oncogenic viruses and their neoplastic processes by the LDH virus has been reported previously (*21, 22*). Of possible relevance to these phenomena are reports indicating that stress may suppress, or otherwise alter, interferon production in virus-infected mice (*25, 26*).

Tumor Enhancement by Virus-Induced or Biochemically Simulated Stress

Cumulative clinical and experimental data suggest that melanomas and certain benign pigmented lesions may occasionally generate immunological responses resulting in temporary or permanent regressions (*27, 28*). It thus appears that such tumors may exist in a fragile histocompatible equilibrium with their hosts, and may therefore be susceptible to alterations in immunological states.

Figure 5B illustrates the influence of acute infection with the LDH virus on the growth of a nonpigmented variant of the B-16 melanoma which, in the noninfected controls, is a slowly growing tumor. In contrast (Fig. 5A), a rapidly growing pigmented variant of this tumor exhibited the same rate of growth in both

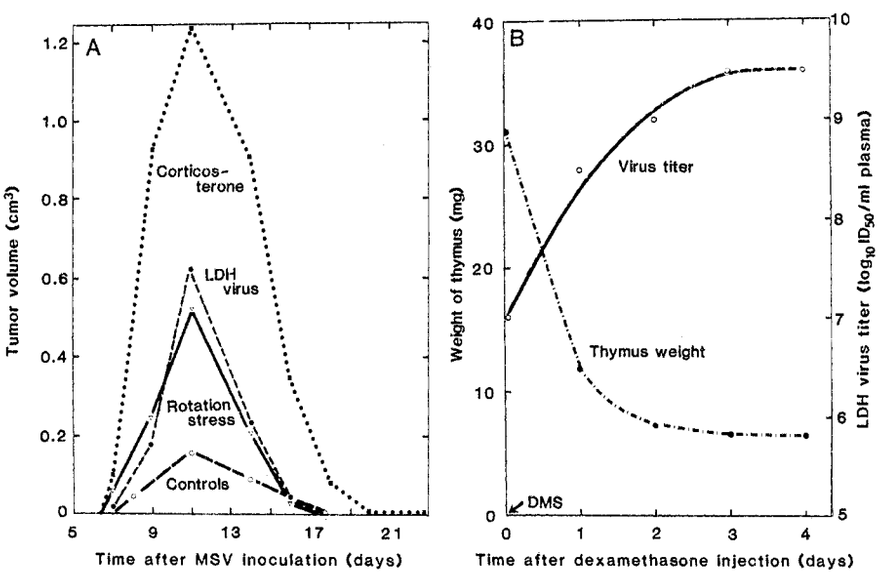

Fig. 4. Effects of various forms of stress on viral-associated phenomena. (A) Enhanced growth of autochthonous tumors induced by the Moloney sarcoma virus (MSV) in BALB/c female mice subjected to stress. One group of mice received subcutaneous implantations in the left hip of a single slow-release pellet of corticosterone on the day of MSV inoculation; a second group was inoculated with LDH virus on the day of inoculation with MSV; a third group was stressed by being subjected to intermittent rotation (45 rev/min, 10 minutes out of each hour, for a total of 72 hours), starting on the day of MSV inoculation. The MSV (0.05 ml) was inoculated intramuscularly in the right hip. (B) Effects of a synthetic corticoid on LDH virus titers and thymus weight. Female BDF mice were infected with LDH virus and 7 days later were injected with a single, slow-release pellet of dexamethasone (DMS). The low virus titers typical of chronic LDH virus infections are increased to a level normally seen only during the acute phase of the infection when endogenous corticosterone concentrations are high.

virus-infected and control mice. The genetic transformation of the tumor from a pigmented to a nonpigmented variant apparently resulted, in this instance, in a tumor with histocompatibility characteristics different from those of the original pigmented tumor. The growth rates of the two tumors in untreated C57/BL mice, the strain of origin, are significantly different (see Fig. 5, A and B). Also, the tendency of the amelanotic variant to undergo spontaneous regression distinguishes it from the more histocompatible pigmented neoplasm. The immunologically suppressible nonpigmented melanoma thus is a suitable experimental model for detecting the influence of subtle immunological modifiers, including anxiety stress, exogenously administered corticoids, and certain viruses. The latter is demonstrated by the effects of acute infection with the LDH virus, which increases corticosterone concentrations and thus modulates host immunocompetence.

Figure 5D indicates that the LDH virus also enhances the growth of the 6C3HED lymphosarcoma. The degree of syngeneity of this tumor with C3H/He mice is imperfect, and acute infection with LDH virus increased the percentage of progressively growing tumors. At 30 days after tumor implantation, five of ten tumors in the non-LDH virus–infected mice had completely regressed, whereas in the virus-infected group, only one out of ten had regressed. Figure 5C shows an analogous enhancement of tumor growth produced by the direct administration of dexamethasone.

Effects of Timing of Simulated Stress on Tumor Behavior

The data in Fig. 6A show that stress-associated impairment of immunological competence may depend on the timing of the application of stress or of stress simulated by the injection of dexamethasone. In this experiment, both tumor suppression and tumor enhancement were observed. When dexamethasone was administered 7 days before tumor implantation the immunocompetence of the hosts was enhanced, with tumor growth being suppressed in comparison with the controls. However, corticoid administration 7 days after tumor implantation resulted in overt immunological impairment, since the tumor escaped host control and grew rapidly with the consequences being lethal for the "stressed" hosts.

In a separate experiment (data not shown), anxiety-stress caused by rotation of C3H/He mice on days 4 through 6 after inoculation of MSV resulted in a significant enhancement of tumor growth. In contrast, mice rotated 3 days before MSV inoculation showed inhibition rather than enhanced growth of the virus-induced tumors. Similar timing effects may account for other instances of stress-induced inhibition of various tumors that have been reported (*3, 29*).

Sequential immunological suppression and enhancement. An experiment conducted by Monjan and Collector (*16*) demonstrated that stress-induced immunosuppression occurred between 1 and 21 days of continuing long-term auditory stress. The observed suppression of T and B cells was undoubtedly a consequence of increased plasma corticosterone. However, the mechanisms underlying the subsequent immunoenhancement are less clear, although other experiments have shown that many humoral factors, including somatotropin and thyroxin, increase in the plasma during long-term exposure to various forms of stress. In any event, corticoid-induced impairment of immunological cellular elements is commonly followed by a homeostatic recovery of these elements, and a subsequent overshoot, leading to immunoenhancement. This phenomenon of alternating immunosuppression and immunoenhancement following exposure to stress may account for many of the conflicting reports regarding enhancement versus inhibition of chemical carcinogenesis, tumor incidence, tumor growth, and regressions in experimental animals exposed to various types of stressful stimuli (*3, 29*).

Effect of stressor timing. Further experimental evidence concerning the importance of the timing of stressful stimuli on neoplastic behavior is provided by the experiment shown in Fig. 6B. The neuroendocrine pathways were again intentionally bypassed, with the physiological effects of stress being simulated by the administration of another potent synthetic corticoid, fluocinolone acetonide (FCA), which is chemically closely related to dexamethasone. The FCA was administered as a single injection of slow-release repository 7, 14, 21, or 28 days after implantation of the 6C3HED

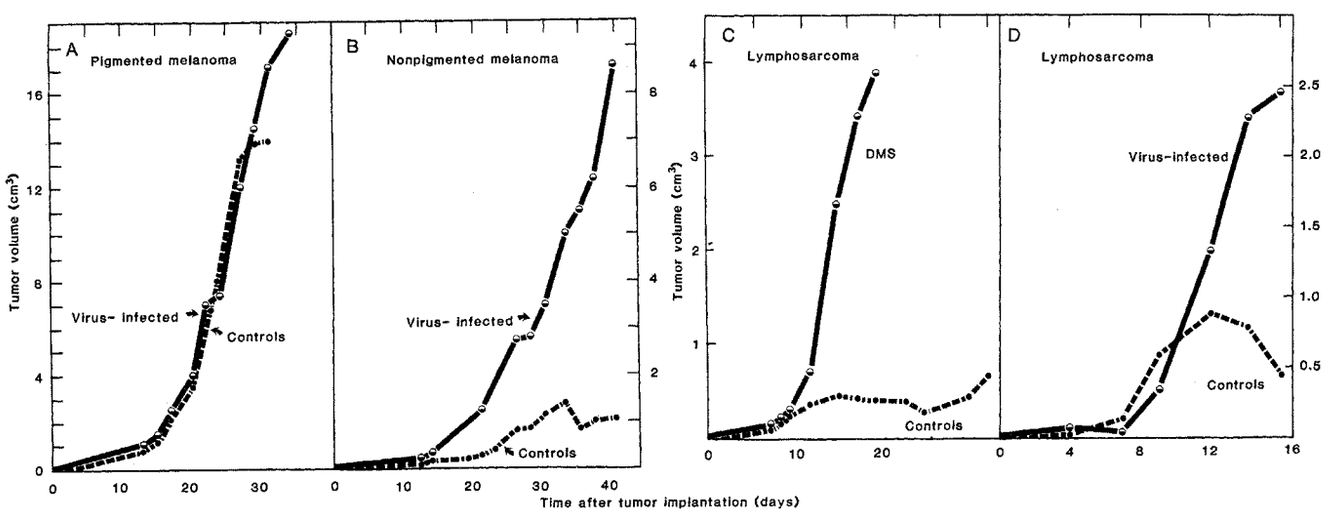

Fig. 5. Factors influencing the growth of transplantable tumors. (A) The lack of influence of acute LDH virus infection on a rapidly growing pigmented B-16 melanoma compared to (B) the virus-induced enhancement of the growth of a partially host-suppressed nonpigmented variant of B-16 origin. (C) Effects of administration of a synthetic corticoid (DMS) on the 6C3HED lymphosarcoma growing in C3H/He mice compared to (D), the effects of an acute infection with the LDH virus on this tumor. These data demonstrate tumor enhancement in mice with corticoid-induced impairment of immune competence. The DMS and the LDH virus were both administered on the same day that the lymphosarcoma was implanted.

lymphosarcoma into C3H/He mice. The growth of this lymphosarcoma in the C3H/He substrain is normally limited, with a high percentage of tumor regressions occurring. When synthetic corticoids were given 7 days after tumor implantation, substantial increases in tumor growth occurred with lethal consequences. The FCA-induced tumor enhancing effects were systematically diminished when the corticoid was introduced at later stages when the tumor was in the process of regressing. If FCA administration was delayed until 28 days after tumor implantation, no enhancing effect on the suppressed tumor growth was observed. Our tentative interpretation of this phenomenon is that only a limited number of viable tumor cells remained at this time, even though a measurable mass, which probably consisted largely of dead tumor cells and connective tissue, persisted at the site of implantation. These data may be interpreted as an indication that the consequences of immunological impairment, in terms of measurable tumor enhancement, are dependent on the number of viable tumor cells available to respond.

Environmentally Induced Stress and Breast Tumors in Mice

In the experiment illustrated in Fig. 7A, groups of C3H/HeJ female mice carrying the mammary tumor virus (MTV) were monitored for mammary tumor incidence and latent periods while housed under two different conditions with respect to chronic stress (*30*). Two groups, one parous (group a) and the other nonparous (group b) were housed in conventional, open-rack facilities and thus exposed to long-term environmental stress, whereas another nonparous group (group c) was housed in the specially designed low-stress facilities previously described (*12–14*). Although all of the mice in these three groups were potential candidates for MTV-induced breast cancer, those in group c, which were partially protected against stress, showed a less than 10 percent tumor incidence at 13 months of age compared with 92 and 68 percent, respectively, for groups a and b (Fig. 7A).

The median latent period for the development of tumors was 358 days for the nonparous mice (group b) housed in conventional facilities, and 566 days for the nonparous mice (group c) housed in the low-stress facilities. The results for group c represent a significant extension over those for groups a and b ($P < .001$). Thus exposure to the noises of cage-cleaning and rack movement, and to the various stress-inducing experimental manipulations of other animals in the same room, as well as drafts and recirculating odors and pheromones, led to an increased incidence of MTV-induced tumors. The mice in group d (Fig. 7A) were similar to those in group c with the exception that the milk-passaged mammary tumor virus had been removed through cesarean section and foster nursing procedures. The mice in both group c and group d were housed in the low-stress facilities.

Aging and Mouse Mammary Tumor Incidence

The experiment depicted in Fig. 7A also offers some useful implications concerning possible relationships between stress, immunocompetence, aging, and the cancer process. For example, a reasonable interpretation of the striking difference between the early slopes of the curves for tumor incidence in the chronically stressed mice, and the slope of the curve for the mice kept under low-stress conditions, is that the diverse slopes express differences in cell-mediated immunological competence or surveillance success in the detection of transformed cells. This is consistent with the thesis that stress impairs vital elements of the immunological process. Applying this principle to the case of the mice with early and high tumor incidence, one may assume that the stressed mice were at a greater risk for tumor development and that transformed malignant cells had a greater opportunity to become progressively growing, irreversible mammary tumors. Immune competence in the low-stress mice was presumably preserved and was thus adequate to prevent most of the transformed cells from reaching overt tumor status.

This interpretation is supported by the steadily increasing mammary tumor incidence observed in the low-stress mice as they moved into old age, since there is persuasive evidence that immune competence systematically decreases as a function of increasing age. The consequences of this immunological decline, and possibly other age-associated changes, are demonstrated by the progressively steeper slope of the curve for mammary tumor incidence in the mice protected from stress. For example, when the mice in group c (Fig. 7A) were between 550 and 600 days of age, a period when immunological competence was probably impaired by aging, the slope of the curve was nearly identical to that of the curve for mice in group a at 200 to 300 days of age. It is interesting that the MTV-free mice depicted by curve d exhibited no mammary tumors even at an advanced age, thus demonstrating that the elimination of MTV largely reduces mammary tumor risk, regardless of stress or age status.

These data indicate that protection against stress offers prophylactic benefits only when a potentially effective immunological system is available. Thus in aged MTV-infected animals with a demonstrably reduced immune capability, protection against stress no longer results in a low tumor incidence. In conjunction with other data, the information derived from the experiments with mouse mammary tumors and stress offers a remarkable composite model for examining the interrelations involving (i) the presence or absence of the cell-transforming mammary tumor virus; (ii) the survival or inactivation of the virus-transformed breast cells in relation to immunological surveillance or competence; (iii) stress-induced impairment of immune competence; and (iv) the time of appearance of overt malignant tumors in the presence or absence of long-term stress. Of special interest is the clear demonstration of the deleterious effects of aging on the ability to prevent the appearance of lethal tumors when MTV is present. The rate of appearance of mouse mammary carcinoma in aged mice protected from stress was identical to that of young adult mice exposed to long-term stress when both groups were chronically infected with MTV.

Another example of an inverse correlation between increasing age and decreasing immunological competence is demonstrated in Fig. 7B, which shows the relative growth of the 6C3HED lymphosarcoma injected subcutaneously into C3H/He mice of three different ages. The youngest mice were the most capable of restraining the progress of this tumor: 80 percent of the tumors eventually regressed in the 7-week-old mice, whereas 74 percent of the tumors in the oldest animals resulted in death ($P < .005$). In logical correlation with this age-associated differential tumor behavior, analogous groups of mice were found to have a corresponding decrease in their thymus weight with increasing age. For example, the average thymus weight of 2-year-old mice was only 36 percent of that of 7-week-old mice ($P < .001$). These data further demonstrate that the normal aging process brings about an

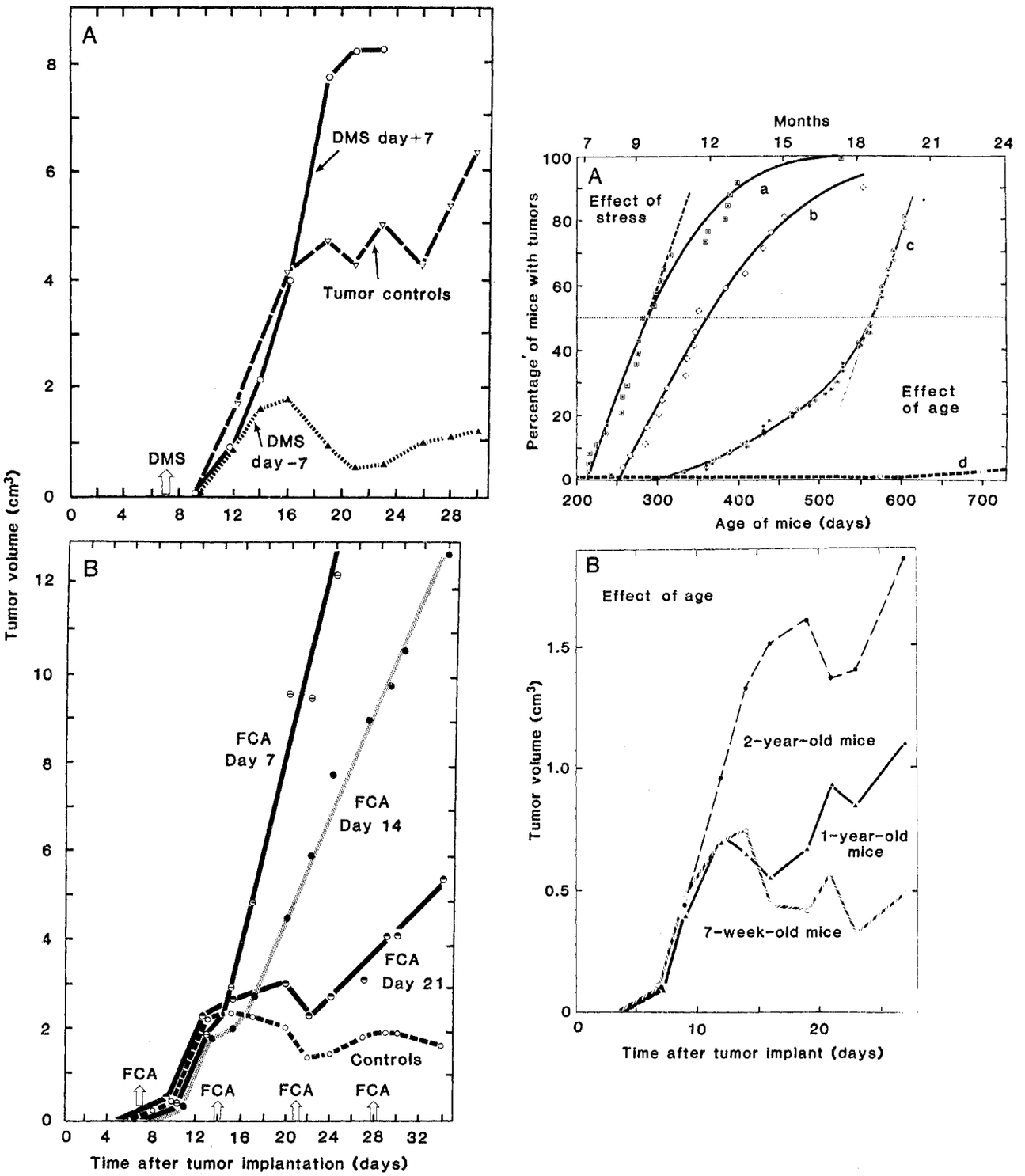

Fig. 6 (left). Effects of timing on the influence of stress on tumor growth. (A) When the synthetic corticoid DMS was injected into C3H/He mice 1 week before (DMS day − 7) implantation of the 6C3HED lymphosarcoma, an enhancement of the immunological competence of the host was inferred from the increased suppression of tumor growth by the host compared with the untreated tumor-bearing controls. In contrast, when the corticoid was injected 7 days after (DMS day + 7) tumor implantation, host immunosuppression occurred, as indicated by the enhanced growth of the tumors. (B) Systematically different tumor growth rates resulting from the injection of slow-release depots of the synthetic corticoid fluocinolone acetonide (*FCA*) at various times after tumor implantation. Fig. 7 (right). Influences of environmental stress and aging on tumor incidence and growth. (A) Mammary tumor incidence and latent periods in genetically susceptible C3H/HeJ female mice housed under two different environmental conditions. Group a consisted of parous mice housed under conditions of chronic stress, while group b consisted of nonparous females housed under the same stressful conditions. Group c were nonparous females housed under conditions of low stress. Group d indicates the absence of mammary tumors in mice genetically the same as those in groups a, b, and c, but not possessing the milk-passaged Bittner mammary tumor virus (MTV). At 400 days of age both groups a and b had high tumor incidences of 92 and 68 percent, respectively. This was in contrast to less than 10 percent incidence for the mice in group c that were housed in protective, low-stress facilities. (B) Use of tumor-host relationships for comparing cell-mediated immune competence in female C3H/He mice of various ages. These data confirm that young mice are more capable of restraining tumor growth than are older mice. This age-associated depreciation of immune competence is analogous to the similar effects of anxiety-stress. Each point is the average tumor volume of ten mice.

immunological impairment that is similar to the damaging effects of stress. Thus, in terms of inducing alterations in tumor behavior, both aging and stress can bring about similar pathological effects.

Conclusions

The forces of stress are limited, and in many circumstances are unable to exert any significant pathological effects. In other cases, however, the physiological and hormonal changes induced by emotional or anxiety stress are capable of shifting an immunological equipoise to produce lethal consequences. Various tumor-host models have been used to illustrate these circumstances.

Although an extensive variety of stimuli and resulting physiological alterations are involved in the stress complex, there are certain common denominators that can account for many of the phenomena. The most conspicuous biochemical factor that is associated with stress in mice, whether it is produced by psychosocial circumstances, environmental factors, mechanically induced anxiety, or viral infection, is the increased concentration of corticosterone in the plasma. Closely related corticoid hormones are involved in other species. The primary harm that corticoids cause when in high concentrations is damage to lymphocytes and thymus elements that are essential for optimum cell-mediated immune defenses and possibly for effective immune surveillance involving NK cells when cancer cells are involved (*31, 32*).

The influences of uncontrolled stress in animal studies, particularly in studies with rodents, call for (i) a more universal consideration of these factors in the design of experiments; (ii) establishment of a low-stress environment for animal housing; (iii) special considerations in the manipulation and handling of experimental animals; and (iv) attention to time factors in terms of minutes, when blood samples are being removed for the establishment of meaningful corticosterone and related values. Because of these largely unappreciated and uncontrolled elements, the question arises as to how much of the present and past work with small animals may be severely flawed. In any event, the information now available calls for a reassessment of the current standards for laboratory animal housing and for techniques related to animal experimentation.

Part of the confusion associated with studies of stress stems from the alternative concepts of direct stress-induction of pathologies as opposed to stress-induced modulation of immune defense capabilities (*3*). There is evidence that stress can bring about an overt pathological state if an incipient or latent neoplasm or infection is either preexistent or enters the picture during a period of acute or chronic stress. It further appears that neoplastic or viral pathologies that are clearly enhanced in the presence of stress are under partial or complete control of cell-mediated immunological defenses. The enhancing effects of stress on these diseases thus occur as a consequence of the adverse effects of stress on specific immunological elements of the host. Such pathological effects of stress are thus indirect (*31*).

Further implications of this working hypothesis are: (i) Stress-associated infectious or neoplastic pathologies will not be observed, despite the presence of stress, if no underlying disease is present. (ii) Even if latent pathologies exist, the enhancing effects of stress will not be observed unless the disease is under partial or complete control of the unimpaired immunological system. (iii) Such adverse effects of stress will be observed only when the immunological defenses of the host and the resident pathological entity are in a state of equipoise that will permit the modulating forces of stress to be manifested by overt disease when the immunological competence of the host is impaired or otherwise altered.

References and Notes

1. H. Selye, *J. Hum. Stress*, **1**, 37 (1975).
2. J. W. Mason, *ibid.*, pp. 6 and 22.
3. V. Riley, in *Perspectives in Behavioral Medicine*, S. M. Weiss, Ed. (Academic Press, New York, 1981).
4. C. D. Turner and J. T. Hagnara, *General Endocrinology* (Saunders, Philadelphia, ed. 5, 1971), p. 382.
5. J. W. Mason, in *Frontiers in Neurology and Neuroscience Research*, P. Seeman and G. M. Brown, Ed. (Univ. of Toronto Press, Toronto, 1974), p. 68.
6. D. Glick, D. Von Redlick, S. Levine, *Endocrinology* **74**, 635 (1964).
7. V. Riley and D. H. Spackman, in *The Pigment Cell*, V. Riley, Ed. (Karger, Basel, 1976), vol. 2, p. 163.
8. D. H. Spackman, V. Riley, J. Bloom, *Proc. Am. Assoc. Cancer Res.* **19**, 57 (1978).
9. R. Ader, *Psychoneuroimmunology* (Academic Press, New York, 1981).
10. C. Holden, *Science* **209**, 479 (1980).
11. J. P. Henry and J. P. Meehan, in *Brain, Behavior, and Bodily Disease*, H. Weiner *et al.*, Eds. (Raven, New York, 1981), p. 305.
12. V. Riley, *Proceedings of the 23rd Annual Session of the American Association of Laboratory Animal Sciences* (Anaheim, Calif., 1972), Abstr. 22 A.
13. V. Riley and D. H. Spackman, *Fogarty International Cancer Proceedings* (Government Printing Office, Washington, D.C., 1976), vol. 28, p. 319.
14. V. Riley and D. H. Spackman, *Lab. Anim.* **6**, 16 (1977).
15. M. M. Jensen and A. F. Rasmussen, Jr., *J. Immunol.* **90**, 21 (1963).
16. A. A. Monjan and M. I. Collector, *Science* **196**, 307 (1977).
17. The most essential features required for protective low-stress animal housing are as follows: (i) No recirculation of noxious air that has been in previous contact with animals; (ii) partial soundproofing of the animal storage shelves; (iii) elimination of animal room vibrations and high-pitched sounds of centrifuges, vacuum cleaners, ventilation fans, and other noisy laboratory or building equipment; (iv) elimination of drafts, air turbulence, and wind-tunnel effects; (v) precise light control to stabilize circadian rhythms and to regulate light intensity exposure; (vi) segregation of males and females with respect to transmissible odors, pheromones, and other stress-inducing signals; (vii) segregation of experimental animals that are experiencing stress from normal or control animals; (viii) introduction of special minimum-stress animal handling techniques and cage cleaning procedures; and (ix) avoidance of drafty, uncomfortable, and stressful wire-bottom cages. Data also indicate that the isolation of animals, with only one animal per cage, is undesirable.
18. V. Riley, M. A. Fitzmaurice, D. H. Spackman, in *Perspectives in Behavioral Medicine*, S. M. Weiss, Ed. (Academic Press, New York, 1981).
19. S. Green, K. Diefenbach, G. A. Santisteban, *Anat. Rec.* **157**, 2 (1967); R. P. Dechambre and C. Gosse, *Cancer Res.* **33**, 140 (1973); W. G. Glenn and R. E. Becker, *Physiol. Zool.* **42**, 411 (1969); J. C. Guilon *et al.*, *C. R. Acad. Sci.* **240**, 1066 (1970).
20. V. Riley, *Cancer Detect. Prev.* **2**, 159 (1979).
21. ———, D. H. Spackman, K. E. Hellstrom, I. Hellstrom, *Proc. Am. Assoc. Cancer Res.* **19**, 57 (1978).
22. W. Turner *et al.*, *Proc. Soc. Exp. Biol. Med.* **136**, 1314 (1971).
23. A. Amkraut and G. F. Solomon, *Cancer Res.* **32**, 1428 (1972).
24. V. Riley *et al.*, *Science* **200**, 124 (1978); D. H. Spackman and V. Riley, *Proc. Am. Assoc. Cancer Res.* **15**, 143 (1974); G. A. Santisteban, V. Riley, M. A. Fitzmaurice, *Proc. Soc. Exp. Biol. Med.* **139**, 202 (1972); R. J. Howard, A. L. Notkins, S. E. Mergenhagen, *Nature (London)* **221**, 873 (1969); V. Riley *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1707 (1976).
25. S. S. Chang and A. F. Rasmussen, Jr., *Nature (London)* **205**, 623 (1965).
26. M. M. Jensen, *Proc. Soc. Exp. Biol. Med.* **128**, 174 (1968).
27. A. J. Cochran, U. W. John, B. P. Gothoskar, *Lancet* **1972-I**, 1340 (1972).
28. E. M. Nicholls, *Cancer (Philadelphia)* **32**, 191 (1973).
29. C. C. Crispens, Jr., *Psychology and Psychiatry* **4**, 169 (1969); B. H. Newberry and L. Sengbusch, *Cancer Detect. Prev.* **2**, 225 (1979); M. S. Hirsch, *Johns Hopkins Med. J. Suppl.* **3**, 177 (1974); J. W. Mason, in *Emotions—Their Parameters and Measurement*, L. Levi, Ed. (Raven, New York), p. 143; W. Pierpaoli, C. Baroni, N. Fabris, E. Sorkin, *Immunology* **16**, 217 (1969).
30. V. Riley, *Science* **189**, 465 (1975).
31. In contrast to the type of stress that is associated with the adrenal cortex and immunocompetence, J. P. Henry and G. A. Santisteban [*Atherosclerosis* **14**, 203 (1971)] have described a number of diseases that appear to be a direct consequence of stress. These include hypertension, arteriosclerosis, nephritis, and diseases of the coronary vessels. Unlike the stress that we have studied, Henry's work has involved activation, through fear and rage, of the sympathetic and adrenal medullary systems whose capabilities for the direct induction of disease may be quite different from those associated with the adrenal cortex and anxiety stress.
32. It has long been known that adrenocorticoids have cytolytic effects on T cells and thymocytes both in vivo and in vitro. In addition, inhibitory effects of adrenocorticoids on virtually every aspect of the immunological response have been described, with special emphasis on those involving cell-mediated immunity. Although much of the experimental data has been derived from rodent systems which are more corticoid-sensitive than the human, many inhibitory actions of corticoids on human immunological responses have been reported, which differ from those of the animal models only in the degree of responsiveness [H. N. Claman, *N. Engl. J. Med.* **287**, 388 (1972); A. F. Burton, J. M. Storr, W. L. Dunn, *Can. J. Biochem.* **45**, 289 (1967); J. D. Baxter and A. W. Harris, *Transplant. Proc.* **7**, 55 (1975)].

33. These studies were carried out in collaboration with my colleagues D. H. Spackman, M. A. Fitzmaurice, and G. A. Santisteban. The work was partially supported by National Cancer Institute grants CA 12188 and CA 16308; American Cancer Society grant PDT-73; by the Eagles Cancer Fund; and the National Science Foundation. The article is an extension and update of a lecture delivered at the meeting of the Academy of Behavioral Medicine Research *in* Boar's Head Inn, Charlottesville, Va., June 1980.