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† During the conduction of this investigation the junior author was a Predoctoral Fellow of the Committee for Research in Problems of Sex, National Academy of Sciences–National Research Council.

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*THE DEVELOPMENT OF SOMATIC MUTATIONS IN MICE WITH AGE**

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Of the many theories to account for the phenomenon of aging, the one which has gained most prominence recently is the somatic mutation theory. This theory proposes that aging is caused by the gradual accumulation of spontaneous mutations in the somatic cells of the body. Evidence concerning this theory has largely been lacking for want of a method for measuring such mutations. Recently an indirect method has been developed[1] in connection with the problem of radiation-induced aging. The method involves scoring the numbers of chromosome aberrations in cells from regenerating livers of mice. The justification for relating this quantity to the numbers of mutations present comes from work with plants[2] where the somatic cells eventually differentiate to form germinal cells whose mutations can be scored in the usual way. Here it is found that the numbers of mutations are proportional to the chromosome aberrations of the somatic cells under a wide variety of circumstances.

Previous work[1, 3] has shown that aberrations in liver cells of mice increase linearly with age at least to an age of 12 months. A single dose of X rays will increase the aberrations to 80 per cent or higher, and these decrease slowly with time over a period of many months. When mice are subjected to continuous low level irradiation by gamma rays, aberrations accumulate more rapidly than for the controls, but calculations show that this is a "multiple hit" phenomenon and thus the chromosomes are capable of self-repair.

The present work was undertaken in an effort to gain evidence for a causal rela-

tionship between spontaneous somatic mutations and natural aging, by scoring the chromosome aberrations in strains of mice having different life expectancies.

Methods and Results.—Female mice of two strains were used: C57BL/6J and A/HEJ. The first of these has been reported[4] to have a life expectancy of 600 days, the second of 395 days. The mice were obtained from Jackson Laboratories when either 6 weeks or about 12 months of age, the latter being discarded breeders. All groups were kept under standard laboratory conditions, and five mice were removed at random from each group at approximately 100-day intervals and sacrificed for scoring of chromosome aberrations.

The cytological methods have been previously described and consisted in injecting the mouse with carbon tetrachloride to destroy part of the liver, and sacrificing the animal 72 hours later at the height of regeneration. Cell squashes were examined microscopically and all cells in either anaphase or telophase were scored as either normal or abnormal. Abnormalities consisted entirely of either bridges or fragments.

The results are shown in Figure 1. Here it will be seen that the aberrations in the long-lived strain increase rather slowly with age, but those for the short-lived strain increase very rapidly. It will be seen that in both cases the mice which had been bred continually from the time they were adult until the start of the experiment appeared to develop aberrations at a faster rate than animals which had not been bred. It is not known whether or not this is a real effect. There were so few mice that mortality curves were not very accurate, but life expectancies found were consistent with those reported.[4]

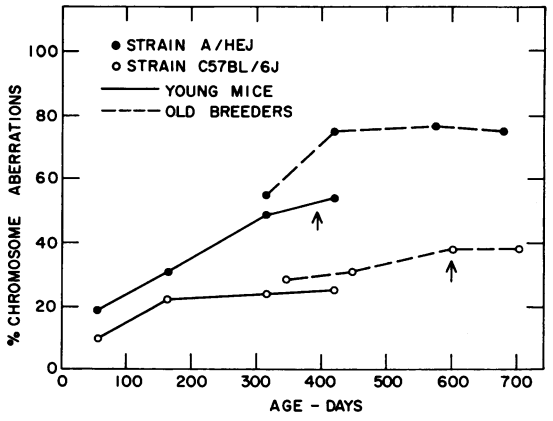

FIG. 1.—Chromosome aberrations in regenerating liver cells in two inbred strains of female mice plotted as a function of age. The median life span of each strain is indicated by the arrows, as reported by Roderick and Storer.[4] In each case, the solid lines represent animals 8 weeks of age at the start of the experiment, and the broken lines old breeding animals about 1 year old at the start of the experiment.

Discussion.—It is interesting to compare this result with previous work. This presents some difficulties, since scoring is somewhat subjective and varies somewhat from observer to observer and even in the same observer at different times. This is why the experiments must be done "blind" and in a rigidly controlled series. Nevertheless, the previous results[1, 3] are entirely in accord with the present ones. The two inbred strains used previously (Carworth Farms strain CF1 and Charles River strain CD1) are intermediate between the two strains used here, both in longevity and in the rate at which the normal control animals develop aberrations in liver cells. Thus, the present results, together with this substantiating evidence, make it seem quite certain that in inbred strains the life expectancy is related to the rate at which spontaneous chromosome aberrations, and presumably mutations, develop in the somatic cells.

Previous work has shown that when the mutation rate is increased by radiation in a variety of conditions, the life span is proportionately decreased. The present work now shows that the same holds true for spontaneous mutations. These facts would make it seem quite extraordinary if there were not a causal relation between somatic mutations and aging.

Summary.—Chromosome aberrations in the liver cells of two strains of mice, one long-lived and the other short-lived, have been measured as a function of age. The development of aberrations is inversely proportional to the life expectancy. This result, together with other recent evidence, makes it seem very likely that somatic mutations play a dominant role in the aging process.

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*REPRESSION OF THE VALINE-ISOLEUCINE PATHWAY IN SALMONELLA**

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Recent investigations have shown that the α-hydroxy-β-keto acid reductoisomerase and the α,β-dihydroxy acid dehydrase enzymes for the synthesis of valine and isoleucine are repressed when wild-type *Salmonella typhimurium* is grown in nutrient broth.[1] Attempts to identify the factor(s) responsible for the repression have shown that valine exerts a partial repression on the reductoisomerase and the dehydrase but that true repression is not obtained with this amino acid.[1] Isoleucine and valine together are no more effective than valine; indeed, isoleucine often antagonizes the effect that valine has on the dehydrase, particularly during the initial phase of growth. A combination of leucine, isoleucine, and valine is also incapable of repressing the pathway.

This report is concerned with the continued investigation of the factors responsible for the repression of the pathway and with the novelty of the regulatory mechanisms.

Methods.—The organism used in this study was wild-type *S. typhimurium*, strain LT-2. All experimental procedures and enzymatic assays have been described elsewhere.[1] Cells were disrupted sonically either by treatment in a 20-kc Bronwill Biosonik for 10 min (Fig. 1) or in a Sonifier (Branson Instruments, Inc.) for 2 min (Table 1). Cell-free extracts prepared in the latter manner possess higher levels of α,β-dihydroxy acid dehydrase activity. Conditions of growth and treatment of the cells for the individual experiments are given with the Table and Figure.