TABLE V. Effects of α-TGdR, 6MP, and β-D-ara-6MP on Production of 19S (IgM) and 7S (IgG) Antibodies.

	Log$_2$ titer					
	Day 5		Day 10		Day 17	
Drug	19S	7S	19S	7S	19S	7S
Controls	8	<1	<1	10	<1	9
β-D-ara-6MP (60)	"	"	"	"	"	"
6-MP (60)	7	"	6	<1	"	<1
α-TGdR (30)	<1	"	<1	"	"	"

Female Swiss mice each received 0.25 ml of a 30% suspension of tanned sheep blood cells on day 0. 6-Mercaptopurine (6-MP), 60 mg/kg, was given once daily for 4 days starting on day 0. The α-D-2′-deoxythioguanosine (α-TGdR), 30 mg/kg/day, and β-D-arabinosyl-6-mercaptopurine (β-D-ara-6MP), 60 mg/kg/day, were given in 2 divided doses daily for 10 days beginning on day —1. All values are from pooled sera from 5 mice.

of the humoral antibody response was not sustained. The decreased effectiveness of β-D-ara-6MP and Me6MPR in prolonging skin grafts between mouse strains differing widely at the strong H-2 locus would imply that these agents would best be employed in individuals given homografts with relatively similar antigenic makeup.

The results of the study presented here, however, indicate that the future design of more potent, non-toxic, and specific immune suppressants for application in organ homotransplantation is possible.

Summary. Six drugs that produced increased skin homograft survivals in mice were tested for inhibition of induction of humoral antibodies. Three of these drugs (6-MP, α-TGdR, and Me6MPR) were found to inhibit humoral antibody formation and three (β-L-6MPR, β-D-ara-6MP, and 6-DMHP) did not. These results implied that it is possible to design specific suppressants of the homograft response.

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Interaction Effects of Environmental Stress and Deuteron Irradiation of The Brain on Mortality and Longevity of C57BL/10 Mice.* (32397)

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Current biological theories of aging can be divided into two general and contrasting categories(1). According to the "stochastic" theories, deviations from environmental steady-state conditions or so-called stress factors throughout the life span may influence the rate of aging, mortality and the total life span. In general support of these stress, rate of living, or wear-and-tear theories of aging, several investigators have presented considerable actuarial data for man and experimental animals indicating that repeated exposure of a population to stress may decrease life ex-

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pectancy and presumably also lead to accelerated aging(2,3,4).

In contrast, the alternative "molecular-genetic" theories of aging have proposed that aging of multicellular organisms is due primarily to increasing failure of non-replicating cells and systems and the rate of failure of such cells is also genetically programmed and analogous to the DNA programmed ontogenetic sequence of early differentiation and growth(1). Age-related changes in such organs as the brain containing non-replicating cells result primarily from the progressive accumulation of defects in DNA. These defects are presumably also determined by intrinsic genetic factors programmed into the genome(1). According to these genetic theories of aging, the environmental or non-genetic factors exert only a very negligible effect on aging. Consequently, mortality, longevity, and rate of aging are completely determined at fertilization(5). In general support of these molecular-genetic theories of aging, the increased incidence of chromosomal aberrations and decreased life span after acute ionizing radiation have suggested an important role for DNA in somatic mutations as well as a similarity between biological aging and cellular changes produced by radiation(6).

Briefly, the time-radiation equivalence studies have suggested that radiation can simulate some of the more specific effects of aging in cells as well as accelerate many of the more obvious structural and functional changes observed normally with aging(7). However, whereas age-associated and radiation-induced changes may frequently share certain time-dependent characteristics, it has also been pointed out that radiation may accelerate aging and decrease longevity simply by representing a non-specific stress factor (6). In order to test the hypothesis that ionizing radiation may represent only a non-specific stress, it seemed appropriate to compare the effects of environmental stress on mortality and longevity between non-irradiated control-stress and brain-irradiated-stress groups of mice. Since the life span of the mouse can be decreased reliably by acute ionizing whole body or head irradiation(8,9), a series of experiments was undertaken to study more directly the interaction effects of environmental stress and deuteron irradiation of the brain on mortality and longevity of C57BL/10 mice. The more specific aims of this study were as follows: (1) to compare differences in mortality and longevity among (a) control, (b) temperature stressed, (c) electrically shocked, and (d) combined temperature and shock treated adult male and female mice, and (2) to evaluate differences in mortality and longevity between these non-irradiated control-stress groups and brain irradiated mice exposed to the same 4 experimental-stress treatments.

Materials and methods. In the past, comprehensive research on age changes in relation to specific environmental factors in higher mammals has been limited considerably by the lack of appropriate experimental designs for evaluation of sources of variance covering the total life span. Specifically, cross-sectional studies confound maturational and generation changes, whereas longitudinal studies confound maturation and environmental treatment effects(10). To minimize some of these design problems, a longitudinal design based on successive cross-sectional age samples of genetically homogenous C57BL/10 mice with similar DNA "programs" was used in the present study. Observations on differences in mortality and longevity among 4 experimental subgroups were made on 6 successive cross-sectional age samples covering the entire life span of the C57BL/10 mouse. Essentially, to attain control over environmental stress factors throughout the life span, all animals were born, raised, and exposed to control, temperature changes, and electric shock in two carefully regulated environmental chambers in accordance with the following experimental design: At 90 days of age a total of 579 adult mice were assigned to 6 main cross-sectional groups. Each main group included 48 male and 48 female mice assigned to 4 treatment subgroups. In each main group, subgroup 1 was maintained at 72°F and 52% relative humidity and served as steady-state controls; subgroup 2 received electric shock for 1 hour each day, subgroup 3 was exposed intermittently to 60°F cold

stress in a second environmental chamber, and subgroup 4 received alternately electric shock and cold stress. Each stress cycle consisted of 4 of these 7-day stress periods or 28 days. Five of the main groups were exposed to 4, 8, 12, 16 and 20 28-day stress cycles, respectively. The animals were then tested in a behavioral test battery, and sacrificed for biochemical and morphological evaluation of the brain, pituitary and adrenals. Group 6 was not sacrificed but was subjected to these 28-day stress cycles throughout the entire life span to evaluate the effects of stress on mortality and longevity.

Irradiation of the brain was performed on 302 additional mice with 20 Mev deuteron beams delivered at a dose rate of 383 rad/sec through an anti-Bragg wheel by the 60-inch Brookhaven cyclotron. These animals were anesthetized and placed in a stereotaxic-type head holder. They received a dose of 500 rads over the dorsal surface of the brain covering an area of 9 $\times$ 5 mm to a depth of 2.5 mm. Except for the calvaria and the dorsal meninges no extraneuronal tissue was irradiated. All irradiated mice were then assigned to 4 main cross-sectional groups and then exposed to the same 4 experimental stress treatments in the 2 environmental chambers in accordance with the experimental design. The effects of environmental stress on behavior in relation to changes in CNS and neuroendocrine biochemistry and morphology have been reported(11,12). The present report is directed primarily to the evaluation of interaction effects of environmental stress and deuteron irradiation of the brain on mortality and longevity. Essentially, age-specific mortality rates expressed as a percent of each experimental subgroup were compared at 4-month intervals in non-irradiated and brain irradiated mice. Comparisons of differences in longevity were based on mean age of death expressed in days among the 4 subgroups in the cross-sectional longevity sample of non-irradiated and brain-irradiated mice.

Results. It is generally accepted that the age-specific mortality rate with increasing age is a rapidly accelerated exponential function(13). An examination of age-specific mortality rates among the 4 experimental subgroups in each of the main cross-sectional main groups at 4 month intervals indicated a significant increase in mortality of the 579 non-irradiated (rho = 1.0, n = 6, $P<0.01$) as well as among the 302 irradiated mice with increasing age (rho = .94, n = 6, $P<0.01$). There was an overall mortality of 10.5% in the 579 non-irradiated mice. In contrast, there was a 25.5% mortality in the 302 irradiated mice. By using the percent mortality of the non-irradiated mice as the overall expected mortality, a Chi-square analysis of differences in mortality between the non-irradiated and irradiated mice was made. This comparison indicated that there was a highly significant increase in the mortality of the irradiated groups ($X^2 = 70.78$, df = 1, $P< 0.0001$). The percent mortality of the control subgroups was then used as the expected mortality for testing the significance of the observed percent mortality among the 4 experimental subgroups of all cross-sectional age samples of the non-irradiated and brain irradiated mice. Surprisingly, the evaluation of mortality among the 4 experimental subgroups of the non-irradiated mice indicated a significant decrease in the mortality of the stressed females ($X^2 = 4.96$, df = 1, $P<0.05$), as well as a significant decrease in mortality among the stressed males ($X^2 = 9.36$, df = 1, $P<0.005$). Even more surprising, an evaluation of the percent mortality among the 4 experimental subgroups of the 4 deuteron irradiated cross-sectional age groups also indicated a significant decrease in mortality of the shock and temperature stress female subgroup ($X^2 = 3.90$, df = 1, $P< 0.05$) as well as an overall significant decrease in mortalaity of the stressed males ($X^2 = 13.93$, df = 1, $P<0.001$). Fig. 1 contains a more complete statistical summary of the main and interaction effects of environmental stress and deuteron irradiation of the brain on C57BL/10 female and male mice mortality throughout their life cycle. In addition to the significant overall decrease in the mortality of the stressed mice, it seems interesting to note that the most significant reduction of mortality occurred in the non-irradiated males ($X^2 = 9.60$, df = 1, $P<0.005$). It

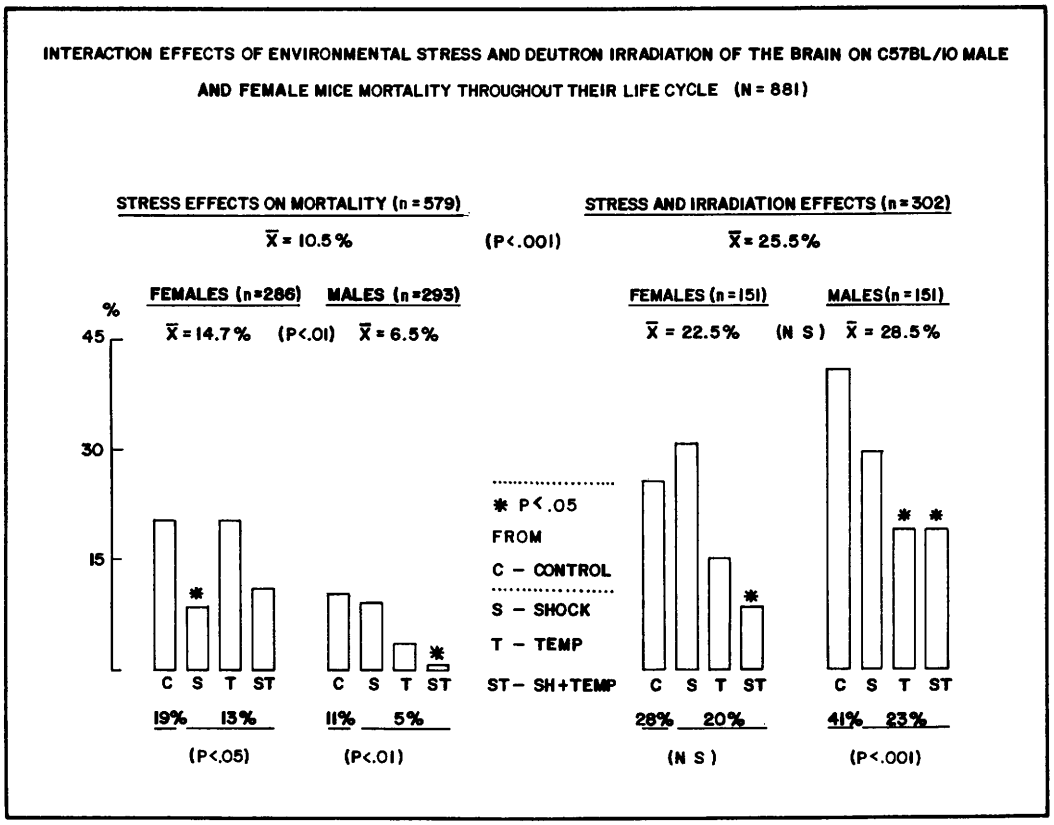

FIG. 1. Chi-square analyses were made to establish significance of differences in percent mortality between: 1) all non-irradiated and brain irradiated mice; 2) controls and the 3 stress subgroups combined as one group, and 3) controls and each of the 3 stress subgroups of the non-irradiated and brain irradiated mice.

also seems noteworthy that the significant difference in mortality between males and females observed in the non-irradiated mice was not found in the irradiated mice. This sex difference in mortality in response to stress was probably eliminated by the significant increase in mortality of the irradiated mice.

An overall analysis of variance was used for evaluating differences in longevity among the non-irradiated and irradiated mice included in the 2 cross-sectional longevity groups. The statistical analysis indicated that there was a highly significant decrease in the longevity of the irradiated mice ($F = 14.22$, $df = 1/161$, $P<0.001$). Newman-Keul's rank test for evaluating differences among combinations of subgroup means indicated that the non-irradiated males attained a significantly higher mean longevity in days than the other male and female mice ($P<0.01$). Figure 2 contains a more complete statistical summary of the main and interaction effects of environmental stress and deuteron irradiation of the brain on C57BL/10 male and female mice longevity.

An examination of the effects of environmental stress on longevity among the 4 experimental subgroups of the non-irradiated animals of the main longevity group suggested a longer life span in most of the stressed male and female mice. However, in view of the significantly higher mortality, this increased longevity was not apparent among the brain irradiated mice exposed to stress.

Discussion. Since the control of environmental factors throughout a life span covering several years is rather difficult to attain even under standard laboratory conditions for higher mammalian species, most previously reported cross-sectional studies have provided only indirect and frequently conflicting evidence on the relative role of genetic and en-

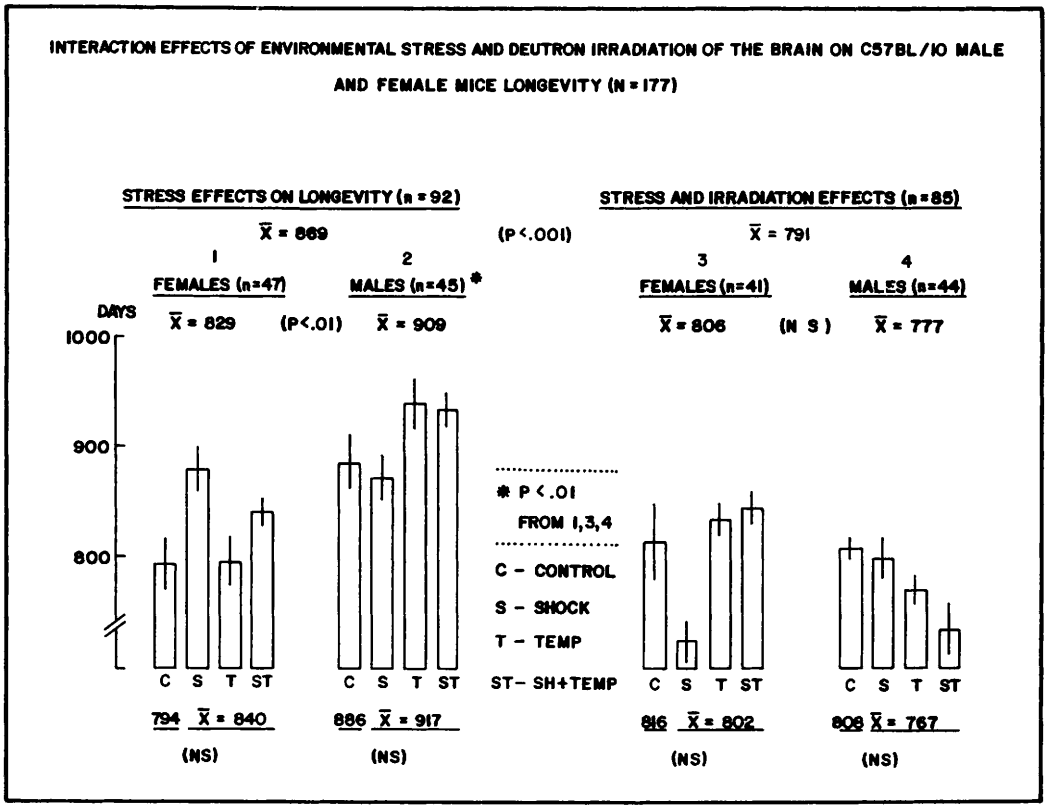

FIG. 2. Statistical comparisons of differences in age at death expressed in mean days (X) longevity among 4 subgroups of non-irradiated and brain irradiated mice.

vironmental factors in determining the rate of aging, mortality and life span. Earlier human stress studies of accelerated aging relied on actuarial mortality data obtained from heterogenous populations exposed to a variety of uncontrolled stress conditions(2). Animal studies have suggested that chronic exposure of young animals to stress results in behavioral, physiological, biochemical and morphological changes similar to age-changes observed in older populations(14,15). Consequently, these actuarial and experimental results have been interpreted frequently in support of the hypothesis that stress results in premature or accelerated aging(16). However, more recently, it has become apparent that only carefully controlled environmental chambers, multivariate designs with appropriate statistical evaluations, and observations on cross-sectional age samples covering the entire life span of a species can permit a more direct and quantitative assessment of both excitatory and inhibitory effects of specific environmental factors on aging(17,10). For example, as part of a more extensive research program on aging, observations were made on stress-induced behavioral changes in all C57BL/10 mice of the present study at 5 successive age levels for correlation with biochemical and morphological changes in the brain, pituitary and adrenals. Statistical evaluations indicated both age-related as well as stress-induced changes in behavior, biochemistry and morphology of the selected organs at the 5 successive age levels(11,12). However, as reported in the present study, it seems interesting to note that the intermittent deviations from so-called optimum steady-state conditions throughout the life span of the C57BL/10 mouse resulted in significantly lower mortality and a longer life span. Consequently, in attempting to interpret the effects of environmental stimulation or stress on aging in accordance with the "wear and

tear" theories, the experimental findings of the present study make it quite apparent that accelerated aging does not seem to result primarily from the accumulation of some residual effects produced by repeated stress exposure.

Perhaps the most compelling evidence against the stress theories of aging is based on the replication of a significantly lower mortality and longer life span of stressed mice whose mean life span had been reduced significantly by brain irradiation. The significant reduction of mortality in the brain irradiated mice exposed to stress also suggests that ionizing radiation may not simply represent a non-specific stress since it would have been reasonable to conclude in the present study that the interaction effects of stress and deuteron irradiation of the brain would have been additive and resulted in a significant increase in mortality and decrease in longevity. On the contrary, the present findings indicate that radiation may represent a rather unique possibility for decreasing the life span independently of stress-induced effects on the organism(6). Finally, as reported previously (11,12), there were significant age-related as well as stress-induced changes in behavior, biochemistry and morphology at all 5 successive age levels in the C57BL/10 mice of the present study. However these stress-induced changes did not accelerate aging since significant stress-induced decreases in mortality and a longer life span were observed in both nonirradiated and brain irradiated mice. These seemingly paradoxical and contradictory findings can only indicate that certain repeated deviations from environmental steady-state conditions at all age levels may result in a variety of biochemical and morphological changes in the organism but still lead to an inhibition rather than acceleration of biological aging. This interpretation of environmental stress effects on biological aging would certainly be consistent with evolutionary theories of natural selection and survival of multicellular populations under fluctuating environmental conditions.

Summary. Observations were made on differences in mortality and longevity among 4 experimental subgroups of 6 successive cross-sectional age samples of non-irradiated and brain-irradiated C57BL/10 mice during their entire life span. The 4 subgroups of each main cross-sectional age sample included 1) steady-state controls, 2) temperature stress, 3) electric shock stress, and 4) combined temperature and electric shock stress. Irradiation of the brain was performed with a 20 Mev deuteron beam delivered at a dose rate of 383 rad/sec through an anti-Bragg wheel by the 60 inch Brookhaven cyclotron. The mice received a dose of 500 rads over the dorsal surface of the brain covering an area of 9×5 mm to a depth of 2.5 mm. All irradiated mice were then assigned to 4 cross-sectional groups and exposed to the same 4 experimental stress treatments in the 2 environmental chambers in accordance with the experimental design.

There was a highly significant decrease in the longevity of the brain irradiated mice. Contrary to expectation, stress resulted in a significant decrease in mortality and a longer life span of both the non-irradiated and brain-irradiated mice. The significant difference in mortality and longevity between males and females observed in the non-irradiated mice was not found in the brain irradiated mice. The significantly lower mortality and longer life span of stressed mice whose life expectancy had been reduced significantly by brain irradiation provided compelling evidence against some current "stress" theories of biological aging. In summary, environmental stress did not accelerate the aging of non-irradiated or brain-irradiated mice. From the non-additive interaction effects of environmental stress and deuteron irradiation of the brain on mortality and longevity of C57BL/10 mice, it was concluded that stress induced decreases in mortality and a longer life span were consistent with evolutionary theories of natural selection and survival of multicellular populations under fluctuating environmental conditions.

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A Possible Role of Hydroxypyruvic Acid Reductase In Growth of *A. niger*. (32398)

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In a study of growth and differentiation in *Aspergillus niger* it has been previously shown that while the operation of the citric acid cycle is essential to the development of spores, growth of the hyphae is dependent on a metabolic pathway that seems to diverge from glycolysis (Embden-Meyerhof), and not upon the pyruvic dehydrogenation system and the reactions of the tricarboxylic acid cycle(1).

With the culture method described below, this mold completes its growth cycle in 80 hours. The lag phase continues to the 20th hour; logarithmic phase begins then and continues until the 58-60th hour. At this point spore formation begins and continues rapidly. Mold pad weight begins to decline at the 80th hour. In a previous paper it was shown that the 40-52 hour interval was critical for spore formation and that the purine analog, 6-ethylthiopurine, if present in the growth medium during this interval would selectively inhibit subsequent spore formation (at 60th hour) without any effect on growth of hyphae or conidiophores. In this paper attention will be focused on the 20-40th hour interval, that is the period when mycelial growth is dominant, prior to conidiophore (stalk) formation. This latter process predominates during the subsequent 40-60 hour period and is specifically inhibited by 6-hydroxy-2-mercaptopurine(2).

In earlier papers the production of hydroxypyruvic acid by this mold was reported and it was postulated that this acid served as a precursor for a series of reactions necessary for hyphal growth(3,4). The data presented below concern further observations on the relationship between hyphal growth and the metabolism of this acid.

Materials and methods. The medium routinely used had the following composition: glucose, 45.0 g; $NaNO_3$, 4.5 g; KH_2PO_4, 3.0 g; KCl, 0.75 g; $MgSO_4 \cdot 7H_2O$, 0.75 g; $FeSO_4$, 0.02 g; water (distilled) to make 1.0 l.; and H_3PO_4, to adjust the pH to 4.0. The inoculum was prepared by mixing 2 mg of spores in 100 ml of a 2% agar solution at 55°C which was then poured into a 20 × 25 cm baking dish and allowed to solidify. Discs were cut from the agar to cover the entire surface of 1.0 ml medium of the depressions (diameter, 21 mm; volume, 1.2 ml) of porcelain spot plates (12 depressions per plate). In contrast with the classical small inoculum