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BIOLOGICAL ASPECTS OF
CANCER AND AGING

BIOLOGICAL ASPECTS OF CANCER AND AGING

Studies in Pure Line Mice

LEONELL C. STRONG



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Dedication

THIS book is dedicated to two Bittners – to my graduate student, John, who discovered the milk factor or virus of Bittner, and to Katherine, my wife in the home and my associate in the laboratory for nearly 50 years.

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Acknowledgments

DURING the years there have been many individuals for whose interest and assistance to me in this investigation I am deeply in debt. I am particularly indebted to Mr. Harold Woodworth for more than 30 years of conscientious work; to Mrs. Lucille Domes and to Mr. Henry Matsunaga for loyalty during the completion of the work.

To the many institutions and funding associations for support I also desire to extend my lasting appreciation.

Foreword

Biological Aspects of Cancer and Aging considers the two problems enumerated in relation to Genetics. There is no attempt to consider other aspects of Biology. The analysis of the several biological characteristics has only been discussed in the text by reference to the aging process (Gerontology).

As a matter of fact, many observations of biological and genetic interest have been made during the extent of this present program. For example, numerous mutations involving either color or morphology have been observed but, due to limited space and facilities, they could not be genetically analyzed. Some have been recorded briefly but many not.

Of particular interest are the morphological markers of birth defects. To cite only a few: (1) polydactylia with its many pleomorphic effects of (2) brachydactylia, (3) syndactylia, (4) luxoid appendages, (5) alopecia, (6) blindness, etc., is of extreme frequent appearance. Several embryological effects of this mutant (Strong's LST syndrome) have been investigated by Forsthoefel (1962, 1963). But many of the physiological effects of polydactylia have only been mentioned casually.

Autopsies of pregnant female mice disclose that many abnormal offspring (including death at several ages) are present which are lost during childbirth.

The present book considers the biological problems of stability and instability. It also discusses the probability that cancer is a result of some aspect of biological instability. That these biological states may have an influence on such problems as spontaneous mutations and birth defects has not been adequately investigated—at least, not in the mammalia.

With the new interest in the investigation of birth defects, it is well to keep in mind that the same techniques that have been so successful in the investigation of aging and cancer, through studies on genetic or biologic stability and instability as outlined in this book, may also be significantly applied.

Introduction

THE history of genetics has been fathomed in one life span. True it is, that certain principles of heredity were developed by Galton and others using statistical methods in the nineteenth century (1805-97). Also, the work of the Austrian monk, Gregor Mendel, upon which the science has been founded in the twentieth century, was done about the same time (1866). But the impetus that developed the science of genetics into the colossus that it is today stems, primarily, from the rediscovery of Mendel's work by three biologists working in three separate countries in the year 1900 De Vries, Correns and von Tschermak).

While a graduate student at Columbia University the present author was impressed by an editorial in the *Journal of Heredity*, "As Genetics Comes of Age" (East, 1922). Now as he approaches another stage in the life process he is concerned with the observations of continued results made possible by the practical application of genetic principles to the problem of cancer research and of gerontology.

From a study of what may be considered intermediate, i.e. the analysis of the morphological and physiological components of the living organism, genetics has expanded in two directions—one, through embryology and the keen interest of pediatricians, cytologists and biochemists, to the earliest evidence of life itself, and second toward senescence and death.

The present series of lectures is upon three fundamental biological problems with a firm basis in genetics. These are (1) the establishment of the inbred experimental animals, especially mice, upon which a very large part of the research program on mammals is based, (2) cancer research and (3) gerontology.

The earliest contributions of the identification of the gene as being involved in various aspects of cancer, that of its role in the transmission of susceptibility and resistance to the transplantable tumor, in its influence of the establishment of the

biological state or states involved in the response to cancer by the carcinogens and even in its role in relation to the origin of spontaneous tumors (somatic mutations), will not be discussed in separate lectures. They naturally belong to a former period and would not be appropriate in *Biological Aspects of Cancer and Aging*. However, these early contributions will be included in the concluding chapter of this book, where a summation is attempted. Without their discussion, in view of recent developments in genetics, the general theme would be greatly impaired.

It is not the intention of discussing the numerous contributions made possible by the two-pronged development of genetics by numerous investigators. Their discussion may be found in reviews, such as Sturtevant (1965) and others. It will be sufficient here to report a series of observations made available by the investigations of the present author and to indicate, perhaps too scantily, the bearing of these observations on modern genetics or, in fact, conversely the bearing of modern genetics upon the present observations of cancer research and of gerontology.

These two problems (cancer and gerontology) will be presented separately although there is bound to be overlapping and there will be an attempt, in the concluding chapter, to try to synthesize the two problems together and to arrive at the general conclusion that cancer and gerontology are fundamentally the same problem. This synthesis will be attempted by the discussion of the phenomena of biological stability and/or instability which unquestionably are involved in the onset and variations of these two phenomena.

Perhaps a few comments on biological stability and/or instability are appropriate now. This concept has had a long history but is usually originally associated with Cannon (1929) through his many physiological experiments and derived concepts. Very many biologists have recognized the fundamental concept that the characteristics of the individual that are gradually acquired through adolescence reach a relatively uniform value during a lengthy adult stage and are gradually lost or atrophied during senescence. The geneticists refer to the uniform biological state as homeostasis (Lerner, 1954). But whether we use the expression physiological regulations, or feedback mechanisms or homeostasis, or the maintenance of constancy,

the same phenomenon is intended. Even the problem was poetically recognized by Shakespeare as "constant in being inconstant". Again he refers to fortune as, "That she [Fortune] is turning and inconstant, and mutability, and variation" (Shakespeare, *Henry V*). How many problems of modern genetics are recognized!

The development of the environmental aspects of homeostasis has been recently discussed by a symposium, *The Development of Homeostasis*, with special reference to "Factors of the Environment", held under the auspices of the Czechoslovak Academy of Sciences, 1960 (Adolph, 1960).

Numerous other articles on homeostasis are available which will be referred to in the concluding chapter of this book.

CHAPTER 1

A Biologist Looks at Gerontology*

I SUPPOSE that when the invitation was extended to me as a geneticist by the President of Palomar College, a lecture on the newest developments in genetics, that is in molecular biology, may have been anticipated. I hope I do not disappoint you in discussing fundamental problems oriented in other aspects of biology but which have not yet reached into the domain of molecular biology. By this I do not mean that the present problems of biology should not be approached by me by the techniques of molecular biology but only that I have found sufficient interest and observations without working yet on the chemistry of DNA, as such, RNA, and the other molecular components of the single mammalian cell.

It might also be expected that after nearly fifty years of cancer research, a talk on this subject would not be out of place. This experience I cannot ignore. But a geneticist, following the application of genetic principles to the cancer problem for so long a time, may be excused in taking up another biological interest, i.e. gerontology.

Cancer research and gerontology have much in common. This can be illustrated by saying that one may start out with a relatively few experimental animals, let us say the mouse upon which so much fundamental biological research has been done. Now some of these mice (if carefully selected) will develop spontaneous tumors of one sort or another. Observations on these mice is cancer research. Some of the mice, however, will escape the dreadful disease of cancer and die of old age. Their study is gerontology. It may be a very significant fact that some mice of these two groups cited above could have been brothers and sisters.

*A lecture presented at Palomar College, San Marcos, California, 22 March 1965.

That is, a very faint line of demarcation could be drawn between cancer research and gerontology. I hope to show how these two fields may be bridged together through the science of genetics.

But the appearance of cancer and approaching old age have other facets of comparison. In both, one has the immediate problem and the eventual one. The cancer patient must be treated by the best method or methods available to modern medicine—the future, by research, must provide better methods of treatment, as well as determining the true nature of the cause of cancer by which prevention and cure may be improved or made possible. In gerontology, civilization is faced with the increasing care of the aging population—and, by research to improve that care and to ascertain the lasting effects of the aging process upon the characteristics of man himself.

Fundamental problems such as: Do parents produce offspring too early or too late in life? Do women have children too frequently? Are modern families too large or too small? Many other problems have been suggested and continue to be suggested from time to time upon which too little fundamental work has been done.

To analyze these problems exclusively in man would be herculean, if not impossible. Impossible, if these effects of the aging process on characteristics of the offspring are small, but cumulative over a period of generations.

If this problem of genetic studies on aging is impossible or very difficult in man with all his uncontrolled and, many times, unpredictable variability, his changing environment, his periodic exposures to cataclysmic wars, etc., it certainly is not impossible in the laboratory using carefully selected experimental animals, such as mice, where all variables are recognized, standardized and kept under control as carefully as possible. Whether the findings in mice or any one species apply to other forms of life including man himself remains for the future to decide.

Let me make a confession. There is very little, if anything, in classical, i.e. historical, genetics to expect to measure the effect of the aging process on characteristics of individuals of the ensuing generations. Classical genetics is involved in the aging process (Strong, 1954).

However, with the development of molecular biology with its new techniques of analysis, several possibilities have opened up

for the study of both cancer and the aging process. Such techniques involve the chemistry and biochemistry of DNA, RNA and transfer and messenger RNA, etc. Already fundamental contributions have been made on the cancer problem—and the application on gerontology is imminent. But these studies are beyond the scope of the present lecture.

The start of my interest in the problem of gerontology was obtained many years ago and it came from the field of cancer research. It was found about 1920 (Strong and Little, 1920) and earlier that a tumor of one strain of genetically controlled origin would not grow in an adult of a foreign strain. The tumor would, however, grow in the newborn of another strain and the same tumor would also grow in a senescent animal or one growing old. Thus the phenomenon of tissue specificity maintains that many of the specific characteristics of the individual are gradually acquired during adolescence, remain at a fairly constant level or plateau during adult life and are gradually lost during senescence. The number of physiological processes that seem to fit into the above scheme appear to be legion.

This changing sequence of events leads to another fundamental concept and that is that perhaps only the mature or adult female individual is in perfect or, at least, partial biological equilibrium where all organs and systems are functioning properly and in balance with each other in an organized whole. During both ends of the life span this equilibrium is only gradually lost during senescence. Accidental death may be superimposed at any time during these changes and perhaps natural death only after the complete normal span has been reached, which may be of rare occurrence.

Now I would like to present the conclusions to the present problem and then to follow through with the presentation and discussion of as much supporting data for which we have time.

The conclusion—that both the origin of cancer and the aging process are the resultant of disturbances of this biological equilibrium which is reached and retained only in the "prime of life" (Strong, 1957).

Among the tools that a geneticist can use, as stated previously, are hybridization, segregation and selection. By these means, the fundamental laws of heredity, the architecture of the germ plasm with the location of the gene or genic material on chromosomes,

which later was proven to be DNA, and many other facts of genetics have been established (Auerbach, 1949; Kit, 1960). These are the experimental tools that were used in this series of experiments that have now covered more years than I like to remember.

Hybridization increases biological variability which can be analyzed in the laboratory under carefully controlled conditions. Inbreeding reduces variability thus leading to equilibrium—at least, equilibrium is to be expected according to the laws of inbreeding (Strong, 1957). There is a concept based upon much experimental evidence that the hybrid individual is in better equilibrium than a pure breeding one. But this technical analysis need not concern us now. Many characteristics, such as age of first litters, litter spacings when the sexes are kept continuously together, reproductive capacity as measured by litter size, fertility, fecundity, the incidence of spontaneous tumors and longevity have all been measured in the same laboratory on the same animals in the same environment, on the same or standard food and water supply, etc. (Strong and Fuller, 1958; Roman and Strong, 1962). Obviously, it would not be possible to discuss all these data in one lecture.

But for presentation of the fundamental principles involved we shall use only (1) age of first litters in one selected strain classified only in reference to the age of the mother at which time she has had her offspring, (2) the incidence of spontaneous mammary gland tumors, (3) the incidence of lung tumors and (4) longevity, i.e. the measure of the life span.

To control the present experiment even more precisely, only one male of a pure breeding strain was mated to several females of another well established inbred C₅₇/St strain. Eight lineal descents were established basing selection only on (1) age of mother at which time her offspring were born and (2) the presence of polydactylia, i.e. extra toes on one or more of the appendages. Thus, in a constant environment and standard food and water, we were able to measure biological characteristics and to resolve the forces that determine them into (1) genetic, i.e. hereditary, (2) maternal age or (3) any other force that may be involved in causation of characteristics of the offspring. The pleomorphic multiple effect gene polydactylia is certainly one of these contributing forces that has an influence on both cancer and the aging

process (Chapter 4) but whether there is time in a single lecture to discuss this problem remains to be seen.

We shall take up (1) the age of first litters through the process of inbreeding.

The first three charts show data on the age of first litters at three levels of inbreeding in one lineal descent of mice with selection toward an early maternal age (<100 days).

Chart 1 shows the data on age of first litters for mice of the first five generations of inbreeding. The largest number of mice had their first litters at about 71 days of age. There is a certain amount of skewness toward late appearing first litters.

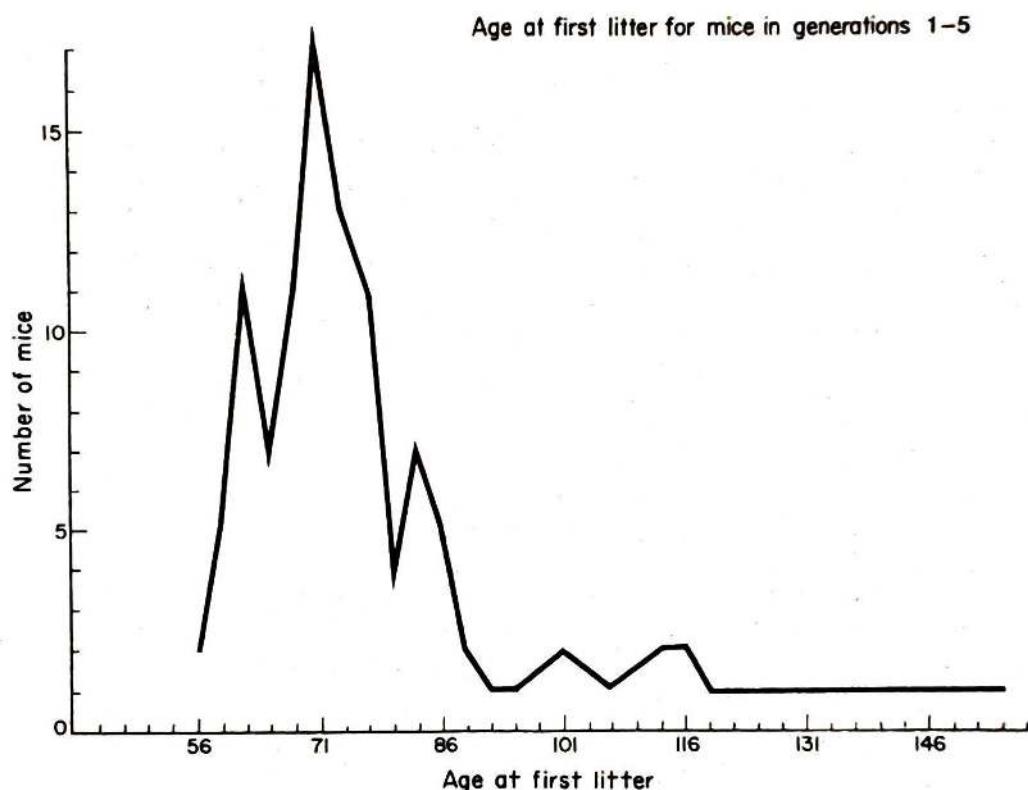


CHART 1. This chart presents data on (1) age of mothers of the <100-day maternal age descent in days at time of first litters for generations F_1-F_5 on the base line and (2) the number of mice in each group on the vertical line.

Chart 2 shows similar data for mice between generations $F_{11}-F_{15}$. Here the largest number of mice have their first litters at about the same age and with approximately the same amount of skewness.

Chart 3 shows data on age of first litters between generations $F_{31}-F_{35}$. Here the distribution is quite similar to the data in the

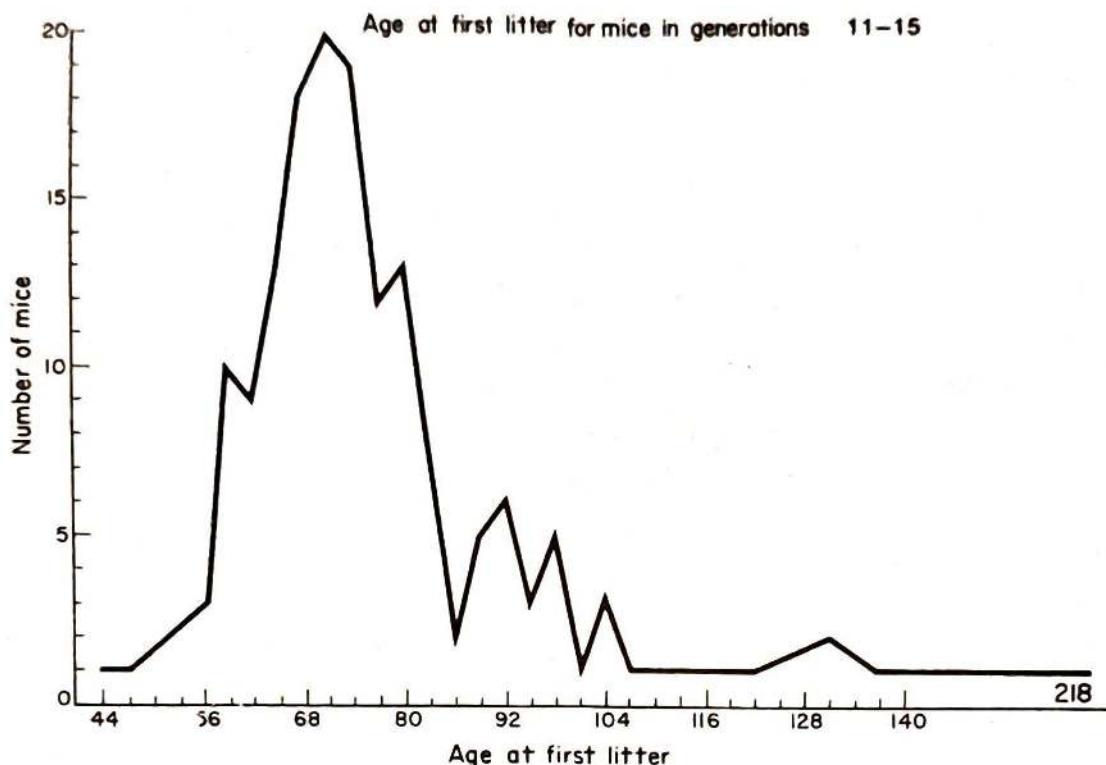


CHART 2. This chart presents the data comparable to the data in Chart 1 but for female mice between F_{11} - F_{15} . Age of mothers is on the base line and number of mice on the vertical line.

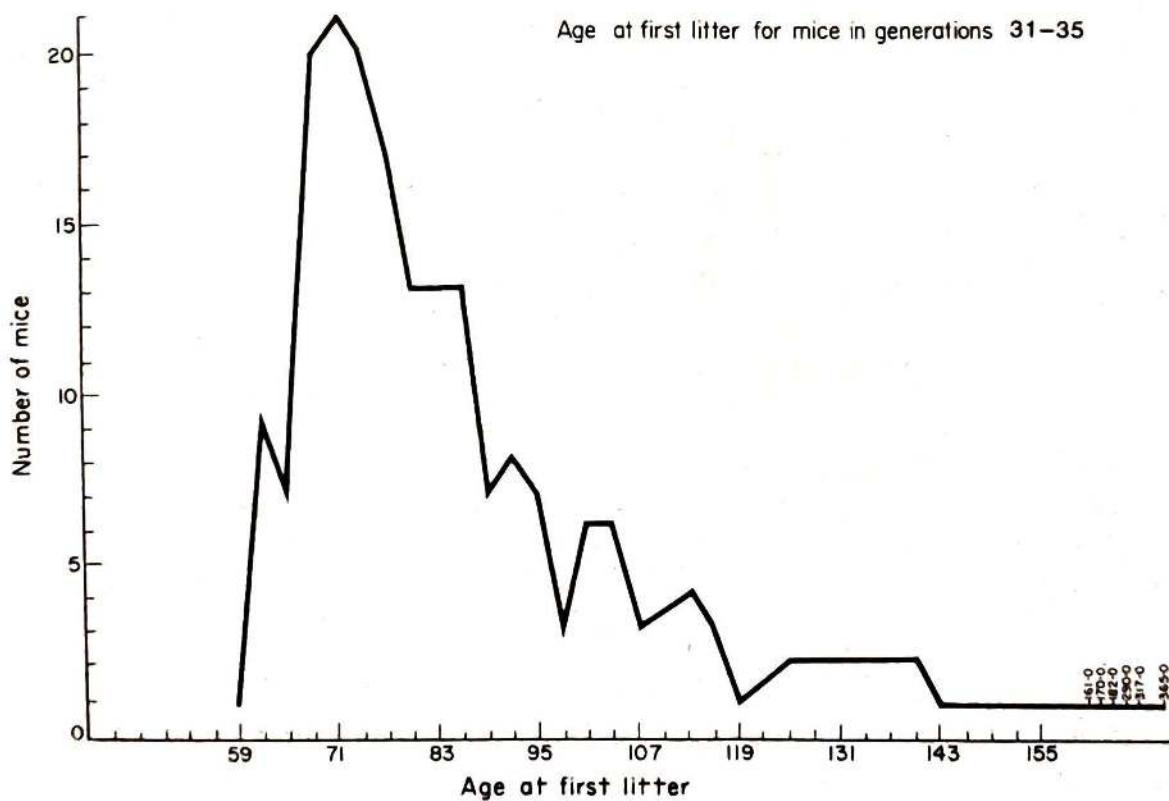


CHART 3. This chart presents the data on age of first litters comparable to the data given in Charts 1 and 2 but for mothers between F_{31} - F_{35} .

first two charts— even after thirty-five generations of inbreeding. The greatest difference lies in the larger number of mice that had first litters at more than 100 days of age.

Chart 4 gives data on the analysis of the logarithms of the averages of ages of first litters through fifty-four generations of

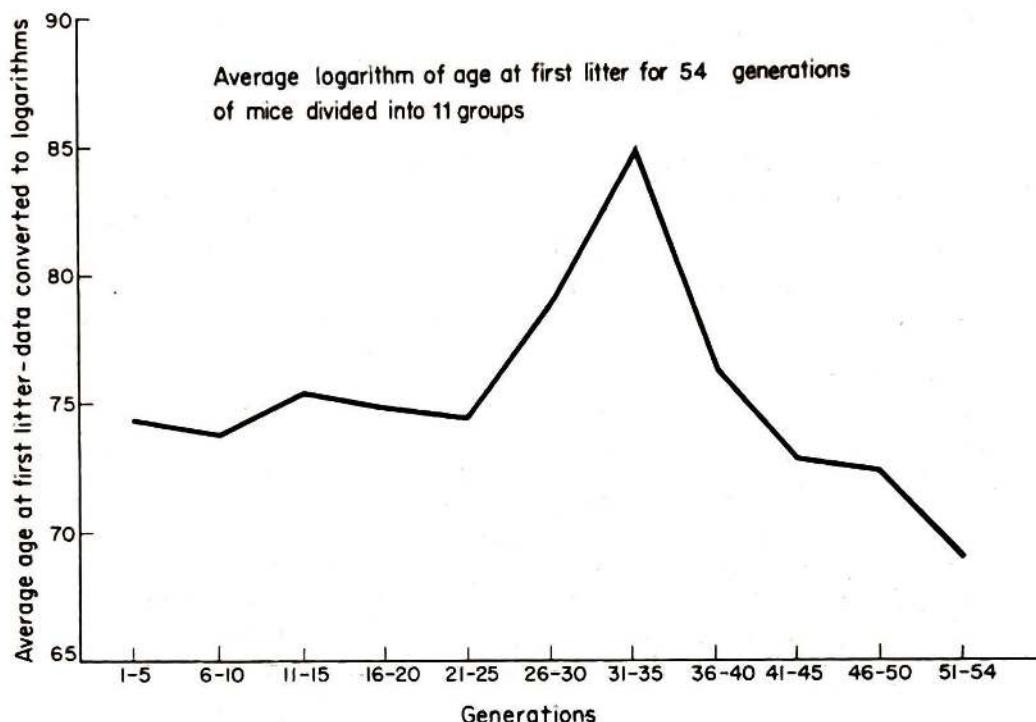


CHART 4. The data on age of mothers at time of first litters of the <100-day maternal age descent are expressed in logarithms. Data on fifty-four generations of inbreeding are given and the entire population is divided into eleven groups of five generations each. Average ages of first litters are given on the vertical line. The generations of inbreeding data are on the base line.

inbreeding. Here it is evident that the average age is very similar for twenty-five generations of inbreeding. Beyond this point of inbreeding there was obtained later and later ages of first litters until a maximum of 93 days was reached in F_{34} . Still later, first litters came earlier and earlier and the lowest value of 71 days was reached after F_{51} generations of inbreeding. Analysis of data on this chart and on others indicates quite convincingly that mice of this descent even after fifty-four generations of inbreeding are still not in biological equilibrium (Strong, Johnson and Rimm, 1965).

Chart 5 shows the analysis of the same data on age of first litters in the same descent but basing the analysis on the median rather than the averages.

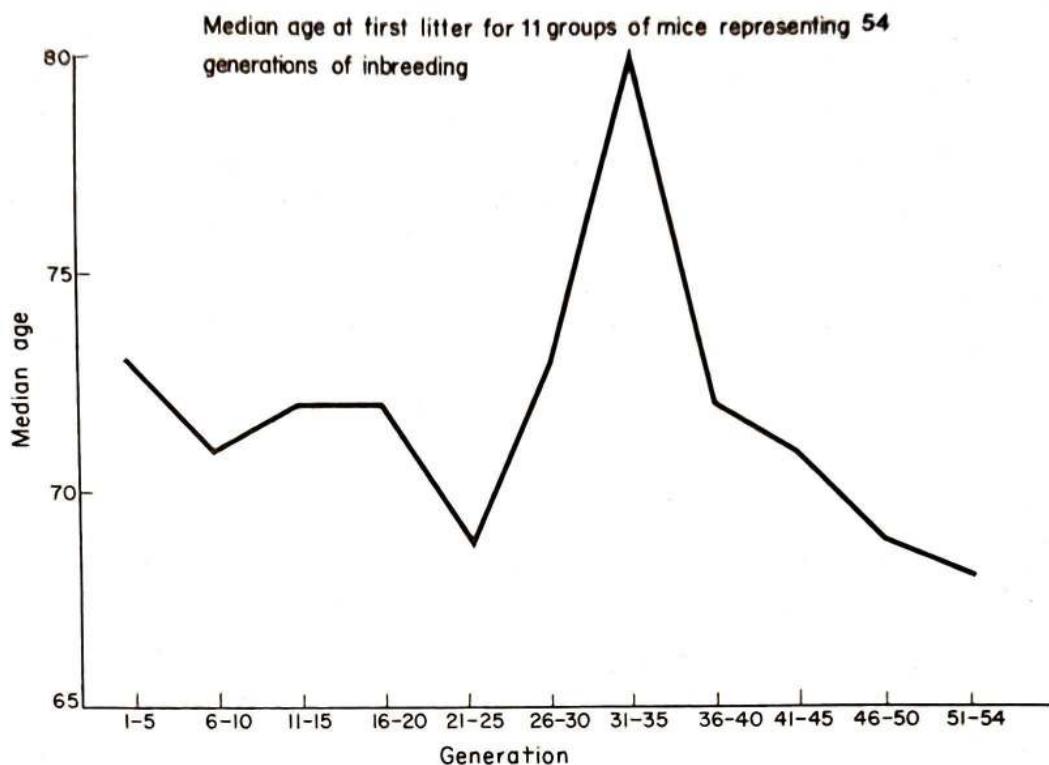


CHART 5. The data on ages of first litters are presented in Chart 5 by analysis of medians. Data on average median age of first litters are on the vertical line; data on generations of inbreeding are on the base line (five generations are grouped together).

Chart 6 shows the presentation of similar data but basing the analysis on the number of mice having first litters at more than 100 days of life. Here it can be seen that the greater number of mice had late first litters between $F_{31}-F_{35}$ when the average or median for the entire class was at a maximum.

Chart 7 compares the standard errors for eleven groups at successive levels of inbreeding. There appears to be a downtrend in the measure of these standard errors toward greater inbreeding. However, the greatest values were obtained between $F_{31}-F_{35}$ and thus there is evidence of variability in age of first litters even after fifty-four generations of inbreeding indicating that these mice are not in biological equilibrium, as stated previously.

The next three charts show data on the incidence of spontaneous mammary gland tumors and longevity in eight collateral sublines that had had a common origin as indicated previously.

Chart 8 presents the data on the age at which spontaneous mammary gland tumors arise in female mice of the successive maternal age descents. Age of onset increases in the first three

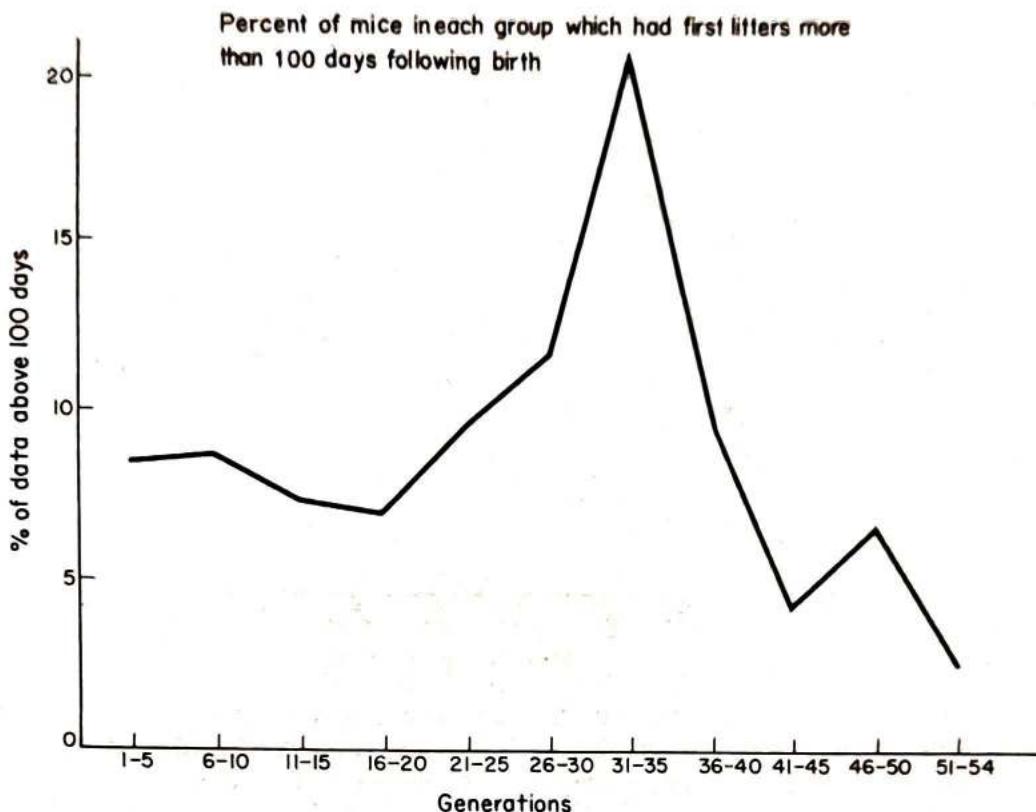


CHART 6. This chart presents the data on the percent of mice in each group of five generations which had first litters < 100 days of age.

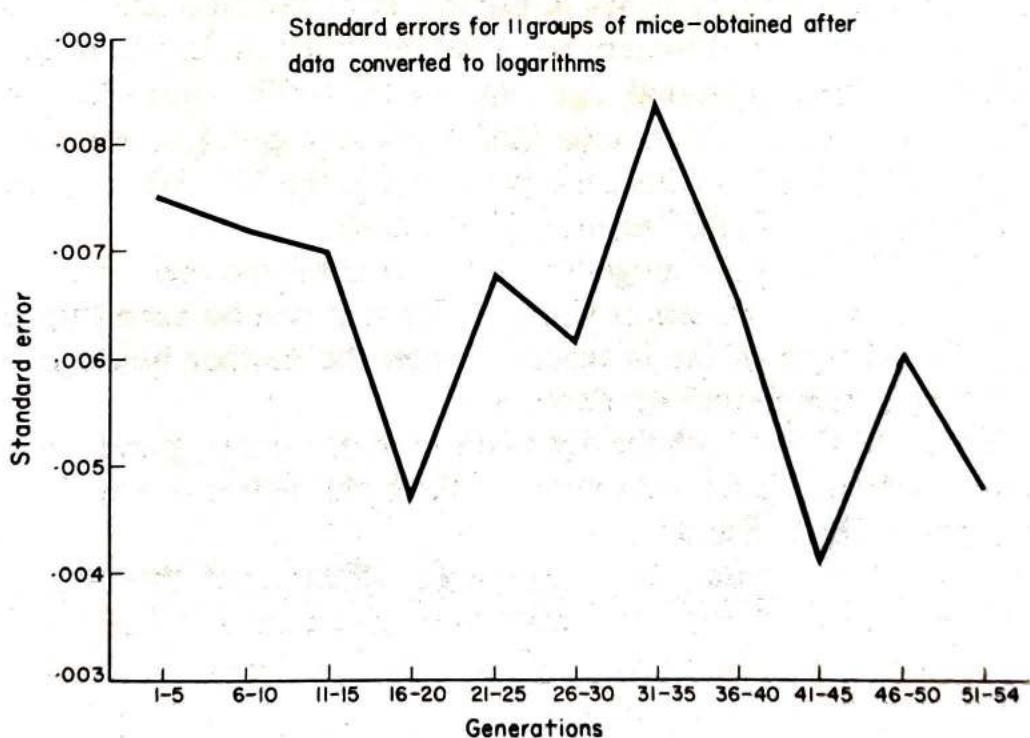


CHART 7. This chart presents the data on standard errors on age of first litters for the eleven groups of mice calculated after the data were converted to logarithms.

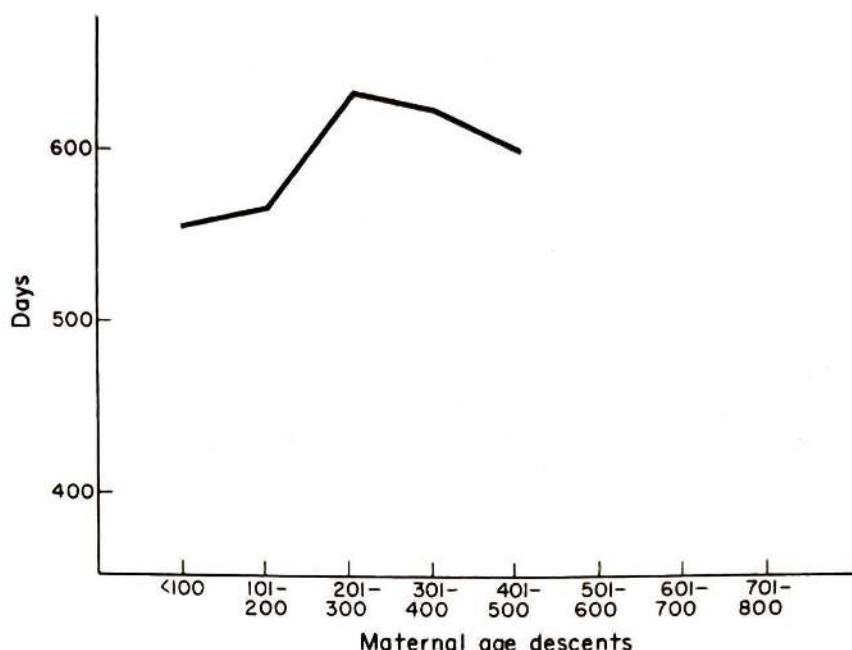


CHART 8. This chart presents the data on the average age of onset of spontaneous mammary gland tumors (vertical line) for female mice of five independent maternal age descents which had a common origin (base line).

classes and then diminishes in the last two maternal age classes. No mammary gland tumors have yet occurred in female mice of the last three maternal age classes (501–600, 601–700 and 701–800 days). It is obvious that mammary gland tumors arise later in female mice whose mothers were in the 201–300 maternal age descent (i.e. in the “prime of life” class).

Chart 9 shows the longevity curve for the same eight maternal age descents as shown in Chart 8. Here it can be seen that the maximal length of life is reached when the mother belonged to the 201–300 maternal age descent.

Chart 10 shows both the age of onset of mammary gland tumors and length of life free of tumors in the eight maternal age classes shown in Charts 8 and 9.

Thus, it is obvious that the female offspring of the 201–300 maternal age descent have mammary gland tumors later in life and when free of a tumor will actually live longer than female mice on either side of those in the “prime of life”.

The next five charts show data on the incidence of spontaneous lung tumors and longevity in the same population of mice as discussed in the preceding three charts.

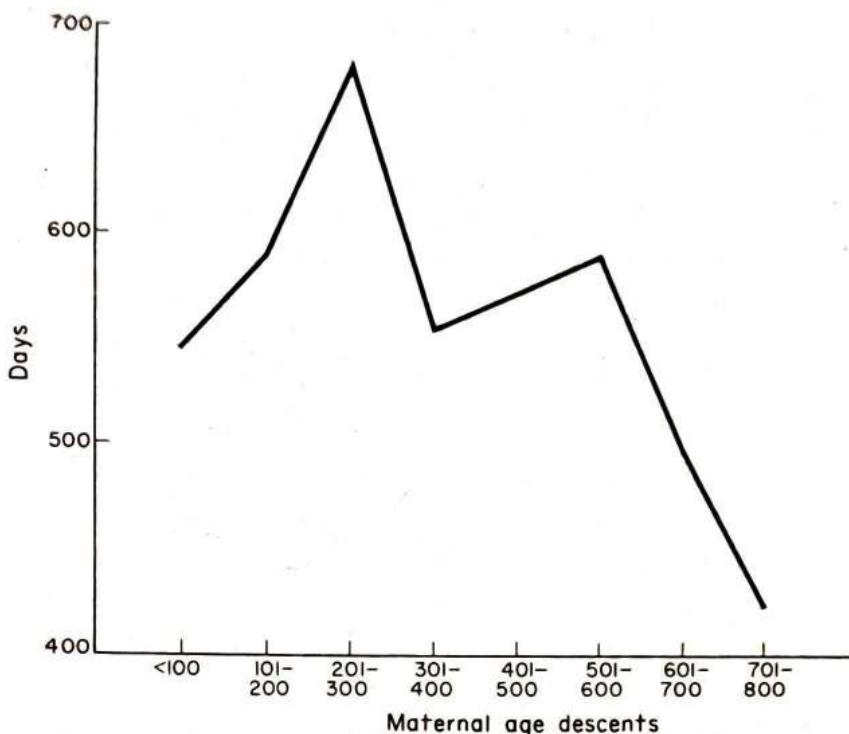


CHART 9. This chart presents the data on longevity (without the presence of mammary gland tumors) on the vertical line in female mice of the eight maternal age descents on the base line.

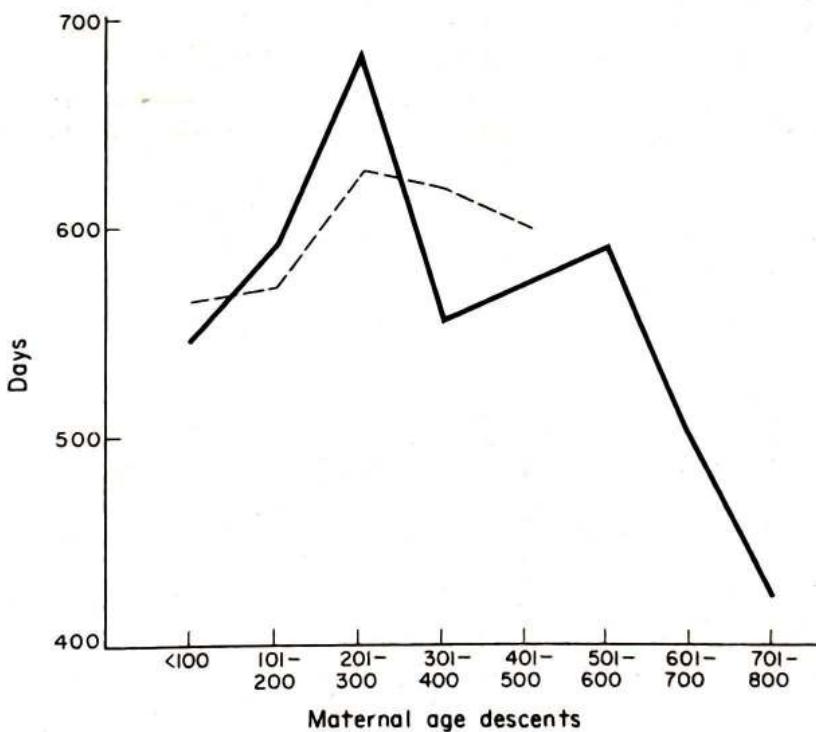


CHART 10. This chart (a combination of Charts 8 and 9) shows data on both the age of onset of mammary gland tumors (dash line) and length of life free of tumors (solid line) in the eight maternal age classes.

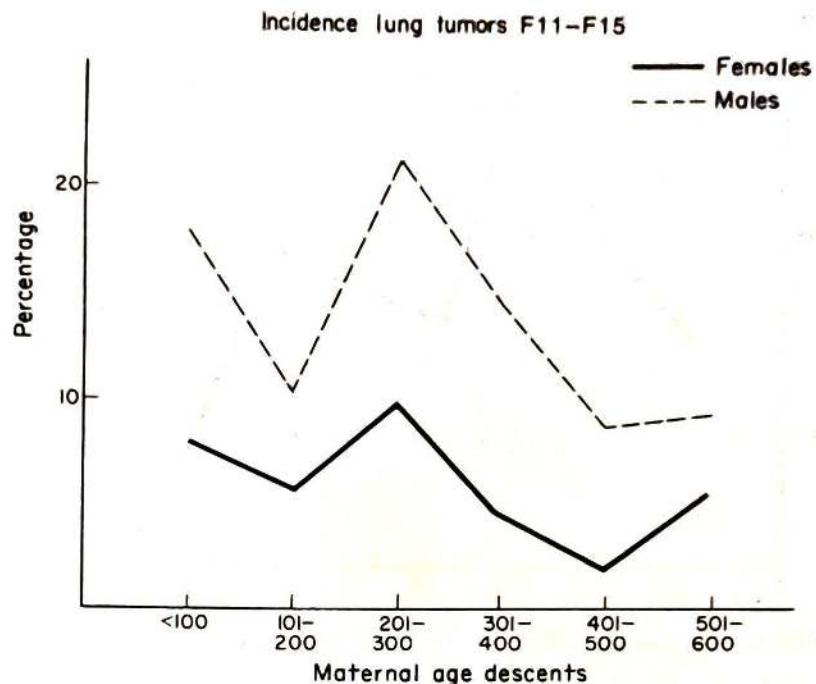


CHART 11. This chart shows the incidence of spontaneous lung tumors in mice of the F₁₁-F₁₅ generations of inbreeding for six maternal age descents. The percentages of mice developing tumors are given on the vertical line; the separate descents on the base line. Data for female mice are on the solid line; data for males are on the broken line.

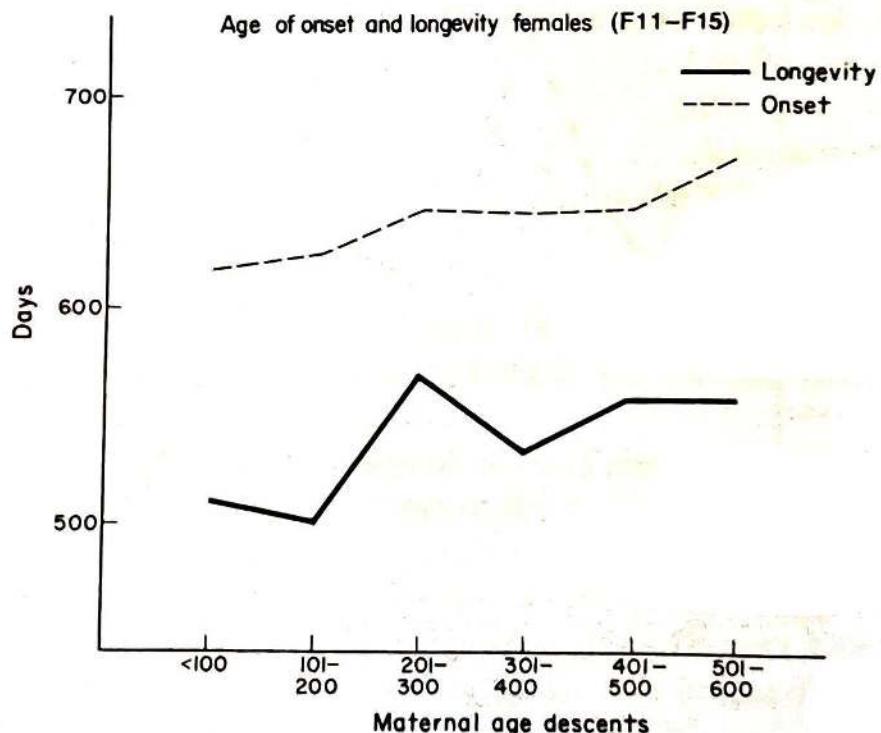


CHART 12. This chart presents data on (1) longevity of females between F₁₁-F₁₅ (solid line) and (2) on age of onset of spontaneous lung tumors (dash line). Age is expressed on the vertical line and maternal age classes on the base line.

Chart 11 shows data on the percentage incidence of lung tumors in both males and females of six maternal age descents in generations F_{11} - F_{15} . It can be seen that males developed more lung tumors than females and that there was a decline in incidence with advancing age of mothers.

Chart 12 shows the age of onset of lung tumors and the measure of longevity in those mice dying free of tumors for the same six maternal age descents between F_{11} - F_{15} . Here the picture is quite similar to the situation with mammary gland tumors—mice of the 201–300 maternal age descent show lung tumors later in life than in the offspring born to young mothers.

Chart 13 shows the percentage incidence of lung tumors in both males and females during the process of inbreeding for the earliest maternal age descent. Lung tumors completely disappear after forty-five generations of selection toward young mothers—

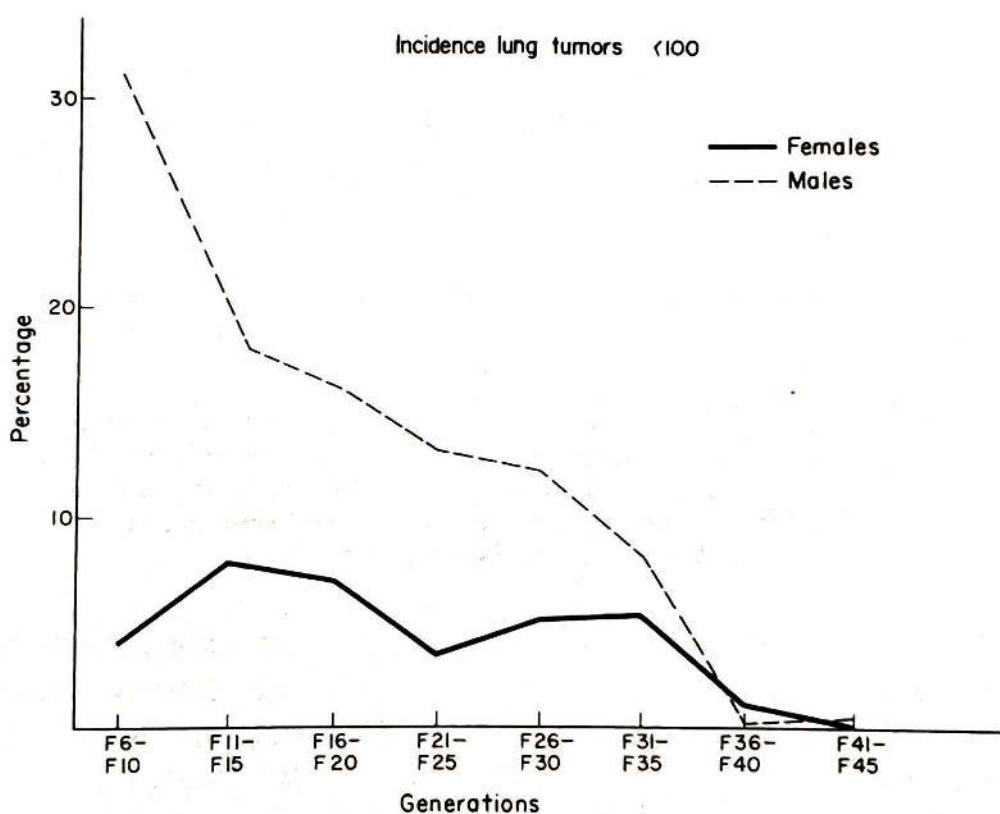


CHART 13. This chart shows the percentage incidence of lung tumors between F_1 and F_{45} of the < 100-day maternal age descent. Percentage incidence of lung tumors is on the vertical line and generations of inbreeding on the base line. Data for females are on the solid line and that for males on the broken line.

and these mice never smoked cigarettes, even when the incidence of lung tumors was high!

Chart 14 shows the comparative data on age of onset of lung tumors and longevity for female mice of the early maternal age descent through forty-five generations of selection toward young mothers. Lung tumors appeared earlier and earlier between F_6 and F_{20} and then came somewhat later and later in life with continued inbreeding.

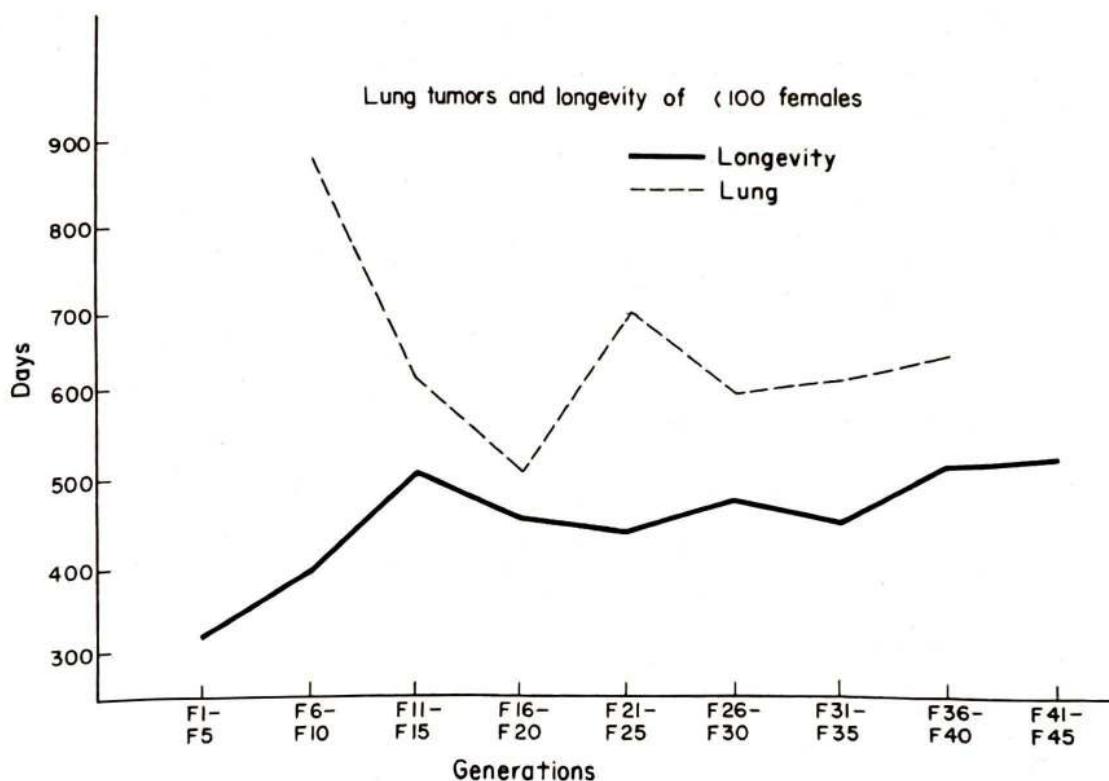


CHART 14. This chart presents data on (1) longevity (solid line) and (2) age of onset of lung tumors (dash line) in female mice of the <100-day maternal age descent during the process of inbreeding between F_1 and F_{45} . Age in days is on the vertical line and generations of inbreeding on the base line in groups of five.

Chart 15 shows similar comparative data on lung tumors and longevity for male mice of the early maternal age descent through forty-five generations of selection toward young mothers. It can be seen that the onset of lung tumors and longevity are both being increased by this selection of young mothers through the process of inbreeding.

Chart 16 shows another facet of this selective process. Here we are dealing with the chemically induced tumors but the same principle also applies to the spontaneous tumors.

It can be seen that even though the different specific types of tumors differ in frequency in different families, the sum total of all tumors remains at about the same level. This is a part of cancer research that needs further study.

But perhaps we have presented enough data for some significant conclusions.

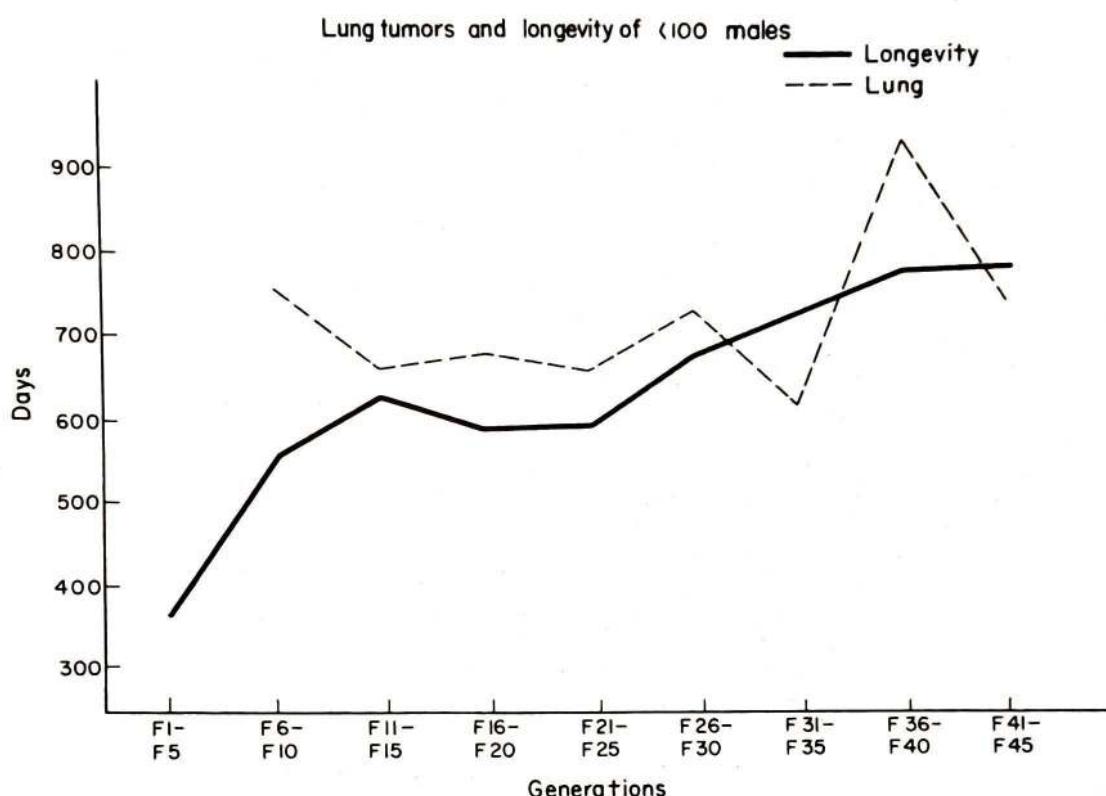


CHART 15. This chart is similar to Chart 14 except that data on males only are presented. Longevity on the solid line and lung tumors on the dash line.

The origin of cancer and the aging process are intimately associated together. Both mammary gland and lung tumors are less frequent and occur later in life in the offspring of the 201-300 maternal age class. Mammary gland tumors appear to be more frequent in the descendants of young mothers and lung tumors in the descendants selected toward young mothers. It appears that the offspring of young mothers and the offspring of old mothers are more variable and hence in smaller degrees of biological equilibrium than the offspring of mothers in the "prime of life", i.e. in the 201-300 maternal age class, and hence the conclusion cited earlier may be at least a working hypothesis that the onset of

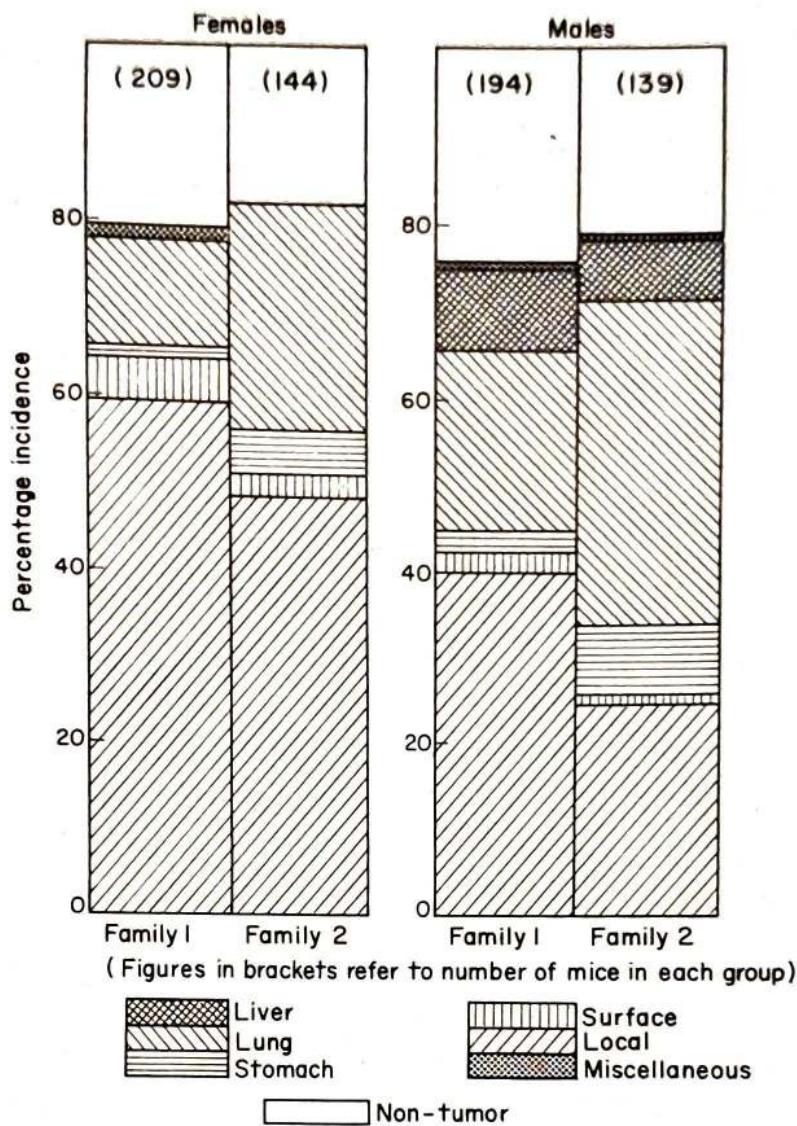


CHART 16. This chart presents data on the percentage incidence of methylcholanthrene induced tumors in males and females of two independent descents of mice, that had had a common origin.

cancer and the aging process are both the resultant of disturbances in biological equilibrium.

We have talked almost exclusively on the lowly mouse. Perhaps a few references to man may not be out of place although no application of the conclusions obtained in mice will be attempted for humans.

Chart 17 shows that the size of the human family is gradually getting smaller and smaller between 1890 and 1950.

Chart 18 shows that longevity is increasing in man between 1600 and 1950.

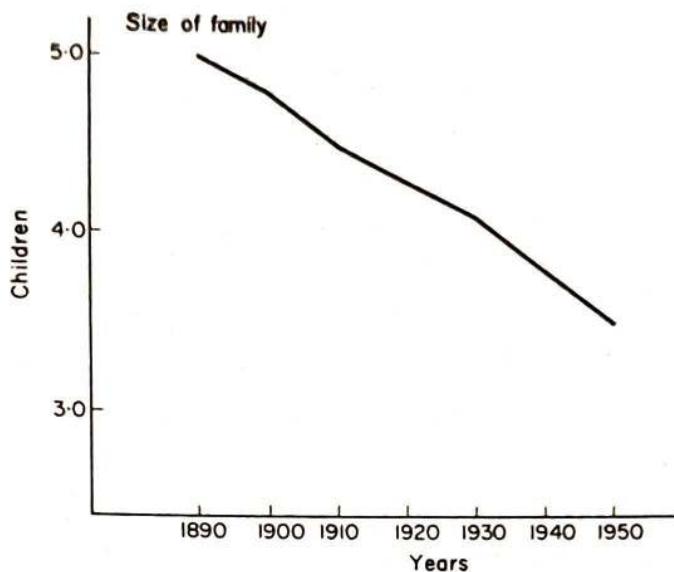


CHART 17. This chart presents data on the decline of the average human family size between 1890 and 1950. Number of children in a family is on the vertical line; time on the base line.

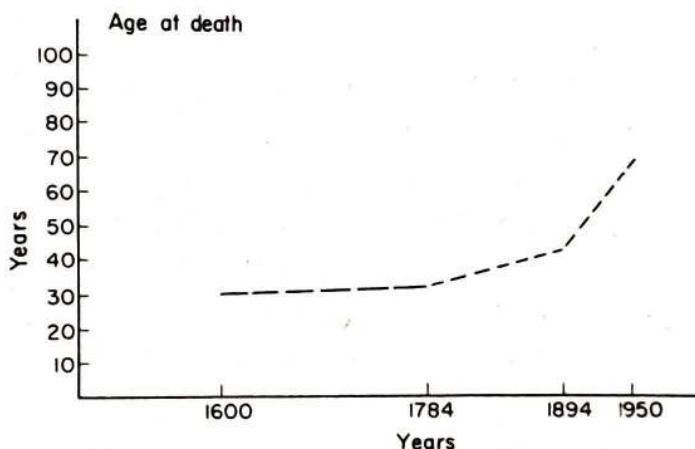


CHART 18. This chart presents the data on the average expectancy of life for humans between 1600 and 1950.

Chart 19 shows two trends in a single family (size of family and longevity). The chart shows ten generations of direct descent in America. The individuals involved were born in the years indicated on the baseline. The original immigrant came to America in 1631 (born 1605), had nineteen children and lived to be 94. The successive generations had fewer and fewer offspring and lived shorter and shorter lengths of time until a plateau of 77 was reached.

CHAPTER 2

Why Did You Originate the Inbred Strains of Mice?*

WHEN I was given this personalized title as to why I had originated the inbred mouse, I first thought it would be a timely topic for a TV script, "This is your life". However, with only a little consideration, it must be concluded that this task was not my full life—but only a part. It was early realized that the mouse, at least, and perhaps other animals as well, should be inbred in order to produce scientific material upon which reproducible results on cancer research, and later on gerontology, could be more profitably pursued. The original plan has fully justified the enormous labor involved.

When I became a graduate student at Columbia University under the sponsorship of Nobel prize winner Professor T. H. Morgan, I carried, particularly, two significant ideas with me from college. The first one was that I had acquired a keen interest in the new emerging science of genetics, and the second was that several individuals of my family (including my father) had developed cancer. It was only natural that I should try to see whether there were any bonds of relationship between these two sets of phenomena.

Soon after residence at Columbia I started to read the literature on cancer research. I was amazed at what depths of despair the cancer investigators had reached following World War I. Reproducible results in such a field as cancer transplantation and immunology in experimental animals were quite unknown. In fact, an investigator sometimes could not even verify his own observations, let alone having another scientist in another laboratory and even in another country do so.

The first indication of what perhaps was lacking in this mentally depressing atmosphere of cancer research was obtained by

*A lecture given at the Animal Care Panel Meeting at The Salk Institute for Biological Studies, La Jolla, California, October 1965.

the teachings of Professor Morgan. He it was, among others, who was applying the principles of quantitative research to biology through the investigation of genetics (Morgan, 1935). These techniques of quantitation had been applied to the mathematical sciences of chemistry and physics sometime previously.

A survey of suitable experimental animals was then made to investigate the problem of experimental cancer and it soon became obvious that there were no animals available that were in such a homozygous or uniform genetic state that reproducible results would be expected—providing, of course, that the genetic constitution of the individual which controls biological variability to a very great extent had anything to do with the characteristics of cancer. One strain of mice which had a history prior to World War I was completely wiped out by an epidemic of paratyphoid at Cold Spring Harbor, Long Island, during my first year there.

Before entering, therefore, into the practical development of inbred or highly homozygous mice, I would like to briefly indicate the tools of investigation that were then available to a geneticist. The tools were hybridization and segregation with or without selection. The laws of inbreeding were fairly well established and presented in *Inbreeding and Outbreeding* by East and Jones (1919). The principle of Johannsen (1909) "that selection within a pure line is ineffectual" (in changing a genetic character) was new and was then being considered as sound.

Between 1918 and the present, a span of some 47 years, I have applied these genetic principles to the common house mouse (*Mus musculus*) three times. The first time was in 1918 in order, primarily, to determine "The Genetic Factors Underlying Susceptibility to Transplantable Tumors" (Strong, 1922). These data were published as a Ph.D. thesis for Columbia University. The descendants of these mice gave rise to the well-known so-called C family. Following hybridization between mice of two partially inbred strains, the descent was divided into several sublines, basing selection only on the degree of susceptibility or resistance to spontaneous tumors of mammary gland origin.

The original F₁ No. S79 was mated to her own brother No. S77 and then to her ensuing son No. S352. The C₃H/St was selected for the earliest appearance of spontaneous tumors and the CBA/St for resistance to such tumors, i.e. a selection toward long life free of any spontaneous tumors. The other C₁₂I/St and CHI/St

sublines showed intermediate degrees of susceptibility to mammary gland tumors. Thus the sublines, all derived from a common genetic origin, could be classified in sequence according to their degree of susceptibility to spontaneous tumors, into the C₃H/St, C/St, C₁₂I/St, CHI/St and CBA/St. These mice, together with their ancestral parental lines A/St and D/St (now inbred), have served the cause of cancer and other biological research to no end and will probably be the source of much research for many years to come.

It would be impossible to enumerate all the contributions to science for which these mice have been partially responsible. I shall enumerate only a few.

Following the completion of the Ph.D. thesis referred to previously, it soon became evident that female mice of the A/St strain developed a very high incidence of spontaneous mammary gland carcinomas. This high incidence of spontaneous tumors continued to appear through the process of inbreeding in mice with a high degree of homozygosity expected by the laws of inbreeding. Divergent selection was then practiced (1) toward the earliest appearance of such neoplasias (A/St) and (2) away from spontaneous tumors by a selection toward resistance to such tumors, i.e. toward long life free of tumors (A₂/St). After several generations of divergent selection of spontaneous carcinoma of mammary gland origin, it was determined that the three frequency distributions for cancer of the mammary gland were identical (Strong, 1940a). The original A/St and the two divergent sublines A/St and A₂/St had the same susceptibility to spontaneous cancer. In other words, Johannsen's (1909) principle that "selection within a pure line is ineffectual" applied to the origin of cancer in experimental animals as well as already established genetic characters, such as the size of castor beans. This finding meant to a geneticist that the genetic constitution of the host was involved in the origin of cancer under carefully controlled experimental conditions.

At the time of the application of Johannsen's principle to cancer research reproducible results could, therefore, be obtained and thus the A/St was made available to other scientists. The various sublines were also made available to all seekers of truth.

A new era of cancer research had been created.

This uniform genetic constitutional state in reference to the origin of cancer has never been changed or altered, providing,

of course, that no new hybridization experiment be introduced. This conclusion has been emphasized by the discovery of Bittner's milk agent or virus on the appearance of mammary gland cancer in mice (Bittner, 1933, 1936).

Following the discovery of the milk factor, as will be discussed in more detail later, it was assumed that "cancer susceptibility" had been changed. However, what was actually found was that an agent transmitted by the mother's milk conditioned a mouse to give rise to cancer of the mammary gland in a carefully determined mouse only with a definite genetic constitution—when the milk factor was restored to the genetically determined susceptible mouse the same degree of susceptibility of spontaneous cancer returned.

These and other observations indicated quite convincingly that genetic factors or genes were involved in the origin of spontaneous cancer in mice.

The same milk factor when introduced into a genetically resistant mouse disappears after several generations.

The C₃H/St and its derived subline C₃HB/St which had been given to John J. Bittner, a graduate student of mine, in 1928, served as the source for the discovery of the so-called milk factor or inciter or Bittner's virus in relation to spontaneous tumors of mammary gland origin. Just when this important discovery could have been made without the availability of suitable controlled animals, produced by the principles of genetics, if ever, is open to discussion. Frankly this discussion belongs to philosophy. The fact does remain that the genetically controlled experimental animal did make this great discovery possible. Even the historical development of the work on Bittner's virus had important genetic implications since its discovery was dependent upon previous research that demonstrated there was a maternal influence on the transmission of susceptibility to mammary gland tumors in mice, as reported by Green (1931), another graduate student of mine.

Again the availability of genetically controlled mice made the analysis of the transplantable tumor possible. Definite genes were shown to be the determiners of cancer susceptibility and cancer resistance by the discovery of segregation and linkage or by the same technique by which morphological and physiological characters were being analyzed in *Drosophila*, maize and other

species (Blakeslee, 1954). This discovery that specific genes were responsible for the successful grafting of tumors in mice was the foundation-stone upon which the field of histocompatible genes have been so profitably pursued.

Another discovery of the nature of tumors arising in F_1 hybrids should be indicated in this present discussion. It was found by Strong and his two graduate students, Bittner and Cloudman, that the genes responsible for the growth of a transplantable tumor derived from an F_1 hybrid were partly derived from one parental or ancestral stock and some from the other. In other words, the F_1 individual is a mosaic of potentialities derived from parents of both ancestral stocks but, what is more important, is that the tumors that arise in an F_1 individual are also of a mosaic nature which are determined by genes (Strong, 1929, 1946; Bittner, 1931; Cloudman, 1932a). Here again is convincing evidence that genes were involved in the determination of cancer characteristics.

Due to time alone, it will be impossible to discuss here, at this appropriate place, the further analysis of the phenomena of transplantation of neoplastic conditions in mice and the sudden appearance of changes (somatic mutations?) in the transplantability of tumors. These were the primary observations of the present author and for which the original inbred strains of mice were developed. These significant genetic observations on tumors were verified by his two graduate students, Bittner (1931) and Cloudman (1932a), and have been verified more recently by many investigators (Strong, 1951a).

At the same time that the above genetic approach to cancer research was being developed, the field of chemical carcinogenesis was expanding, particularly by the stimulus supplied by the two Japanese researchers, Yamagiwa and Itchikawa (1915), with coal-tar paintings in rabbits and by a group of English chemists headed primarily by Sir Ernest Kennaway (1924a, b) on the fractionation of coal-tar products. It was soon found that a great number of pure chemicals could be shown to be carcinogenic when injected or applied to a suitable experimental animal. The opinion was commonly expressed that a "chemist could produce cancer at will in practically every animal used in an experiment". Limitations to this approach when used exclusively were soon apparent.

It became necessary, therefore, for me as a geneticist to accept this challenge as to the nature of the origin of cancer.

The second approach to the preparation of suitable experimental animals to investigate the possible relationship of chemistry to genetics in cancer research was instigated in 1938.

Here the same techniques of hybridization, segregation and selection were employed as were done in the early experiment of 1918 on the incidence of spontaneous tumors. The selective force now used was the injection of a very large dose of one of the most powerful carcinogens, 1 mg of 20-methylcholanthrene dissolved in 0.1 cm³ of sesame oil at a very critical or susceptible period of life (60.0 ± 4.0 days) and the retention of the most resistant pair of mice in each generation as the parents for the production of the succeeding generation. To visualize the experimental approach one must keep in mind four items to which a fifth was later added. These were: (1) increased variability of individuals through the process of hybridization, (2) the administration of a very large dose of a chemical already known to have an effect on many biological characteristics, (3) the retention for breeding purposes of some animals being exposed to an insult of injection of a powerful carcinogen over many weeks and even months, and (4) the population was undergoing reduced variability through the process of inbreeding. Following this procedure it was deemed wise to continue the experiment into (5) the continuation of the experimental procedure of injecting methylcholanthrene in the experimental mice after a high degree of homozygosity of genetic determination had been reached. Whether the carcinogen is undergoing a series of metabolic changes during this long time within the animal body should be of additional interest, but except for one series of observations near the end of the experimental approach does not interfere with the analysis of the unexpected results obtained. These observations were on the incidence of chemically induced cancers and the modification of many other biological characteristics (Strong, 1940a).

The late appearing phenomenon that may have been due to an altered metabolite of the injected 20-methylcholanthrene several months previously were on the production of several clear-cut germinal mutations.

In the early hybrid generations following a suitable outcross,

only spindle cell sarcomas were obtained at the site of injection of 20-methylcholanthrene. This is what one would expect from the literature. However, it was soon found that with only a small amount of selection of partially resistant mice, their offspring developed fewer spindle cell sarcomas and rather developed squamous cell carcinomas of the skin and rhabdomyosarcomas of muscle origin, each with a different definite frequency distribution (Strong, 1941a). With further selection toward resistance to chemically induced tumors these three types of locally appearing tumors (at the site of the injected carcinogen) no longer appeared. Then tumors began to arise all over the surface of the body, such as in the mouth and lips, on the eyes, ears, anus, genitalia, etc. (Williams and Strong, 1944). With further selection toward longevity the appearance of these "surface spread tumors" was suppressed and internal ones then appeared, first in the lungs and then in the stomach and in the liver and finally in the ovary (Strong, 1944a, 1945b, 1945c, 1946e). With further selection toward resistance to chemically induced tumors, no tumors arose in spite of the fact that an overwhelming dose of a very powerful carcinogen had been injected at a susceptible period of a mouse's life (Strong, 1948a). Normally this discovery of a resistant-to-cancer state should have satisfied a geneticist. I had taken mice and by the application of genetic principles developed a resistant-state-to-cancer by using the most powerful tool the chemists had been able to develop at that time. Surely this resistant-to-cancer state should provide material for the analysis of the mechanism of resistance that these mice obviously possessed.

But I did not stop at this time. With continued selection toward more resistance following the production of this cancer-resistant state, cancer of the various specific types came earlier and earlier and finally I had a colony of mice that were more susceptible to locally appearing tumors than they were in the early hybrid generations (1948a). These highly selected mice even developed a high incidence of mammary gland tumors, which were not evident in the early hybrid generations of this outcross.

Now, if the first part of this selective experiment was genetic, and it very well may be since variations were taking place in the direction of selection, or when many heterozygous pairs of genes were theoretically becoming homozygous, what then was the nature of biological change in cancer in the second series where

variations of tumor types were taking place counter to the trend of genetic selection? I believe these results may also be genetic. One of these tumors (one involving the stomach) became hereditarily established by a mutation on the "B" tagged chromosome following its first appearance with the injection of methylcholanthrene (Strong, 1944b, 1947a, 1947b, 1949a). These facts have been established for the production of a large number of neoplastic conditions by carefully controlled quantitative methods, but the critical analysis of the phenomena has never been completed.

One other series of genetic phenomena occurred in mice in the midpoint of the selection experiment toward resistance to chemically induced tumors that should be of extreme genetic interest but unfortunately, as far as I know, has never been duplicated. This was the appearance of many germinal mutations in the descendants of mice that had been injected with 20-methylcholanthrene for a long enough period for them to have developed internal tumors, particularly, of ovarian origin (1949b). This discovery brings the origin of cancer and the production of germinal mutations by the same inducing agent very close together (Strong, 1949c). If these are valid observations, they will be verified; not by a cursory observation but by an investigator who will take the time and patience to repeat the above selection experiment as outlined here in detail.

But quite aside from the scientific contributions that the originator of this concept of the origin of inbred mice has been able to make, the animals thus produced are available to other investigators for their particular needs. Not all of these strains with peculiar and unique biological characteristics are available now. Without federal or other funds for the protection of scientific material for future science, these strains could not all be continued. Survival of these has always been too much on a personal basis. They still are.

I cannot leave this second attempt to produce a variety of inbred strains of mice that had had a common origin without indicating a conviction that has developed over the years and which will be emphasized more emphatically at the conclusion of the ensuing third extensive experiment of hybridization, segregation and selection.

This is that the biological (or genetic) response to an environmental or external influence (or insult) is quite different on

heterozygous mice following the hybridization of two well-established strains of mice than it is when the individuals have reached a high degree of homozygosity. Whether this difference is quantitative or in fact even qualitative can only be determined by considerable more research than I have been able to spend on it. I shall continue, however, to report facts, as I discover them.

But before I can outline the third attempt to develop a variety of sublines that have had a common genetic origin, I should like to discuss briefly some commonly accepted ideas on genetic phenomena. One of the foundation-stones of genetic segregation is that it occurs at random. Mendelian analysis is based upon this concept. Now as a corollary to this concept, it may be stated that if segregation is not random, then there must be some secondary or inciting reason for variations to be occurring.

A series of A B C D E and F or 1 2 3 4 5 and 6 are not random (Charts 20 and 21).

A series of descending or ascending orders such as A B C D E and F again are not random. And even if the values change, such as increasing between A B and C and then decreasing between C D E and F, the distribution is also not random. A series of A C E B D and F or one of 1 3 4 2 5 and 6 are random; and is what would be expected if genetic segregation alone is responsible for the measuring of a biological character through the process of inbreeding. Consequently, even if a series of sublines had a common genetic origin one would not expect, by segregation of genetic determiners alone, to have the variation being measured have a linear arrangement or sequence, in the separately segregating sublines. When this does occur then one should look into the possibility of another mechanism outside of an exclusive segregating or genetic one as the cause of biological variation.

The third attempt to produce a series of genetically related sublines was as follows: a male mouse of the Brpb/St subline with polydactylia derived from the second series of inbreeding (i.e. the second series exposed to 20-methylcholanthrene being the selective force) was outcrossed to female mice of the C₅₇/St pure strain.

Separation of the various sublines was based upon maternal age selection, beginning in the F₁ generation.

This outcross has given rise to seven fundamental or separate descents. Selection was continued, as stated previously, on

CHART 20

NOT RANDOM

A B C D E and F or 1 2 3 4 5 and 6

$A < B < C < D < E$ and $< F$ or $1 < 2 < 3 < 4 < 5$ and < 6

$A > B > C > D > E$ and $> F$ or $1 > 2 > 3 > 4 > 5$ and > 6

$A < B < C > D > E$ and $> F$ or $1 < 2 < C > D > E$ and $> F$

RANDOM

A C E B D and F or 1 3 4 2 5 and 6

CHART 21

NOT RANDOM

A B C D E and F, etc, or 1 2 3 4 5 and 6, etc

etc. 1

or

etc.

D

B C E
B A E

3 5

26,

RANDOM

A C E B D and E etc. 1 3 4 2 5 and 6 etc.

CHARTS 20 and 21. These two charts illustrate series of variables or characters that are either random or not random in order to discuss the implications involved in mendelian segregation which occurs at random.

maternal age or age of mother at which time she had had a litter. These sublines are designated as: < 100 maternal age, 101-200, 201-300, 301-400, 401-500, 501-600 and 601-700. Several additional sublines have been derived from one or the other of these primary sublines, for various reasons.

The discussion of the many biological observations that have been investigated in this single but elaborate experiment has occupied me for several years—and the end is not in sight. For those interested a preliminary monograph in the form of a progress report has been prepared, and numerous papers published.

Here, we are obtaining evidence that biological characteristics, such as age of first litter, reproductive capacity, fertility, fecundity, longevity and incidence of spontaneous tumors are not randomly segregating out in the separate sublines. Many of these biological characteristics show a linear sequence associated with increasing maternal age.

I can only indicate a few fundamental conclusions derived from this third series of hybrid mice undergoing the process of inbreeding.

1. There appear to be at least three forces in determining the biological characteristics being measured in this selection experiment. These are (a) the segregation of an unknown number of unidentified genes, (b) the presence or absence of at least one specific gene, *lst* and its normal allele *LST*, and (c) maternal age or age of mother at the time she has had her offspring.

2. Some of the various sublines appear to be in biological equilibrium and any selection away from this norm of the subline cannot, therefore, be continuous. Eventually a compensatory change takes place which brings the subline again to another equilibrial state.

3. The development of the equilibrial state in the various sublines does not take place at the same rate in the various sublines but is somehow or other associated with maternal age.

4. Even after fifty-five generations of inbreeding of brother-to-sister matings, the early maternal age descent (<100 maternal age descent) is not in equilibrium as far as age of first litters, reproductive capacity as measured by litter frequency and other characteristics are concerned (Chart 22).

5. The 201–300 maternal age descent is in equilibrium even after twenty generations of inbreeding.

6. Thus it can be concluded that the descent between 201–300 maternal age descent is in equilibrium but the descents with earlier maternal age and perhaps later than 201–300 maternal age are not. (Definite conclusions on later than 201–300 maternal

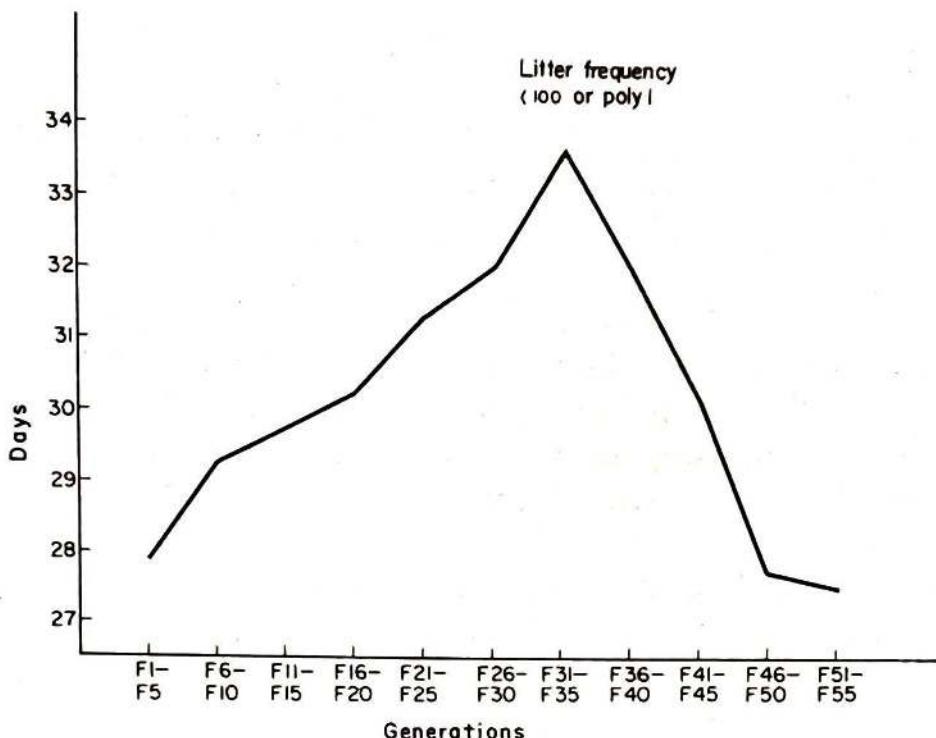


CHART 22. This chart presents the average litter frequency during the process of inbreeding in an early maternal age descent (< 100 days). Litter frequency in days is given on the vertical line and generations of inbreeding are given on the base line.

age descents are provisional due to the few generations of inbreeding to which these mice have been subjected.)

7. Spontaneous cancer (particularly of mammary gland origin) is associated with a constitutional state of the host which is not in equilibrium, i.e. unstable.

8. Longevity of offspring is also influenced by stability or instability since offspring of the 201-300 maternal age descent have fewer tumors than the early maternal age descent and when they do, have them at more advanced ages. The 201-300 descent mice also live longer than mice of the other maternal descents.

9. The polydactylia gene, in addition to having pleomorphic effects on several morphological characteristics such as luxoid appendages, alopecia, blindness, etc., also has an effect on what could be called biological stimulation, or increasing the "tempo" of biological activity. Its normal allele (LST) reduces biological variations including the appearance of spontaneous tumors.

These are only a few of the fundamental conclusions derived from the genetic techniques of hybridization, segregation and selection toward or away from an inciting influence.

In the first case the selection was toward or away from the incidence of spontaneous tumors of mammary gland origin. In the second, the inciting influence was the presence of a powerful carcinogen, 20-methylcholanthrene. And in the third, they were maternal age and the presence of the polydactylyia gene (lst).

In closing I would like to emphasize the purposes pursued for the development of genetically controlled inbred mice.

In the first place, this approach to scientific research was called for by the principles of quantitation in biology.

In the second place, the approach was needed by which a geneticist working in the field of Mammalia could make contributions in his chosen field.

In the third place, the availability of inbred mice has made possible innumerable contributions to science by investigators around the world.

In the fourth place, they will serve as scientific material for the indefinite future.

The end has fully justified the means.

In the Nobel prize lecture of 1934, Professor T. H. Morgan stated: "The most important contribution for medicine that genetics has made, in my opinion is intellectual."

CHAPTER 3

Genetics in Relation to Cancer Research and Gerontology*

MADAM PRESIDENT, Mr. Chairman, members, guests and friends of the Women's Auxiliary of The Salk Institute for Biological Studies and The American Association of University Women, it is a signal honor and pleasure to meet with you this evening and to discuss some aspects of genetics.

With the founding of The Salk Institute for Biological Studies and the other centers of higher learning in San Diego, the citizens of this community, particularly, had the opportunity of hearing about some of the wonderful contributions that were being made in the science of molecular biology, which was formed by combining the science of genetics to those of biochemistry and physics. I nearly mentioned mathematics in this welding or wedding of scientific interests but this is not exactly so since genetics, from the days of Gregor Mendel, has always been a mathematical science.

Restricting comments pertinent to the fields of cancer research and of gerontology, it may be said that there have been three major contributions from the science of genetics that have had a tremendous bearing on the status of present research. The first of these contributions was the production of highly inbred experimental animals, particularly mice, where biological variability had been reduced to a minimum by a system of restricted inbreeding thus leading to the production of reproducible results; that is, the introduction of the principles of quantitative research where all the variables are recognized and kept as nearly constant as possible. This procedure laid the foundation upon which many observations were made and conclusions were reached upon which continued progress was possible. The second contribution of genetics was the production and control of biological states that

*A lecture presented to The Women's Auxiliary of The Salk Institute for Biological Studies, 28 February 1966.

differed in cancer susceptibility and cancer resistance. This contribution made possible the discovery that the female sex hormone, estrogen, was involved in the origin of several kinds of cancer in mice. For example, in cancer-susceptible strains such as the A/St and C₃H/St, cancer of mammary origin occurs only in the female; never in the normal male (Strong, 1935, 1937, 1938). But when the female sex hormone is injected into males, then they also will develop cancer of mammary gland origin. Again, the use of genetically controlled strains of mice made possible the discovery of the Bittner (1933) virus in the origin of mammary gland cancer. This discovery was made by J. J. Bittner, a graduate student of mine from the University of Michigan, also a brother to my wife.

To enumerate all the discoveries made possible by the use of these carefully controlled experimental animals would consume much more time than is allotted here.

The third contribution of classical genetics to the cancer problem was that the use of these animals made possible the pointing of science to the gene within the nucleus of the cell as being involved in the mechanism of the origin of cancer. The principles involved here were similar to the ones used in the determination of the architecture of the germ plasm through the phenomenon of free assortment and linkage—or those principles which were the foundation-stones of classical genetics. It is this third aspect of genetics that has been so extensively and fruitfully developed into molecular biology, particularly in reference to DNA as the biochemical unit that makes up the gene. But the evidence for the incrimination of the gene in the origin of cancer is just as convincing from classical genetics as it is from modern molecular biology.

Now there is a new set of phenomena that is unfolding as to the nature of the biological mechanism involved in the origin of cancer. This is biological stability (and instability). I hope that I do not become too technical in the discussion of this very important subject.

The first contribution of classical genetics, as mentioned previously, was the establishment of uniform strains of mice with well-established uniform biological states. This was accomplished through the process of restricted inbreeding, usually by brother-to-sister matings over many generations.

Recently, two new forces have been introduced into this genetic work on cancer. These are (1) maternal age selection and (2) the investigation of a pleomorphic gene, known as LST.

1. Several sublines from a common genetic origin have been continued by selecting the offspring born to mothers and fathers at a given chronological age. These sublines now differ from each other in many characteristics. Cancer susceptibility appears to be correlated with maternal age. For example, in order to develop a strain that will develop a high incidence of mammary gland cancer one needs to select a descent from a mother when she is less than 100 days old at the time her offspring are born (Johnson and Strong, 1966). Again, a selection toward early mothers over a number of generations of inbreeding eliminated cancer of the lung in the offspring (Johnson and Strong, 1966). And these mice did not become resistant to lung cancer by stopping cigarette smoking—they never smoked, even when they had a high incidence of lung cancer!

One of the most interesting facts that is coming out of this present experiment is that there is continued biological variation or instability of several characters when there is a high susceptibility to cancer—and it may very well be true that this unstable biological state may be responsible to the increased hazards of developing cancer.

2. The second new observation is that cancer susceptibility or resistance is somehow or other dependent upon the presence or absence of the LST gene. In very many biological characteristics so far mentioned this gene seems to be involved. A conversion of LST to lst, a procedure that appears to be simple by genetic manipulation of suitable matings by selection, always leads to a lower threshold of biological activity. For example, mice with lst have their first litters fully 31 days later than their close relations bearing LST (110.6 days compared to 78.8 days) (Chapter 4). Litter size is reduced in mice bearing lst and the mice bearing lst actually live longer than mice with LST as indicated in Chart 23. In spite of the fact that these mice bearing lst live longer—they actually have fewer cancers of mammary gland origin (42.4 percent penetrance, compared to 6.2 percent). This difference is 26.2 times the probable error which is, of course, a very highly significant finding.

In a short time, there has been presented evidence that gene-

ticists working alone or working in collaboration with other scientists in other branches of science have indicated many forces bearing on the mechanism of the origin of cancer. Emphasis on one aspect of the problem or another differs among scientists themselves but to a geneticist, trained in the classical genetic schools of Morgan and of Castle, the problem of cancer may be summarized as follows:

Cancer is the resultant of a particular genetic state at a particular chronological period of time in the life of an individual. Extrinsic forces such as viruses and intrinsic forces such as hormones may play a very significant initiating role—but in the last analysis it is the cell, controlled to a great extent by the genes it contains, that must be involved either directly or indirectly to carry the neoplastic state through succeeding cell divisions. This scientific genetic state is influenced tremendously, if not exclusively, by the activity of specific genes. Such a one is the pleomorphic LST. Biological stability and especially its counterpart, instability is of paramount importance in the origin of cancer. There is some evidence that LST may be involved in the "tempo" of biological change and hence determine instability (Chapter 4). There may, however, be other specific genes that are not yet recognized.

To me, who has spent so many years in cancer research, the actual problem of the origin of cancer is just being determined. The solution appears imminent.

Just a few more comments on cancer may not be out of place. Where is this concept of the origin of cancer leading? The answer may not be too far off. For one thing, I would like to make the statement that I have seen so many mice with cancer that I appreciate one more that does not develop cancer. I believe that the reason why a mouse does not develop cancer is the possibility that it may have a mechanism of resistance to cancer. Somewhere in its genetic makeup or in its physiological or biochemical entity wholly or partially under gene control that resistant mechanism is there—and someday someone is going to find it. And when they do—they will have the controlling mechanism of cancer. Unfortunately, all too little work is being done on this negative aspect of cancer research.

But time is moving on and I must get to gerontology.

The first point to be raised is, why are cancer and growing old discussed together—even through a common bond of genetics?

The answer is simple. If one starts out with a group of experimental mice—some will develop spontaneous cancer. Their study is cancer research. The remainder, and they may be brothers or sisters of the cancer probands, do not develop cancer but die eventually either naturally in old age or otherwise. Their study is gerontology. Added to the problem of death studies on the aging process or senescence are also included in gerontology.

But to orient the problems of gerontology with possible genetic implications more precisely, several questions may be asked. Do women have children too early—or too late? Do they have children too frequently? Do women have children long after they should be doing something else? These are old sociological problems. Their investigation is usually neglected or ignored, sometimes being set aside with quasi or pseudo so-called explanations. Even a too restricted concept of genetics with its extreme view that most, or if not all, biological characteristics are genetically determined, evades the issue since the distribution of genetic determiners through cell division or reproduction is controlled by segregation and recombination and these genetic phenomena are not supposed to be influenced by the aging process. That one genetic phenomenon, mutation, increases with age, as was shown by Blakeslee (1954) in the Jimson weed, has had little effect upon the problem now before us.

As just formulated and presented these questions on behavior apply to the human subject. But I am a mouse doctor and I propose now to investigate these age changes and characteristics of the offspring in one species only, the common house mouse. I shall use maternal age analysis only, rather than parental, since the survey of literature discloses the need of this emphasis. It is the aging mother rather than the aging father that may influence significant characteristics of the offspring. At the time this survey of the literature on parental age was made for a symposium at the New York Academy of Sciences in 1954 (Strong, Editor) the problem of aging with implications to genetics was oriented but the data already obtained by several investigators appeared to be meager.

Whether these findings on the mouse can be applied to man or any other species remains to be seen.

Instead of investigating different biological characteristics, such as age of first litters, reproductive capacity as measured by

litter size, litter frequency, fertility and fecundity, longevity and incidence of spontaneous cancers in separate experiments in separate laboratories as is being done all over the world, I considered the possibility of investigating as many characteristics as possible in one group of mice in one laboratory.

The method of obtaining the data in the present experiment is quite different from those used in the scattered randomly obtained data referred to previously—and in fact, to that being continually produced by man himself. Man sings the popular tune of "What come naturally" glibly but scientifically does little about it. In our laboratory we let nature take its course and try to measure the results. A female with its own brother is born in the same litter. At weaning time brothers and sisters are put into separate breeding boxes and kept continually together until one of them dies. I must confess, however, that I do not know whether mice have any concepts of mores or other sociological habits or not but certainly we are measuring untrammeled nature itself.

The individual approach to analysis of the data obtained is being aided by suitable statistical methods but leaves something unresolved. The present resolution of the multicharacteristic complex obtained on mice has produced so many data that they cannot be completely analyzed until further facilities of a computer system have been made available. The original concept of doing the work in one laboratory has also had to be changed. The work was started in 1949 at The Yale University School of Medicine, New Haven, Connecticut. Then in 1953 the program was transferred to Roswell Park Memorial Institute, Buffalo, New York and finally in 1964 to The Salk Institute for Biological Studies, San Diego, with temporary facilities in Del Mar. However, these changes necessitated by circumstances under which scientific research is continued in the United States has probably not invalidated the conclusions reached.

The pedigrees and the mice and the complete data obtained during this 17-year time period are now housed here and the experiment is being continued—as long as there will be suitable facilities. The end is not in sight.

The experimental approach to the problem of gerontology has been as follows:

A single male mouse of one strain was mated to several female mice of another strain and the offspring have been continued

through many generations of inbreeding by a selection of maternal age—or the age of the mother at which time she has had a litter. It must also be mentioned that these mice have a variable incidence or penetrance of polydactylia, i.e. more than five toes on a hind foot or more than four on a front foot. Polydactylia is the resultant of at least one pleomorphic gene LST, which was referred to previously in relation to the origin of cancer.

Now a complete listing of all the biological characteristics investigated during this extensive investigation would be impossible with the time now at our disposal. I shall mention, therefore, only a few. The results are presented in a series of charts.

Chart 23 on longevity with LST and its normal allele *lst* has already been referred to in the discussion of cancer but will be discussed again later.

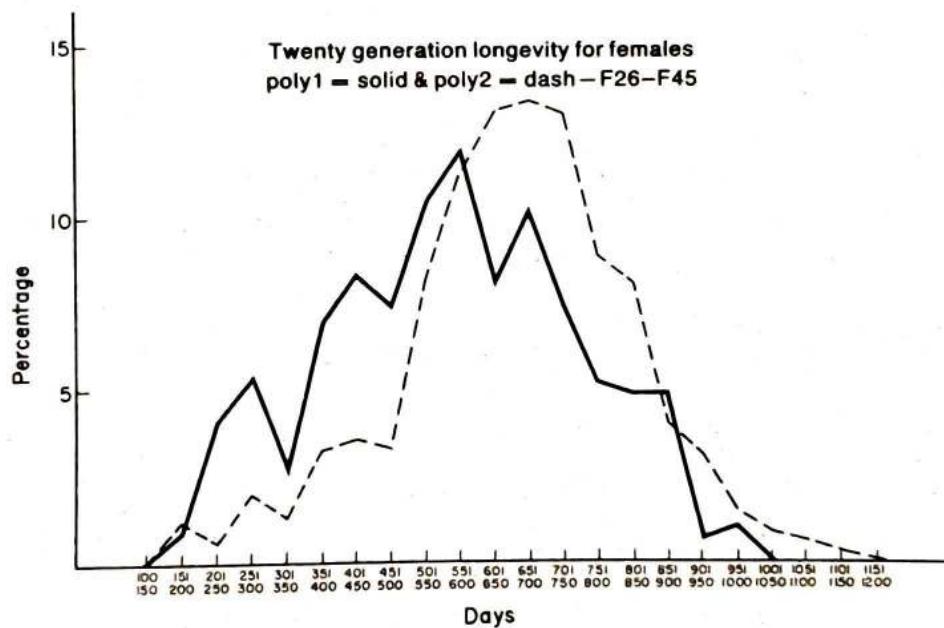


CHART 23. This chart gives the data on longevity for female mice between $F_{26}-F_{45}$. The Poly 1 descent (with high penetrance of polydactylia) is on the solid line; data on longevity for the Poly 2 descent (with low penetrance of polydactylia) are on the dash line. Duration of life expressed in days is on the base line and number of mice on the vertical line.

Chart 24 shows the age of first litters born to female mice of the seven independent descents from a common origin. It can be seen that a selection toward older and older females causes an earlier and earlier appearance of first litters. Thus it can be seen from these data that the mouse species is in equilibrium when the

CHART 24

Maternal age class	Age first litter
< 100	76.7
101-200	75.2
201-300	73.9
301-400	70.6
401-500	70.3
501-600	67.6
601-700	63.7

CHART 24. This chart presents the data on the average age of first litters expressed in days for female mice belonging to one or the other of the seven independent maternal age descents that had had a common origin.

selective pressure of maternal age descent is applied to it, as far as this character of age of first litter is concerned.

Chart 25 presents the same data on age of first litters graphically as were given in tabular form on Chart 24.



CHART 25. The data on average age of first litters are presented graphically in this chart. These data are the same as given in Chart 24.

Chart 26 presents similar data on age of first litters in still another manner. Here the data are presented on a generation of inbreeding basis. It can be seen that the earlier maternal age descents are having first litters later, whereas the later maternal age descents have first litters earlier. As a matter of fact, the data

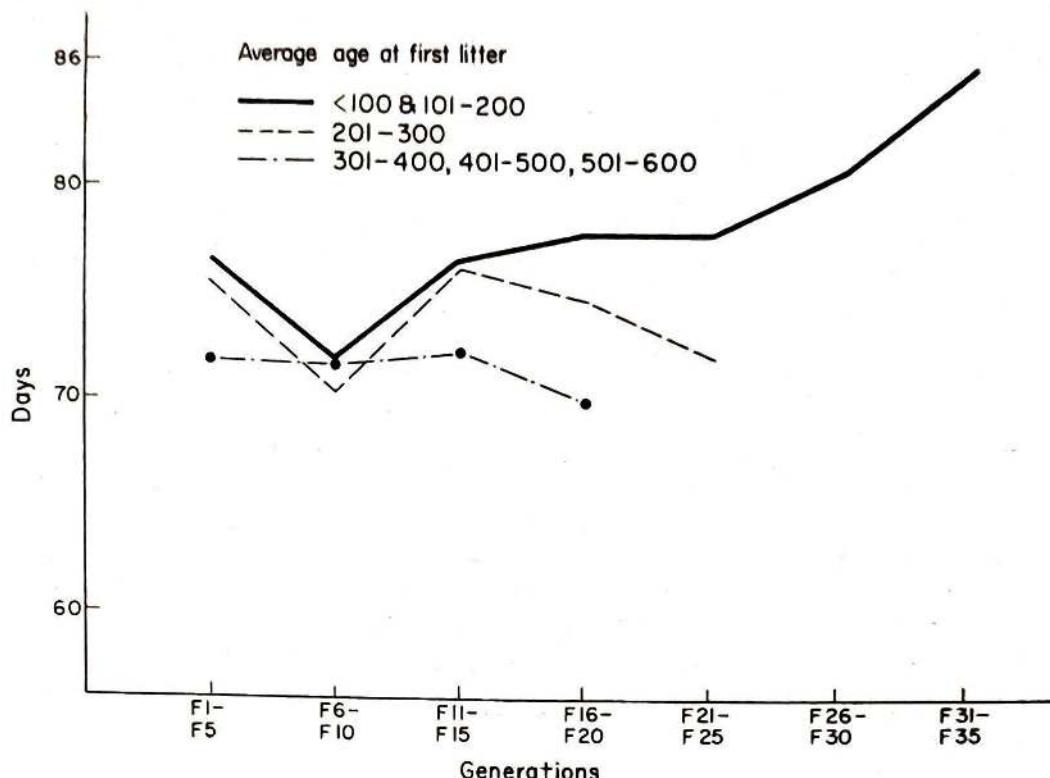


CHART 26. This chart gives the data on age of first litters on a generation of inbreeding basis. The two early maternal age groups <100 and 101-200 are totaled together and 301-400, 401-500 and 501-600 are averaged together. The data are further grouped into five successive generations of inbreeding.

for the combined 301-400, 401-500 and 501-600 are extremely uniform, whereas the data on the earlier descents are variable. I believe that this concept of stability and instability is very important and I shall, therefore, emphasize it throughout this presentation.

Chart 27 shows the analysis of the numbers of litters born to females through the successive generations of inbreeding for the <100 maternal age class. For comparison, Chart 28 is given for similar data obtained on the 201-300 maternal age descent, i.e. when the females were in the prime of life. Since the 201-300 class has only reached twenty-five generations of inbreeding, one should keep in mind that during this same period in the <100 maternal age class there is continued variability in this character. Now Chart 28 shows that the descents from females in the prime of life are quite uniform. Here again is stability and instability but now the stability is characteristic of a descent from the prime of life and instability with an early maternal age descent.

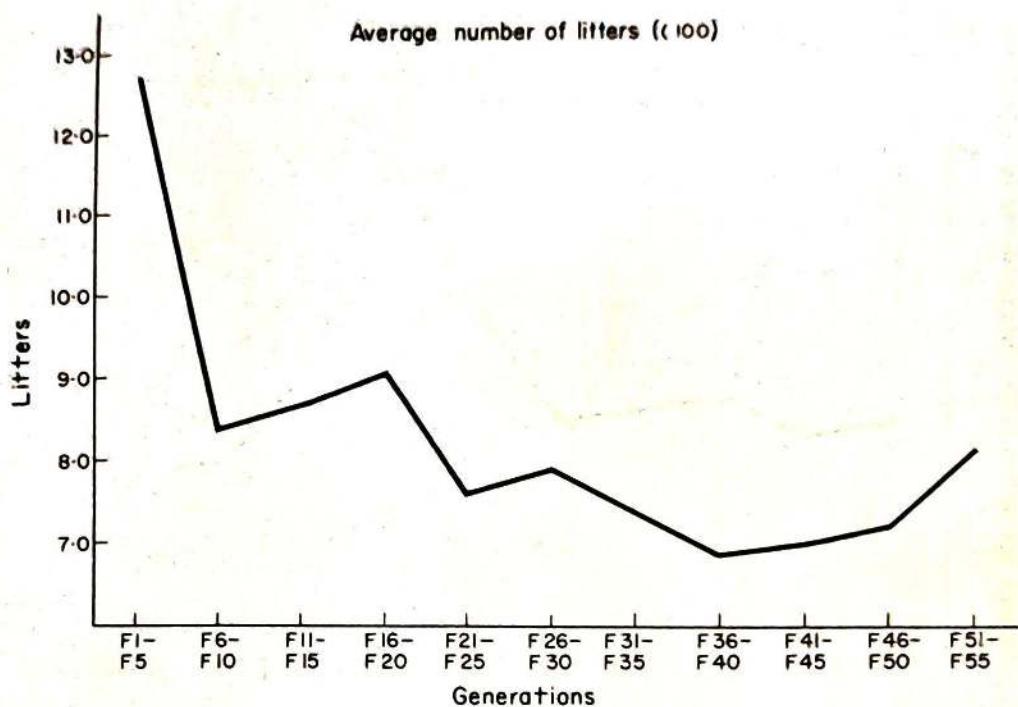


CHART 27. This chart shows the data on the average number of litters per mouse in the < 100-day maternal age descent during inbreeding or between F_1 and F_{55} .

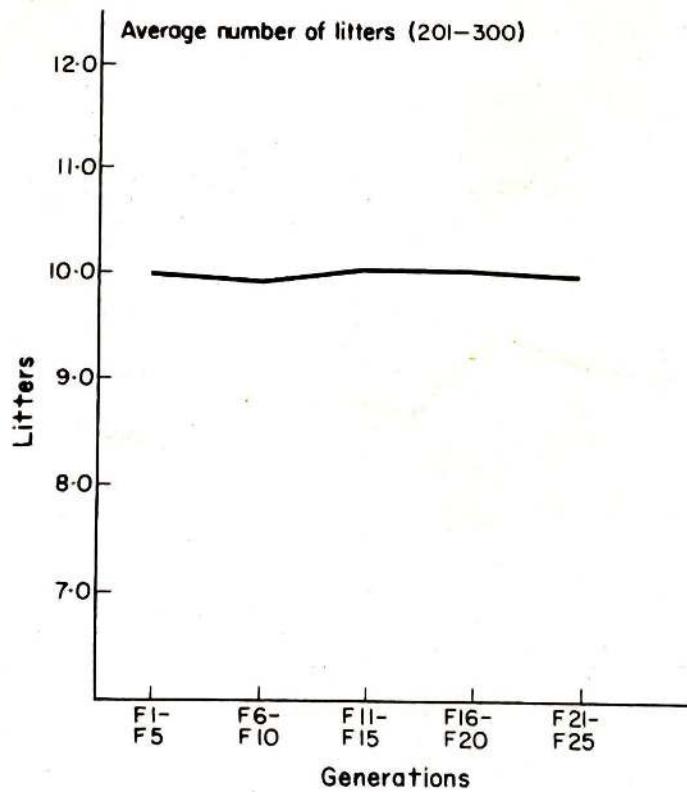


CHART 28. This chart shows the average number of litters per mouse in the 201-300-day maternal age descent during inbreeding or between F_1 and F_{25} .

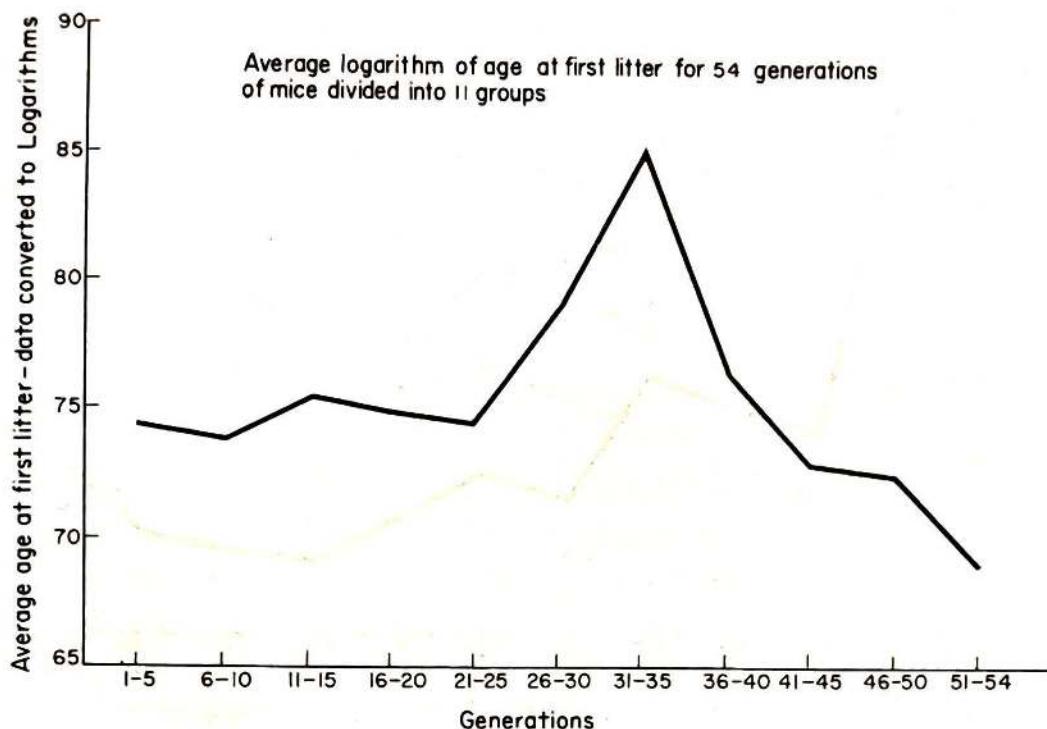


CHART 29. Average of first litters for female mice of the < 100-day maternal age descent during the process of inbreeding or between F_1 and F_{54} are given in this chart.

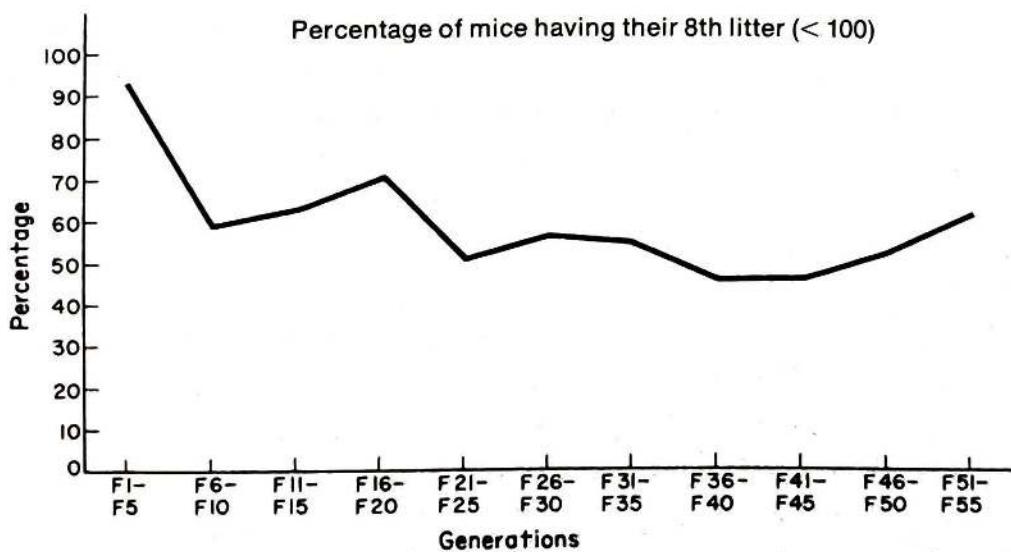


CHART 30. This chart shows the average percentage of female mice having an eighth litter in the < 100-day maternal age descent during inbreeding or between F_1 and F_{54} . Percentages of total female mice having an eighth litter are on the vertical line and generations of inbreeding (grouped into classes of five) are on the base line.

Chart 29 returns to the analysis of the age of first litters in the early maternal age descent. It can be seen that this variability or instability has continued at least through fifty-four generations of inbreeding—or long after the mice are supposed to be inbred or pure by the accepted laws of classical genetics.

Chart 30 shows the percentage of mice having eight litters for the early maternal age descent—again continued variability through twenty-five generations of inbreeding.

Chart 31 shows a similar analysis on number of mice having eight litters for the class in the prime of life (between 201–300 days)—again good stability.

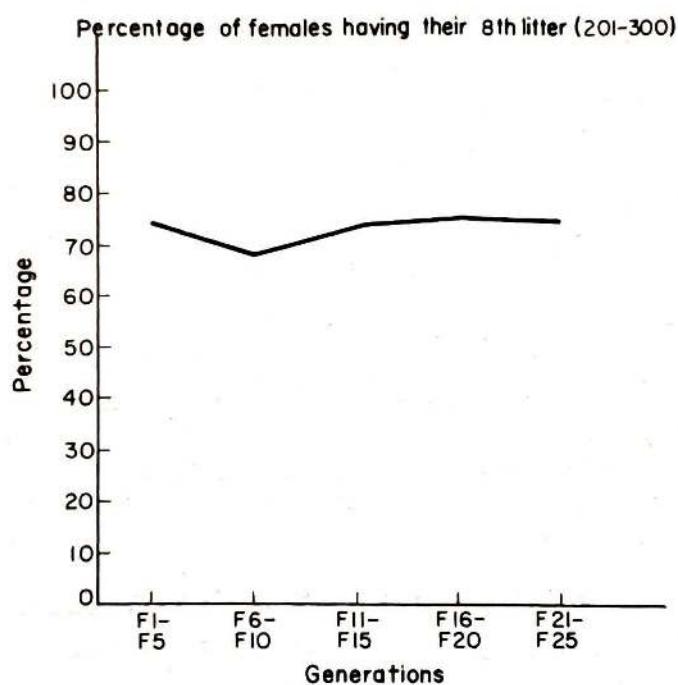


CHART 31. This chart shows the percentage of mice having an eighth litter in the 201–300-day maternal age descent during inbreeding. Percentages of mice are on the vertical line and generations of inbreeding (grouped into five generations) are on the base line.

The next section will be on the analysis of longevity.

Chart 32 presents the data on the age of death of offspring from the early maternal age descent through forty-five generations of inbreeding. The data for females are on the solid line, those for the males on the dash line. It can be seen that both males and females are living longer and longer in succeeding generations—the males, however, outliving their own sisters. As far as can be determined from the survey of the literature, the mouse is the only

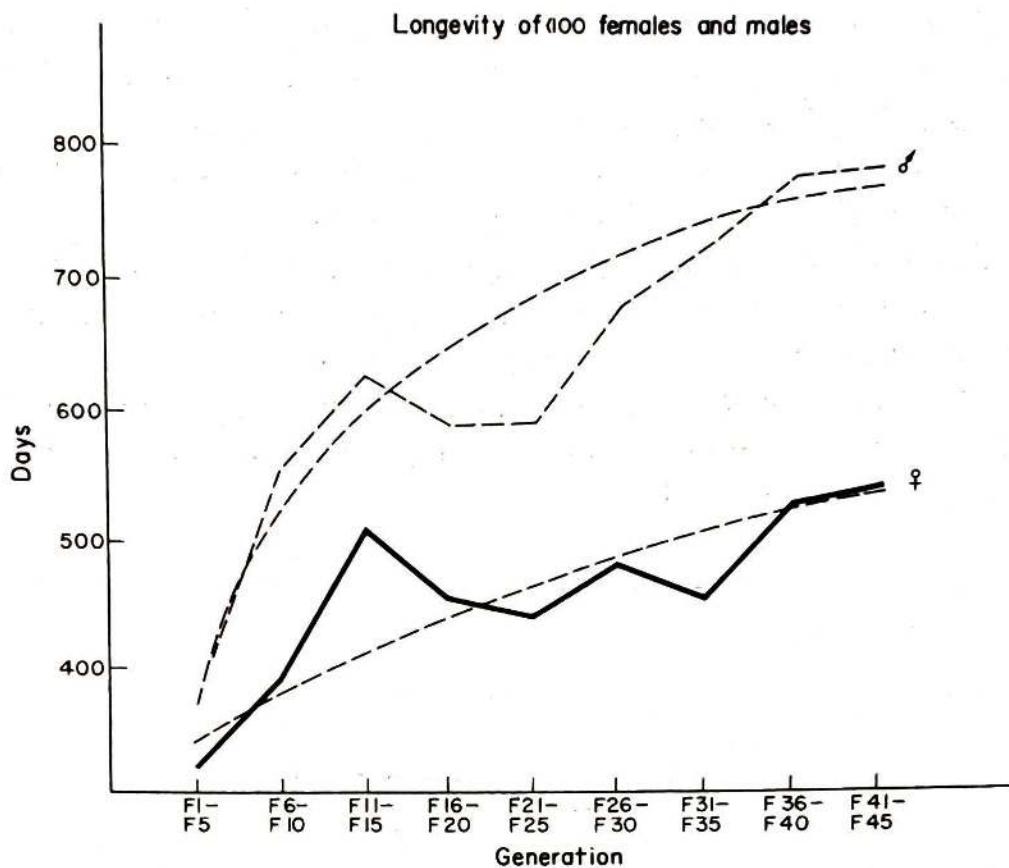


CHART 32. This chart shows the data on longevity for female mice (solid line) and male mice (dash line) of the < 100-day maternal age descent during the process of inbreeding. Smooth curves are drawn through the actual points. Longevity in days is on the vertical line and generations of inbreeding (in groups of five) are on the base line.

species in which the male outlives the female. Here again you see continued variation; in this case, an improvement of longevity by a selection toward young mothers.

Chart 33 shows the data on longevity of females between F_6-F_{15} generations of inbreeding belonging to the different maternal age descents. It can be seen that female mice born to mothers between 201–300 days or in the prime of life outlived female mice belonging to all other maternal age descents, either born earlier to young mothers or later to old mothers.

May I recall Chart 23 which showed the frequency distribution of longevity for female mice with or without the gene LST. Female mice with LST outlived female mice without LST.

The following section contains further data on the incidence of spontaneous cancer of mammary gland origin in females of the different maternal age descents.

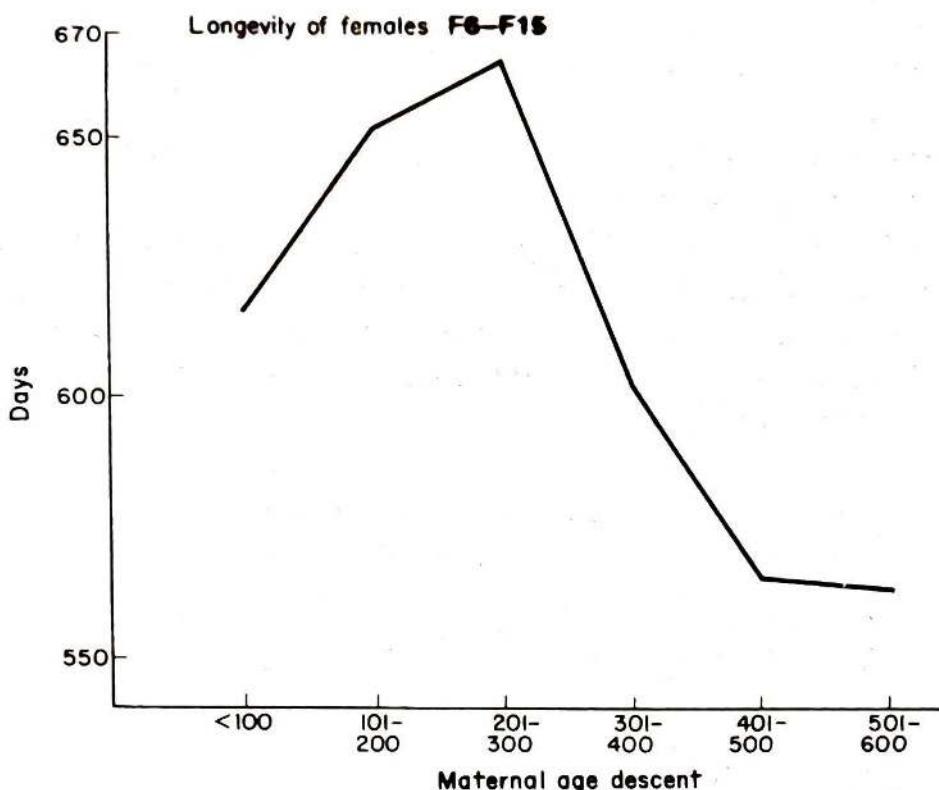


CHART 33. This chart shows the data on longevity for female mice between F_6-F_{15} for the six independent maternal age descents which had a common origin. Longevity in days is on the vertical line and six maternal age descents are on the base line.

Let us continue on the origin of cancer in the several classes of mice indicated previously.

Chart 34 presents the data on longevity for non-tumor bearing mice for both females and males for five maternal age descents.

CHART 34

Maternal age class	Age death non-tumors	Age days M.G. tumors	Percent M.G. tumors
<100	♂ 662.1 ♀ 592.6	♀ 552.3	♀ 31.5
101-200	653.0 658.3	590.9	15.5
201-300	680.6 667.7	618.7	14.0
301-400	658.2 639.8	594.1	13.5
401-500	660.5 624.4	581.4	6.5

CHART 34. This chart shows data on age of death of non-tumor-bearing mice (both female and male), the average age of onset for spontaneous mammary gland tumors and percentage of female mice developing mammary gland tumors for five independent maternal age descents.

The chart also includes the age of onset and percentage incidence of cancer of the mammary gland for the same maternal age descent.

Cancer of mammary gland origin becomes less frequent in descendants from older mothers.

Chart 35 gives the same data graphically as was presented tabularly in Chart 34. Here it can be seen again that the age of onset of mammary gland cancer is quite similar for female mice of all descents—but the percentage incidence decreases with advancing age of the mothers.

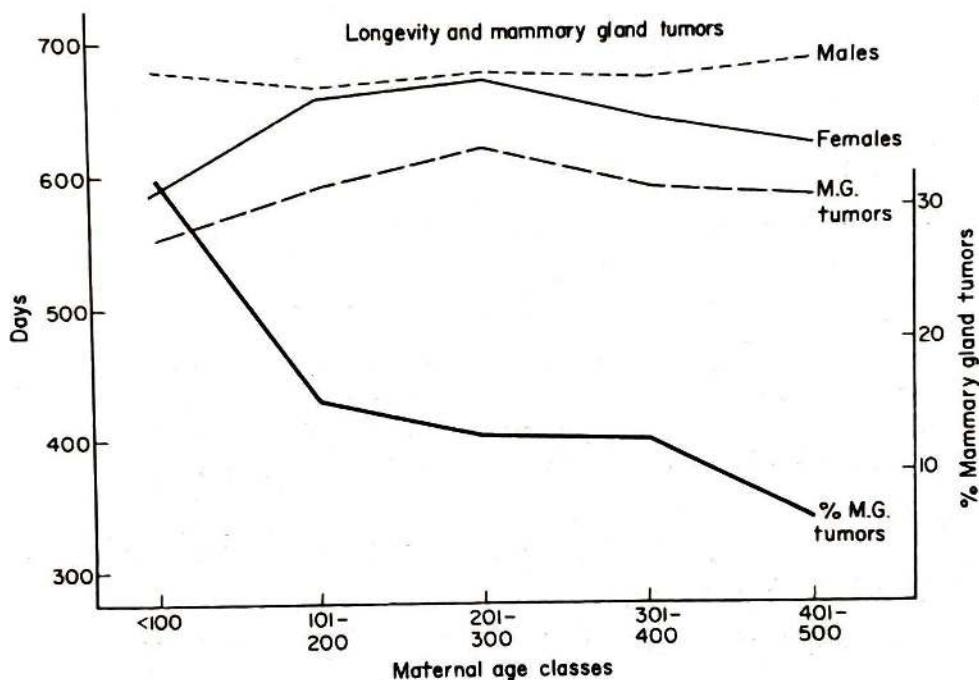


CHART 35. This chart shows longevity of males and non-tumor-bearing female mice and age of onset of mammary gland tumors in five independent maternal age descents (scale in days on left of chart); and percentage of female mice developing spontaneous mammary gland tumors (scale to the right of chart).

Chart 36 presents data on the percentage incidence of mammary gland cancer after several more generations of inbreeding than was encountered in the earlier hybrid generations. Here, after several further generations of selection toward mothers in the prime of life, there were obtained fewer cancers than were obtained in the earliest and the latest maternal age groups.

Chart 37 presents the data on the incidence of cancer of mammary gland origin through twenty generations of selective

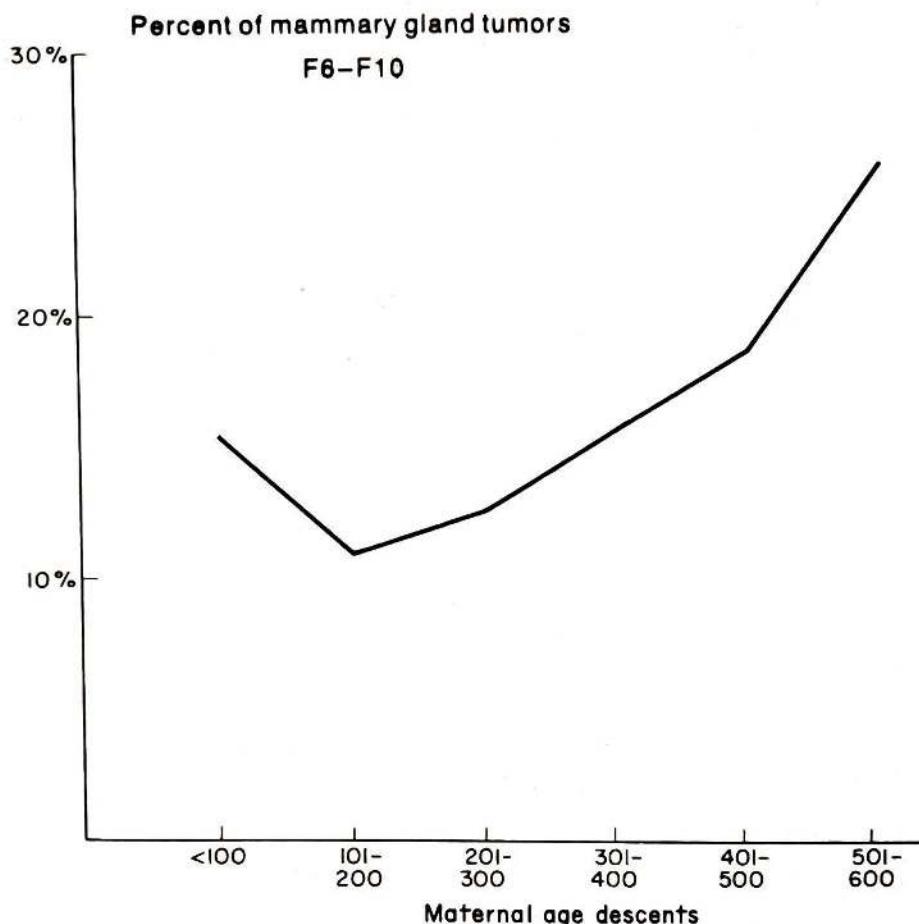


CHART 36. This chart presents the data on the percentage of mammary gland tumors in female mice between F_6 and F_{10} for the six independent maternal age descents which had had an original common origin.

inbreeding. Cancer in the 201-300 maternal age class stayed at a uniform level of about 10 percent, i.e. showing stability, whereas in the < 100 maternal age descent cancer became more and more frequent, i.e. the incidence of cancer continued unstable or variable.

Chart 38 shows the data or percentage incidence of cancer in the early maternal age descent through forty-five generations. The data continue to show an increasing tendency to develop this particular type of cancer. What the nature of the period of low incidence between F_{20} and F_{35} is remains unsolved.

Chart 39 presents the data on the age of onset of cancer of mammary gland origin in the early maternal age descent through forty-five generations of inbreeding. Thus beginning in F_{15} cancer is coming later and later in spite of the fact that according to

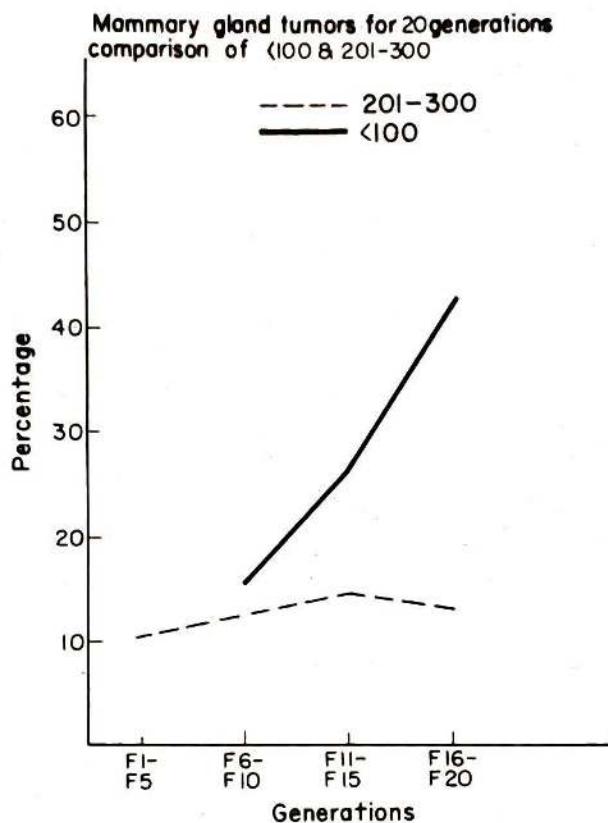


CHART 37. This chart compares the data on the percentage of mammary gland tumors between F_1 and F_{20} for the <100-day maternal age descent (solid line) and the 201-300 day maternal age descent (dash line). Percentages of mammary gland tumors are expressed on the vertical line and generations of inbreeding on the base line.

Chart 38 it is becoming more frequent thus again indicating continued instability.

The next section will be briefly on lung cancer.

Chart 40 shows the incidence of cancer of the lung in females and males of the early maternal age descent as referred to previously. It can be seen that this neoplastic lesion is more frequent in males than in females—but that the lesion is disappearing in both sexes by a selection toward young mothers.

Finally, as to the presentation of the actual data we come to the discussion of the LST gene. It has already been mentioned that the loss of this pleiomorphic gene reduced the incidence of mammary gland cancer from 42.4 percent to about 6.2 percent. This gene also influences most of the biological characteristics referred to in this presentation. But time does not permit a complete analysis. I shall mention only one fact. Mice with LST have their

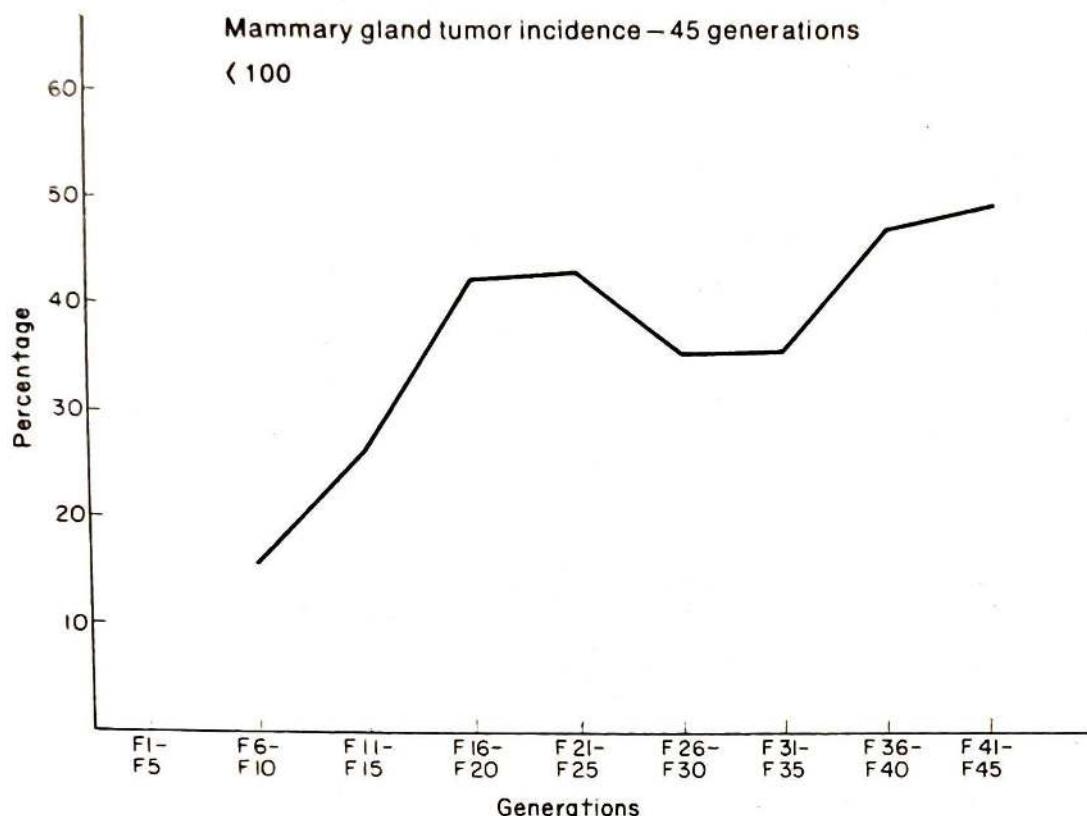


CHART 38. This chart shows the percentage incidence of spontaneous mammary gland tumors in female mice of the < 100-day maternal age descent between F_1 and F_{45} . Percentage of tumors is on the vertical line and generations of inbreeding (in groups of five) are on the base line.

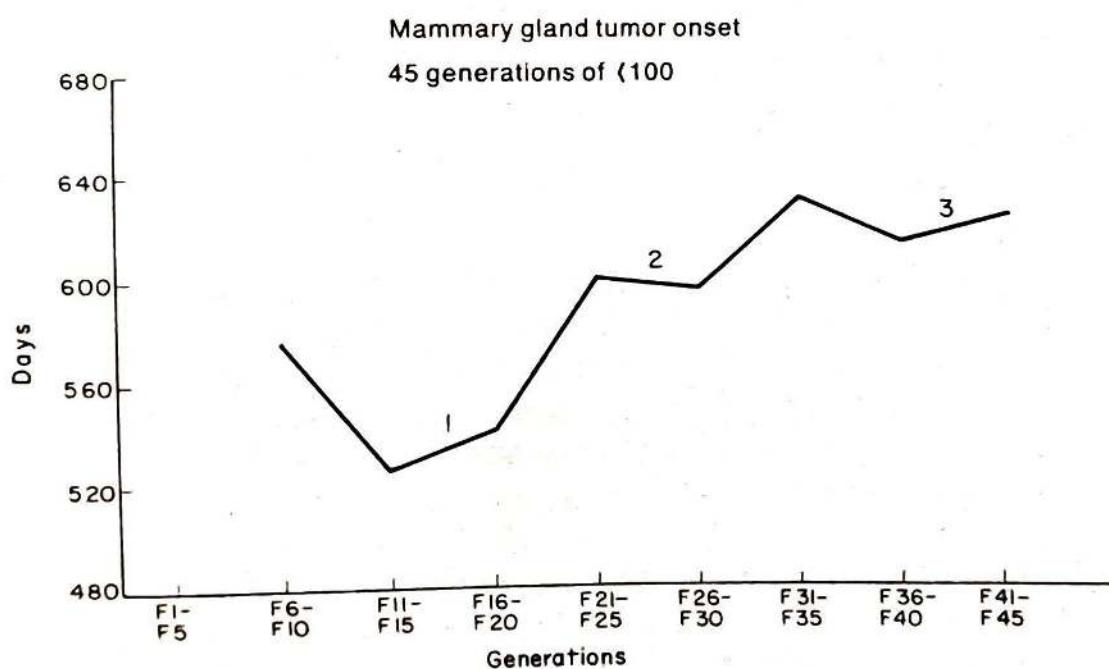


CHART 39. This chart shows the average of onset of mammary gland tumors in female mice of the < 100-day maternal age descent during inbreeding. Age of onset is on the vertical line and generations of inbreeding (in groups of five) are on the base line.

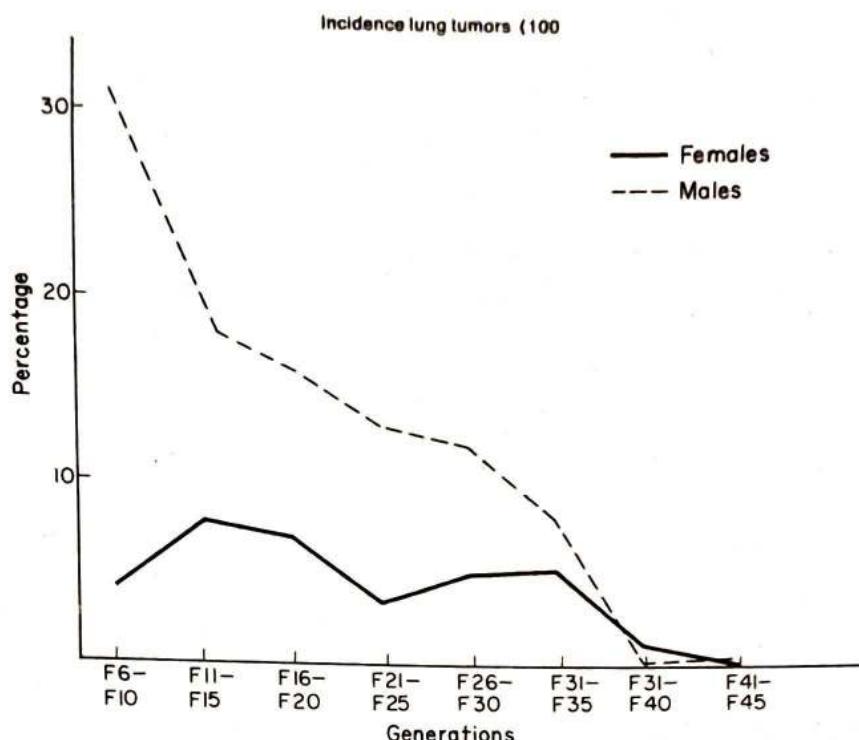


CHART 40. This chart shows the percentage incidence of mice developing spontaneous lung tumors in the < 100-day maternal age descent. Females are on the solid line and males on the dash line. Percentage incidence of tumors is on the vertical line and generations of inbreeding (in groups of five) are on the base line.

first litters at 78.8 days; mice with 1st belonging to the same maternal age class in the same generation of inbreeding have their first litters at 110.6 days. This is a difference of 31.8 days (Chapter 4).

I shall now attempt to bring together the data which I have tried to present, perhaps too scantily, but I hope not too inadequately. We have discussed some principles of classical genetics, some observations on cancer research and some data obtained on the aging process particularly in association with the characteristics of succeeding generations associated with maternal age selection.

The more research I have been fortunate to do in this ambitious program, the more I become convinced that a fundamental problem in biology is being oriented—perhaps not indicating the crux of the real essence of the problem but at least skirting around the margin.

When one discusses the observations of cancer research and the aging process one always comes up with the concept of how

much these two phenomena have in common. Differ so greatly as they do, perhaps on the surface, fundamentally they are very similar.

I shall discuss these differences and similarities briefly under four headings as follows: (1) specific genetic constitutions, (2) biological stability and instability, (3) sensitivity to maternal age selection and finally, (4) responsiveness to the pleomorphic gene LST and its normal allele *l*st. (1) In the first place, they both depend upon a specific genetic constitution for their expression. These genetic states may not necessarily be the same. Considering the possibility of thousands of different spatially related genes being obtained at the formation of the fertilized egg which gives rise, by development, to the individual, one cannot conceive a standard end result. (2) Be this as it may, both the onset of cancer and the aging process are no doubt the end result of biological stability and instability. Only a small fraction of the supporting data leading to this conclusion could be presented here. (3) These biological states of stability and instability are both receptive to maternal age selection. In both sets of biological phenomena, cancer and physiological characteristics the maternal age effect is more pronounced on a homozygous than on a heterozygous genetic constitution. It would be trite indeed to discuss here the genetic influence on stability and instability—one of the most important building stones of the science itself. (4) Lastly, both the onset of cancer and the aging process are influenced, if not controlled, by the gene LST or its normal allele *l*st.

This brief summation may appear to lead to utter confusion. I do not think so. Perhaps the future line of approach to analyze these fundamental biological problems may be clarified by some additional thinking.

There is a principle in logic that is usually referred to as a razor of Occam (*ca. 1300–49*). This paraphrased is “of two-solutions to a problem choose the simpler”. The original was *Essentia non sunt multiplicanda praeter necessitatem*.

The following is one attempt to synthesize a solution or a program of research to continue the investigation.

The LST gene is a controlling gene that not only has a pleomorphic effect on the development of several morphological characters but also on several physiological ones as well. Its primary effect is on a control of biological tempo or rate of

physiological activity or change. At critical periods in the life of the individual disturbances of several natures result in biological instability. Some of these disturbances could result in cancers or derangements leading to age changes and eventual death. The normal allele of "lst" reduces the chances of continued biological instability and hence an animal with this "lst" gene will have fewer tumors and actually live longer.

This then is a philosophy that keeps a scientist young.

CHAPTER 4

Studies on the Pleomorphic Gene LST and its Allele lst

POLYDACTYLIA is of widespread appearance in many species of animals. The present character that has been the source of interest for many observations in the fields of genetics, cancer research and gerontology first appeared in 1939 in a mouse of the Brpb/St strain following the subcutaneous injection of 20-methylcholanthrene into its mother at 60 days of age. In fact, two mice (one female and one male) among a litter of eight showed polydactylyia. It was the male mouse (No. S31251) with this new mutant that gave rise, by hybridization, to an extensive descent. Many of the various sublines from this first polydactylous mouse have been used in numerous research projects which need not concern us now. It is the present intention of discussing particularly one descent only, with, as far as known or genetically analyzed, a single gene "LST" for polydactylyia, together with a single subline derived from LST but containing the normal allele, lst.

Ideally, it would be desirable to work with isogenic strains in order to compare the effects of LST with lst. However, this has not been done. All that one can say is that the two strains, now highly inbred, LST with sixty-five generations of restricted brother-to-sister matings, and lst in F_{50} , had twenty-nine generations of restricted inbreeding in common and are, therefore, genetically very similar, but there is a possibility that residual heterozygosity leading to delayed segregation even after this length of inbreeding may be involved in the morphological and physiological differences ascertained in mice of the two descents. There is also the possibility of mutation producing differential differences, but this factor must be slight indeed since the average mutation rate in mice is approximately 1 in 26,000 individuals.

The polydactylyia syndrome manifested itself early as being pleomorphic in expression. For example, a single mouse showed all the following deviations from the normal or one or more in any

combination; (1) reduction or absence of the tibia, (2) torsion of the remaining tibia, (3), polydactyla on one to four appendages, (4) alopecia, (5) open eyelid, (6) mild anemia, (7) hydrocephaly and (8) semilethal. Polydactyla appeared more frequently on the right hind foot than on the left. When the character showed on three appendages, it did so more frequently on the left front than on the right front. A further discussion of the pleomorphic effects of this character need not concern us here.

Forsthoefel (1962) has investigated the "Genetics and Manifold Effects of Strong's Luxoid Gene in the Mouse, Including its Interactions with Green's Luxoid and Carter's Luxate Genes". Forsthoefel concluded that,

only one mendelian factor causes the various effects observed in the Strong luxoid mutant. The penetrance of Strong's luxoid gene in heterozygous condition in the strain of origin (DP) is 0.9566; in the C₅₇Bl/10 strain, its penetrance is 0.85.8. Strong's luxoid gene is not an allele of either Green's luxoid or of Carter's luxate gene, nor is it linked with these genes. In the heterozygous condition, Strong's luxoid gene causes preaxial polydactyly of the feet, especially of the hind feet, and favors the presence of an interfrontal bone. In homozygous condition, Strong's luxoid gene causes preaxial polydactyly of all 4 feet, reductions and duplications of the radius, reductions and rarely duplications of the tibia, reduction of the pubis, modifications of the skull, abnormal proportions of the parts of the brain, open eyelids at birth, temporary dorsal alopecia, a posterior shift of the umbilicus, anomalies of the genital phalli, ectopia of the testis, and sometimes anemia. Strong's luxoid gene interacts additively with Carter's luxate and Green's luxoid genes on body parts each affect independently. The reduced tibia in mice heterozygous for both Strong's luxoid and Green's luxoid gene frequently is duplicated.

Later Forsthoefel (1963) reported on "The Embryological Development of the Effects of Strong's Luxoid Gene in the Mouse". Forsthoefel concluded:

The skull and brain anomalies in Strong luxoid homozygotes are a result of deficient development of the anterior part of the head, including the facial processes, first observed at 12 days in the embryo. The failure of the eyelids to close before birth are a result of deficiency in their development induced by tension exerted on them by the upward-pushing mesencephalon first observed at 12½ days. Excessive lateral extension of the eyes by the outward-pushing diencephalon is also a factor. Polydactyly can be observed in the forelimb at 11½ days and in the hind limb at twelve days. By 12½ days, the apical ectoderm is considerably enlarged preaxially around a prominent extra lobe of the foot-plates. The pattern of blastematal condensation for skeletal elements in both limbs combines deficiencies with duplication. Deficient skeletal elements, especially the radius, tibia, and pubis, lag in chondrification and ossification. Studies on the localization and the amounts of ascorbic acid and alkaline phosphatase activity in both fore and hind limbs showed a

lag in chemodifferentiation in the deficient skeletal elements, specifically in the radius and tibia. Deficient development of the anterior half of the phallus was first marked at 13½ days. Stratified epidermis fails to form between the root of the umbilical cord and the phallus, leading to a weakness of the belly wall in the midline. The failure of a normal first coat of hair to form on the dorsum of the homozygotes is not due to failure of formation of follicles for the various kinds of hairs in this region, but probably results from a defect in their structure which prevents them from persisting after eruption. The structural defect very likely results from deficient development of the first hair follicles. The most fundamental effect of Strong's luxoid gene found in this investigation appears to be a retardation of development of parts of the head, integument, limbs, and belly.

The single descent of mice reported here has been called Poly 1 and was developed by restricted brother-to-sister matings only basing selection on two counts as follows: mothers were less than 100 days of life at time of production of young and the presence of polydactylia in both parents. The descent consists, therefore, of parents from first or second litters only. The sexes were kept continuously together until one of them died or developed a spontaneous tumor. Numerous observations have been reported in various publications.

For the present experiment it can be pointed out that in this Poly 1 descent the penetrance of polydactylia rose from 2.63 percent in the F_1 to the high value of 93.6 percent in F_{33} . Beyond this point of inbreeding, polydactylia has somewhat stabilized off at about 88 percent.

Of the numerous observations already published on this descent, we shall refer only to age of first litters, "The Effects of Fifty-four Generations of Inbreeding on Age of First Litters. Selective Influences of Early Maternal Age and Polydactylism" (Strong, Johnson and Rimm, 1965).

The conclusion from this study was that the

age of first litter in mice selected for early maternal age is variable and not normally distributed (i.e. it does not have the characteristic bell-shape curve). To facilitate the study of the effect of inbreeding on days to first litter, the data were converted to logarithms. The average age of first litter (geometric average, since the data were converted to logarithms) did not decrease or increase steadily with increased inbreeding. The variability of age at first litter gave some slight indication of decreasing as homozygosity increased. There seemed to be no relationship between sterility and homozygosity; that is, after forty generations of inbreeding there were no appreciable increases in sterility. After fifty-four generations of brother-sister matings, with selection for early maternal age and polydactylism, a relatively stable or predictable response with respect to age at first litter was not attained.

In F_{29} of Poly 1 descent with a polydactylia penetrance of 93.21 percent, a daughter was born that produced a progeny with a penetrance of only 1.33 percent polydactylia. She was, therefore, set apart as the progenitor of Poly 2 which was continued by a selection of the descent from young mothers (litters 1 and 2 as was being continued in Poly 1) but also a selection for normal feet. Mice of this Poly 2 descent produced many unexpected results some of which (reported in 1962) are in an article published in *Lavori Ist Anat. Istol. Pat. Perugia*, entitled "Mouse number 46801" (Strong, 1962). The conclusions arrived at from the study of this descent were as follows:

The penetrance of polydactylia increased from 2.6 percent in the F_1 to a high of 93.6 percent in the F_{33} following a cross between a single polydactylous male of the Brpb/St inbreds and female mice of the C₅₇/St strain. There was a gradual reduction of reproductive capacity as measured by age of mothers at the time of the production of successive litters during the process of inbreeding. The average values for litter spacings between litter one and litter eight for a single descent of mice have been: between F_{11} - F_{15} , 29.7 days; between F_{31} - F_{50} , 32.9 days. The descendants of mouse 46801 have produced a variety of biological and genetic variables including (a) an increased reproductive capacity as measured by litter spacings, (b) several new point mutations some at well established loci and others at new, yet unresolved, loci and (c) a reduced penetrance of polydactylia. These new point mutations and low penetrance of polydactylia continue to arise in succeeding generations from mouse 46801. A "mutator gene" associated with the reduction of polydactylia (some change in 1st) appears to be involved in the above manifold biological and genetic characteristic.

It is the purpose of the present paper to make a comparative analysis of the data bearing upon several biological characteristics in mice of these two inbred strains, Poly 1 and Poly 2. From the beginning it should be kept in mind that the two strains have had twenty-nine generations of inbreeding (restricted brother-to-sister matings) in common and that the two strains differ especially with one major difference—Poly 1 has a high penetrance of polydactylia and Poly 2, an extremely low penetrance. According to the genetic and embryological evidence provided by Forsthöefel (1962, 1963) this polydactylia difference is one gene difference, i.e. LST compared to 1st.

We shall consider the same morphological and physiological characteristics that have been used in numerous publications in the past.

Results

The results will be presented in a series of charts.

Chart 41 presents the data on the penetrance of polydactylia in the Poly 2 descent between F_{27} and F_{55} . The data are compared together in groups of five successive generations and it can be seen that polydactylia is becoming less frequent with a selection of mice with normal feet.

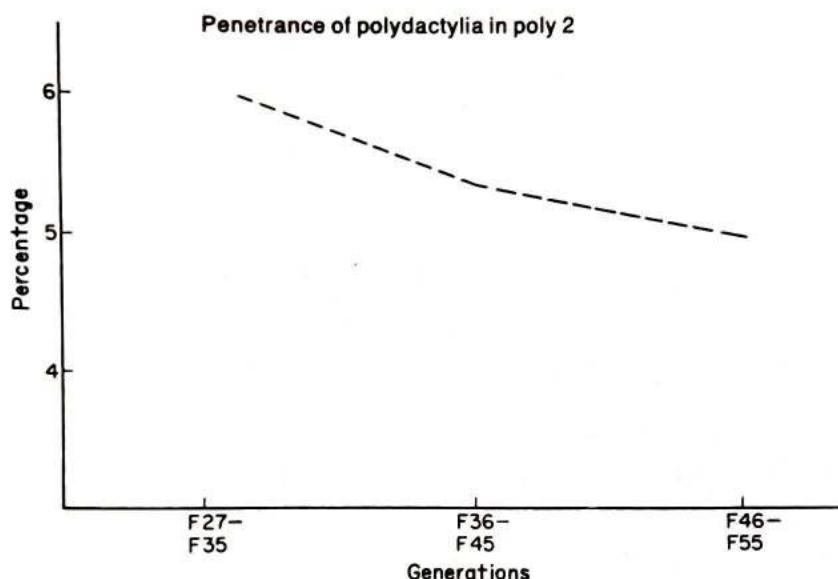


CHART 41. Penetrance of polydactylia for twenty-eight generations of Poly 2, $F_{27}-F_{55}$.

Chart 42 compares the data on penetrance of polydactylia in the two descents Poly 1 and Poly 2 between F_{16} and F_{55} . Poly 1 has been produced by a constant selection toward polydactylia and Poly 2 by a selection toward normal feet but only after there had been a sudden loss of polydactylia in the descents of mouse number 46801, belonging to F_{29} .

Chart 43 compares the data on age of first litters for the two groups through the process of continued inbreeding. The penetrance of polydactylia is expressed in percentage for the five generation groups that were combined. It can be seen that the penetrance of polydactylia has changed in mice of Poly 1 from 91.6 percent in $F_{31}-F_{35}$ to 81.0 percent in $F_{51}-F_{55}$. During the same period of inbreeding polydactylia in mice of Poly 2 decreased from 6.0 percent in $F_{31}-F_{35}$ to 4.3 percent in $F_{51}-F_{55}$.

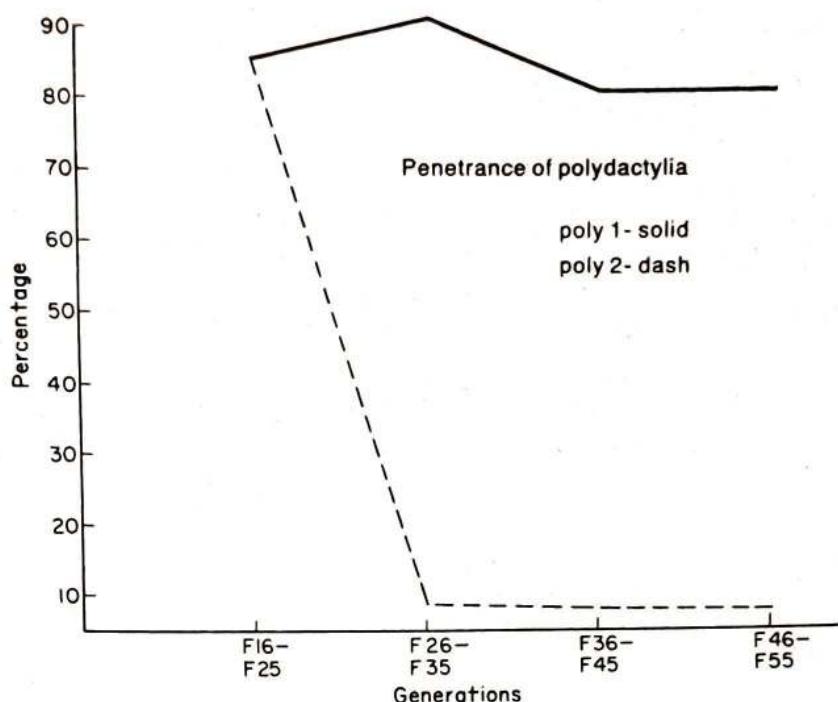


CHART 42. Comparison on penetrance of polydactylia of Poly 1 and Poly 2 for thirty-nine generations, F₁₆-F₅₅.

Thus it can be seen that in F₅₁-F₅₅ mice of Poly 1 with a penetrance of 81 percent polydactylia had first litters at 71 days whereas mice of Poly 2 with a penetrance of 4.3 percent polydactylia had first litters at 91 days.

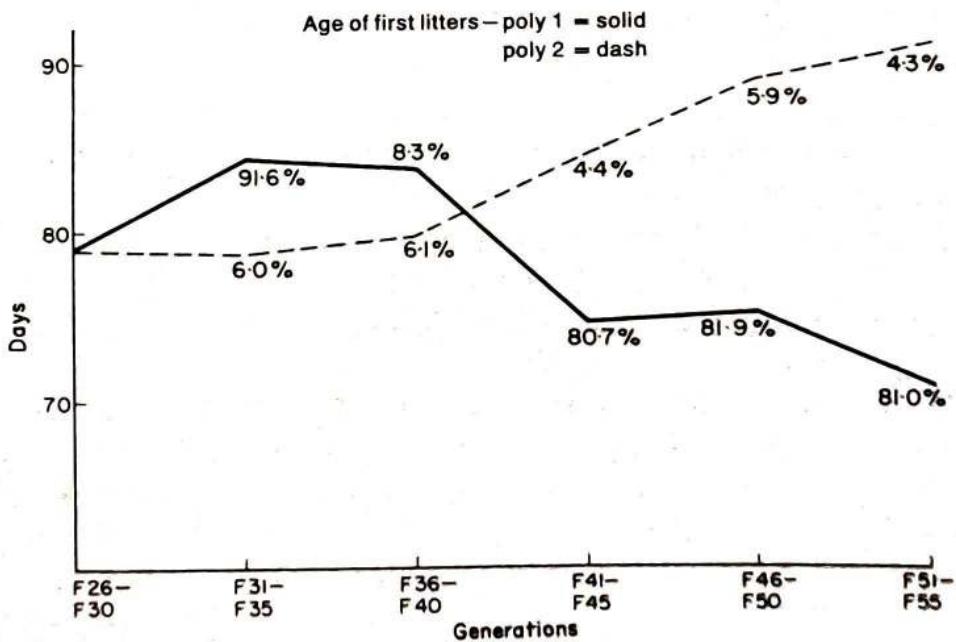


CHART 43. Comparison of Poly 1 and Poly 2 for twenty-nine generations, F₂₆-F₅₅.

Chart 44 compares the data on litter spacing or frequency in mice of the two descents when the two sexes were kept continuously together. It can be seen that between F_{51} - F_{55} mice of Poly 1 with 81.0 percent penetrance of polydactylia had subsequent litters spaced 27.5 days apart, whereas mice of Poly 2 with 4.3 percent penetrance had litters spaced 30.9 days apart. Also, it should be noted that the Poly 1 descent shows continued variation even after thirty-five generations of inbreeding. This concept of biological variability and equilibrium or stability shall be emphasized from now on in this presentation.

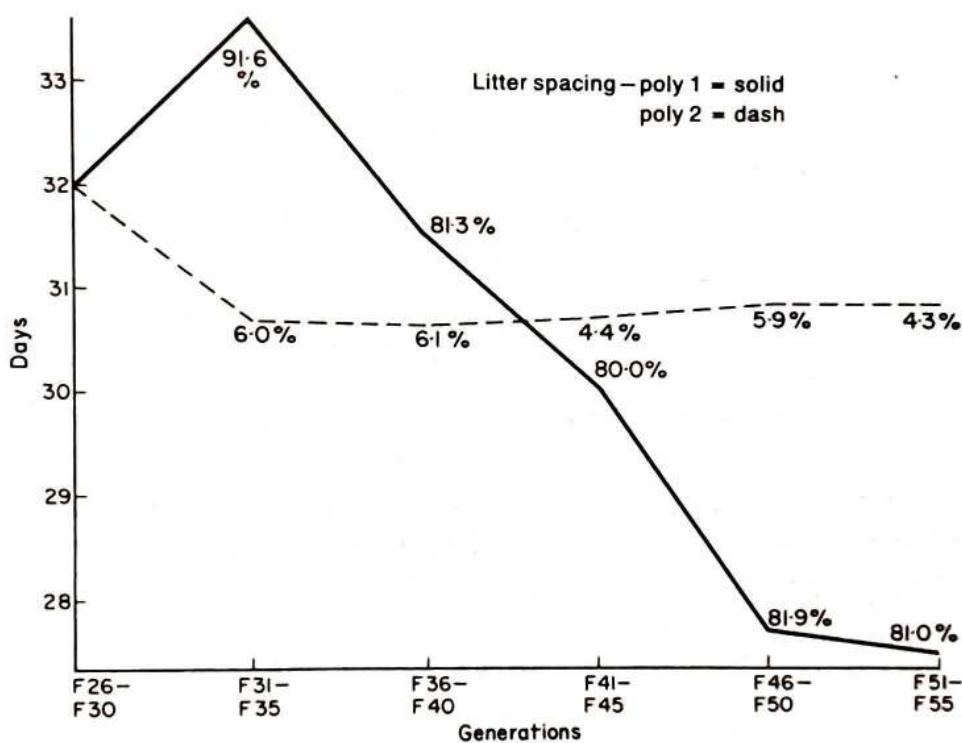


CHART 44. Comparison of Poly 1 and Poly 2 for twenty-nine generations, F_{26} - F_{55} (litters 1-8).

Chart 45 gives the data on age at which the mice had eighth litters. Here again the Poly 1 data are on the solid line and the data for Poly 2 on the dash line. It is clear that the Poly 1 descent continues to show variability in this character—changing from a high of 320 days in F_{31} - F_{35} to a low of 262 days in F_{51} - F_{55} , thus showing continued variability, whereas mice of Poly 2 descent had their eighth litters at approximately 295-305 days of age being a very small difference. Also it can be seen that, in F_{51} - F_{55} ,

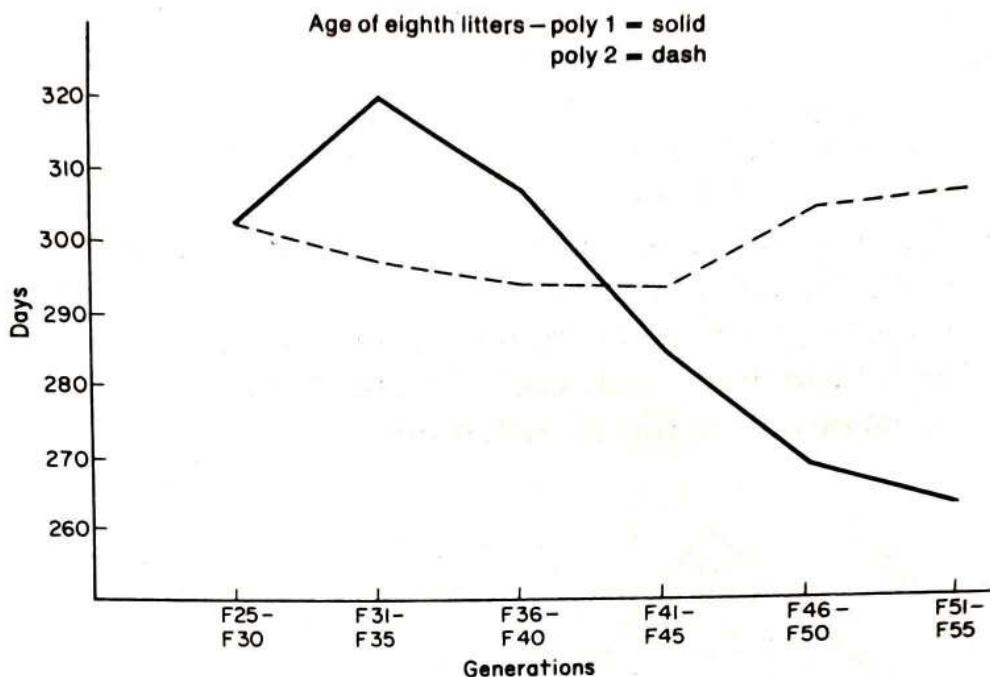


CHART 45. Comparison of Poly 1 and Poly 2 for thirty generations,
 $F_{25}-F_{55}$.

mice with LST had their eighth litters much earlier than mice of Poly 2 descent with 1st

Chart 46 shows the data on the percentage of mice in each descent that had eighth litters. In this character the mice of Poly 1 descent, although between $F_{46}-F_{50}$ there seems to be very little difference between the two descents in the number of mice having eight litters.

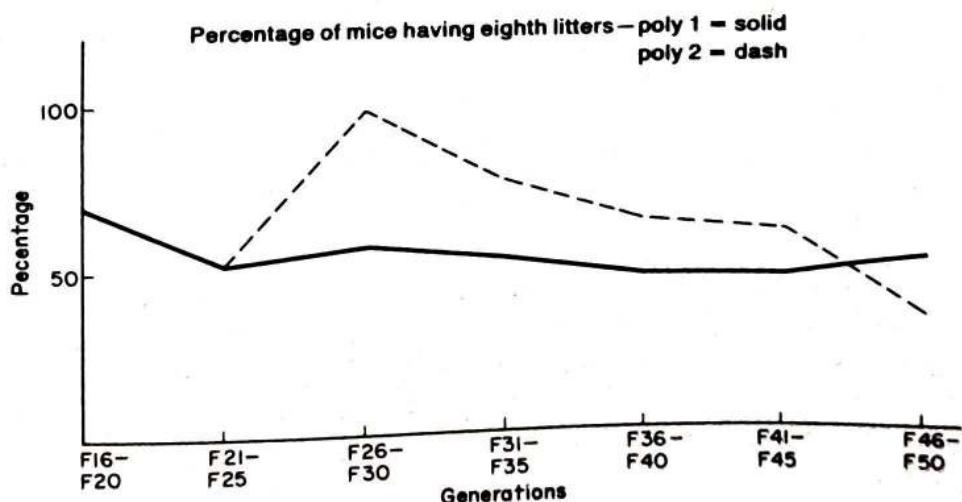


CHART 46. Percentage of mice having eighth litters, Poly 1 and Poly 2 for thirty-four generations, $F_{16}-F_{50}$.

Chart 47 presents the same data on number of mice having eight litters in the two descents but presented in a different manner. Here the data are given as a scattergram with straight lines drawn between the different points of succeeding generations of inbreeding. The same conclusion appears to be obvious as the one reached from Chart 46.

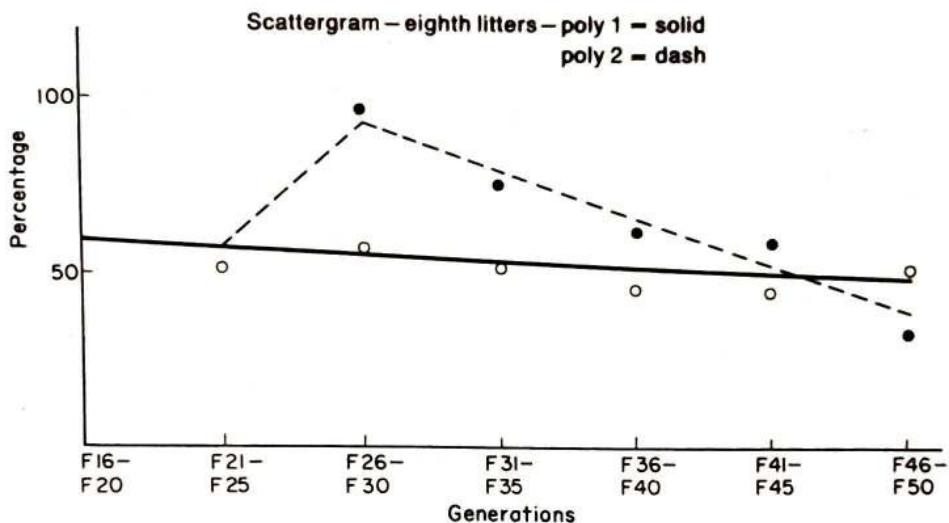


CHART 47. Percentage—scattergram.

Chart 48 shows the data on litter size for mice of the two descents between $F_{26}-F_{30}$ and $F_{51}-F_{55}$. At $F_{26}-F_{30}$ litter size

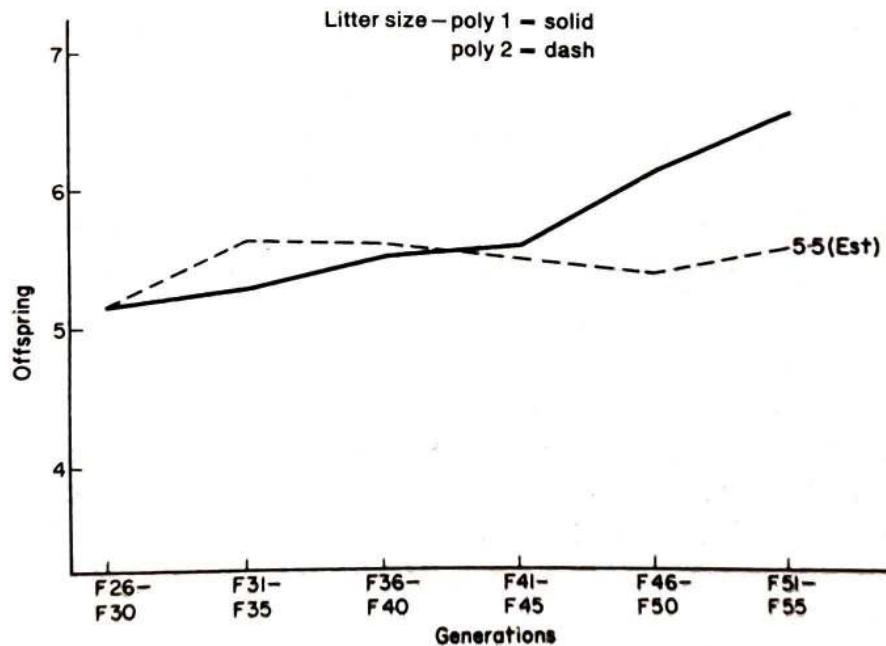


CHART 48. Comparison of Poly 1 and Poly 2 for twenty-nine generations, $F_{26}-F_{55}$.

was the same for mice of the two descents even though Poly 2 (dotted line) had been derived from Poly 1 (solid line) by a radical change in the penetrance of polydactylia. In Poly 1, litter size is increasing between these two levels of inbreeding $F_{26}-F_{30}$ and $F_{51}-F_{55}$. Litter size in Poly 2 appears not to have changed during the same extent of inbreeding as experienced by mice of Poly 1. Here again is stability of a biological character with 1st and continued variability with LST.

Chart 49 presents the same data as in Chart 48 but in different form. In each case, a straight line was drawn between successive

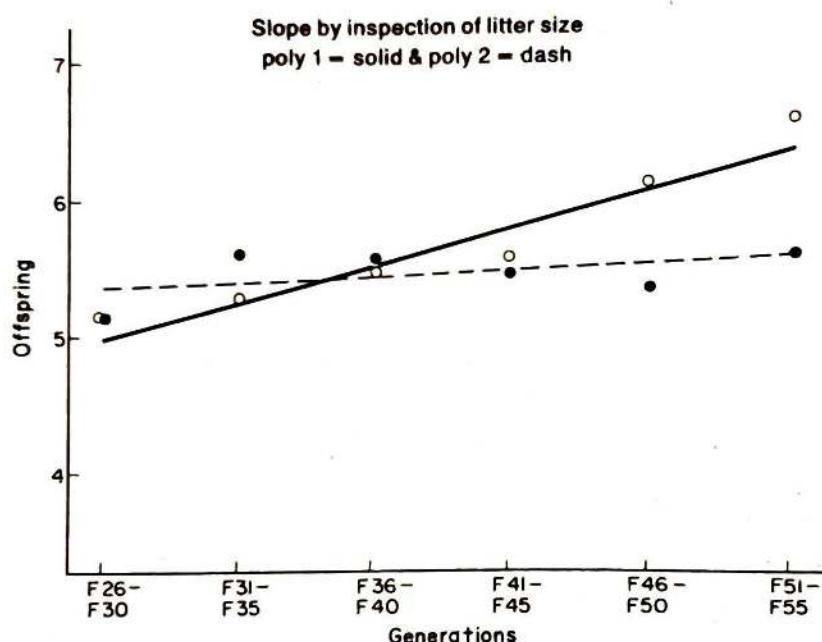


CHART 49. Comparison—scattergram.

points on average litter size during the process of inbreeding. The same conclusion is again possible as obtained from Chart 48—continued variability (i.e. an increased litter size in mice of Poly 1) but stability in mice of Poly 2.

Chart 50 shows the same data as on Charts 48 and 49, except here instead of projecting a straight line through the data for Poly 2 the chart shows a common point of origin in $F_{26}-F_{30}$ for both descents. It should be emphasized, although the difference is not very large, that, in $F_{15}-F_{55}$, Poly 2 mice with 1st have smaller litters than Poly 1 with LST.

Chart 51 presents the data on fertility (total number of offspring) for mice of the two descents. In this character the mice of

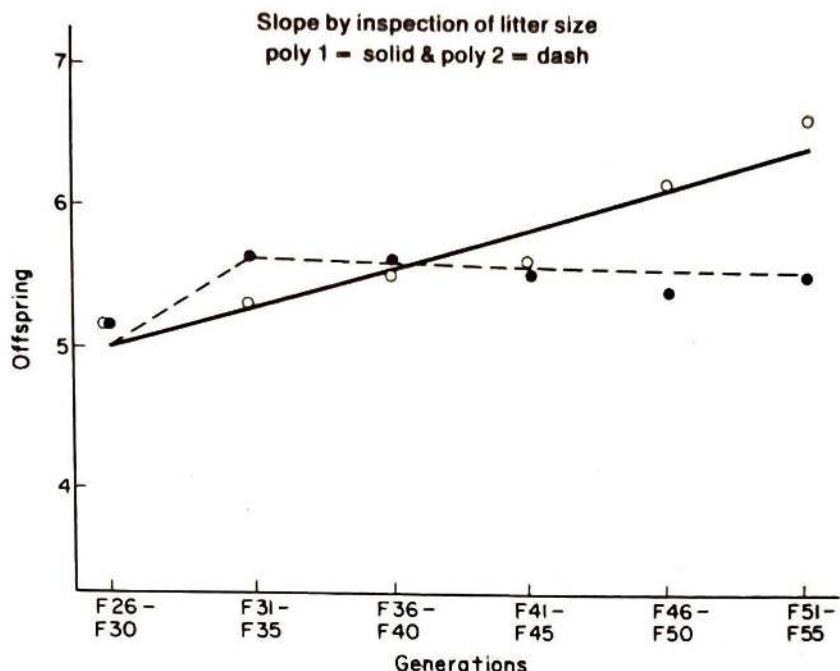
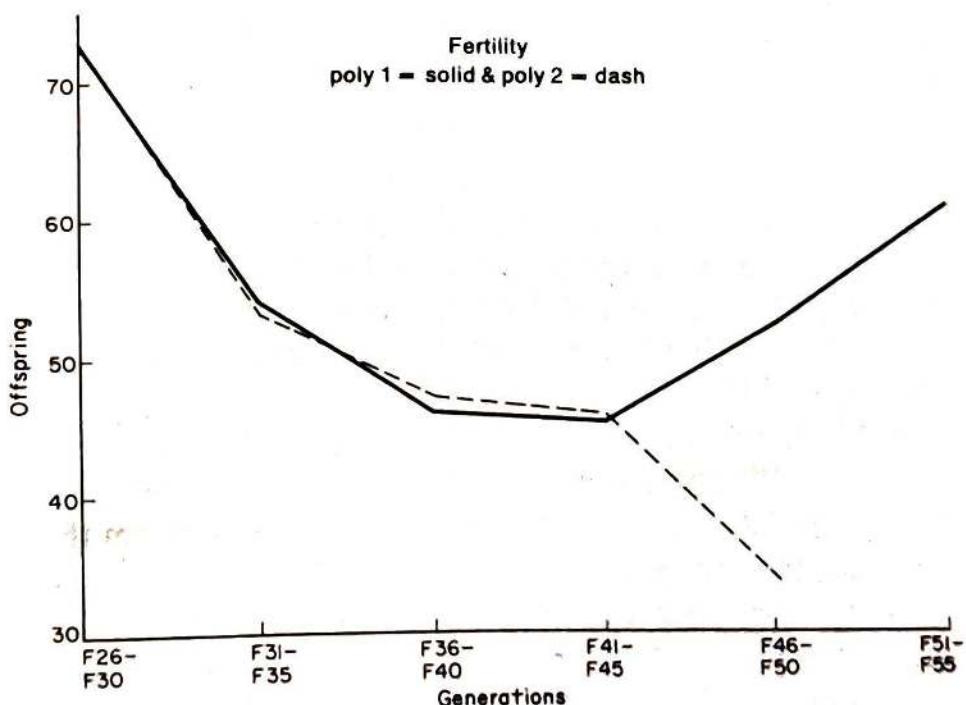


CHART 50. Comparison—scattergram.

the two descents show remarkable similarity in fertility between F₂₆-F₃₀ and F₄₁-F₄₅. After this degree of inbreeding the two descents appear to deviate. Mice of the Poly 2 descent (with 1st)

CHART 51. Comparison of Poly 1 and Poly 2 for twenty-nine genera-tions, F₁₆-F₅₅.

continue to lose fertility, in a continued uniform trend, whereas, mice of Poly 1 (with LST) tend to reverse the downtrend in fertility and actually improve in this character. By F_{46} - F_{50} the two sublines are quite different, Poly 1 mice produced fifty-two young on the average whereas mice of Poly 2 produced only thirty-three.

Chart 52 shows the comparative data on fecundity (total number of litters produced) for mice of Poly 1 (solid line) and

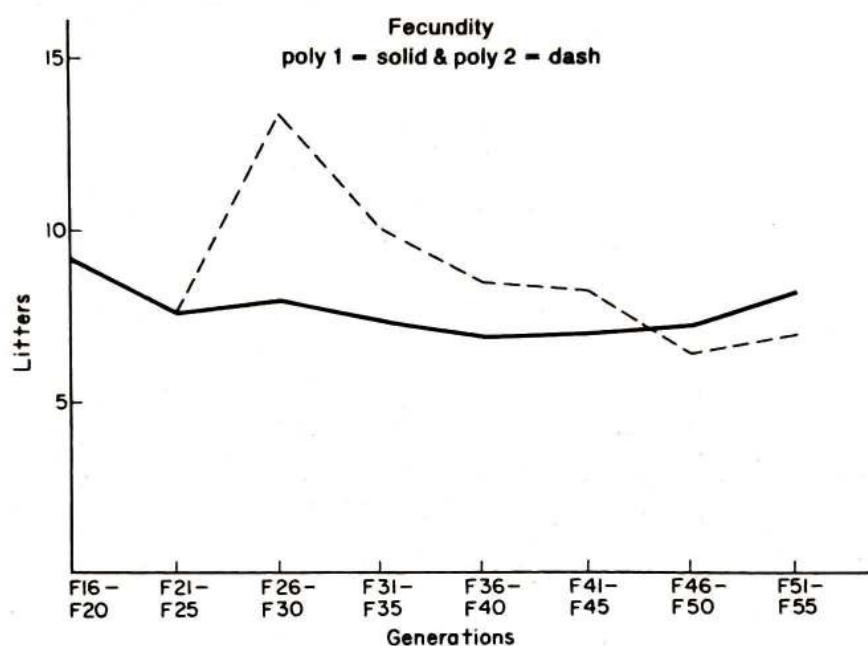


CHART 52. Comparison of Poly 1 and Poly 2 for thirty-nine generations,
 F_{16} - F_{55} .

Poly 2 (dash line). In fecundity it appears that mice of Poly 2 deviated from their strain of origin in F_{26} - F_{30} . With continued, although separate or independent, inbreeding the mice of the two descents appear to have very similar values for fecundity between F_{46} - F_{50} and F_{51} - F_{55} .

Chart 53 shows the same data as given on Chart 52 but presented in a different manner. Here the data are presented as a scattergram. The same conclusion was drawn as from the data of Chart 52 that mice of Poly 2 deviated in the characteristic of fecundity from mice of Poly 1 at the time of their origin. With further inbreeding, but on an independent basis, the mice of the two strains eventually show very similar values for fecundity.

Chart 54 gives the data on longevity for female mice of the two descents (Poly 1 on solid line and Poly 2 on dash line).

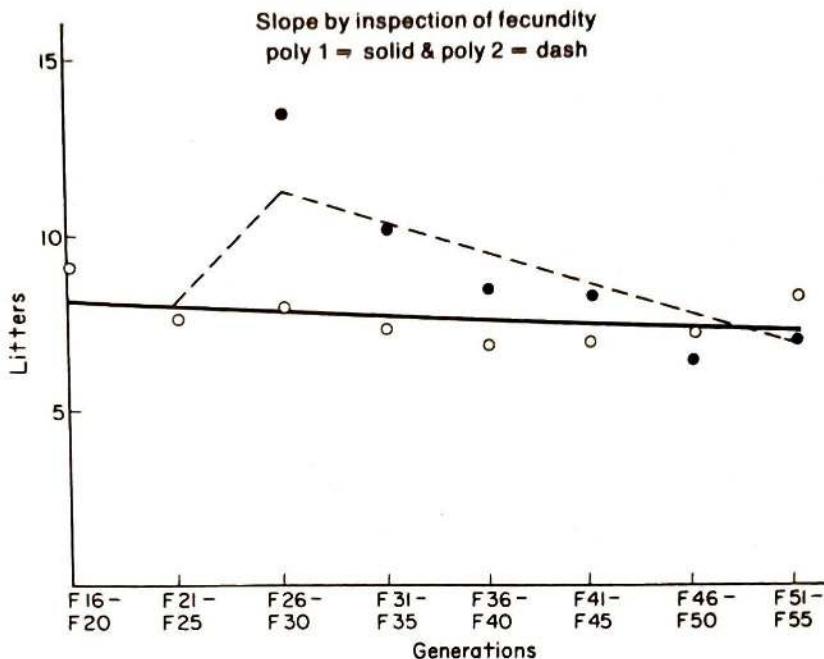


CHART 53. Comparison—scattergram.

This character increased significantly from 590 days to 700 days in female mice between F₂₆-F₃₅. The difference between the two values is $10.2 \times P.E.$ There has been a decline in longevity in female mice for both descents which is still a significant difference—the female mice of Poly 2 outlive the female mice of their ancestral strain, Poly 1, between F₃₆-F₄₅ by a value which is $6.4 \times P.E.$.

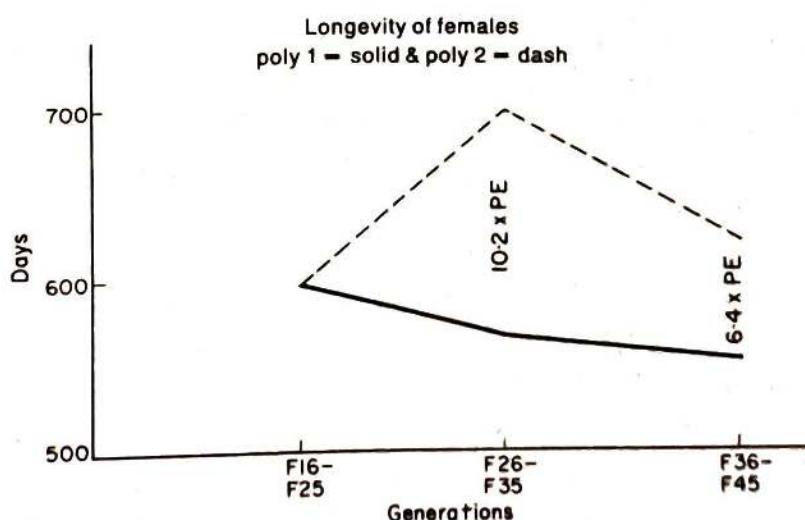
CHART 54. Longevity (freedom from tumors). Comparison of Poly 1 females and Poly 2 females for twenty-nine generations, F₁₆-F₄₅.

Chart 55 presents the data on longevity for female mice of the two descents in another manner. Here there is a comparison of the data on longevity between $F_{16}-F_{25}$ and $F_{26}-F_{45}$. It can be seen that the female mice of the Poly 1 (solid line) descent die earlier in $F_{26}-F_{45}$ than do female mice of the Poly 2 descent. Female mice of the Poly 2 (dash line) descent actually are improving in longevity. The difference in longevity between female mice of the two descents is $2.8 \times P.E.$ — a value which is not significant

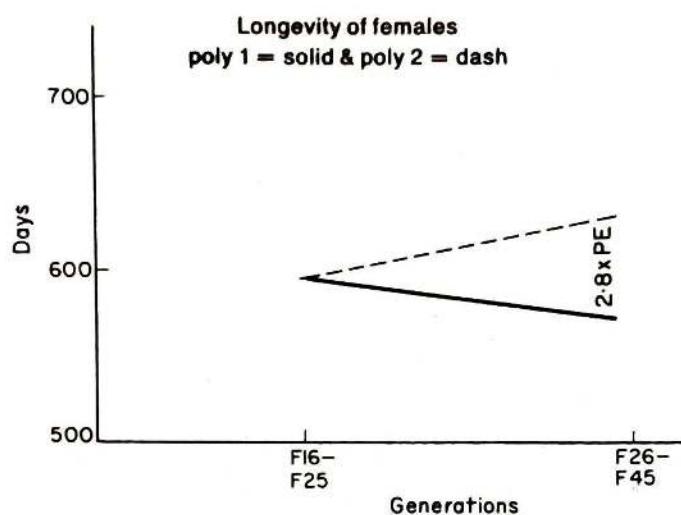


CHART 55. Comparison of Poly 1 females and Poly 2 females on a two group basis, $F_{16}-F_{25}$ and $F_{26}-F_{45}$.

but obviously is approaching significance (difference assumed significant when a value of $3 \times P.E.$ is obtained).

Chart 56 presents the data on longevity of female mice of the two descents between $F_{26}-F_{45}$ by another method. Here the data are given by the frequency distribution method. Data for female mice of the Poly 1 descent are again given on the solid line and for female mice of Poly 2 on the dash line. Here the curve of distribution for female mice of Poly 2 is somewhat to the right of that for female mice of Poly 1 — hence it can be concluded that female mice with 1st (Poly 2) outlived the female mice of Poly 1 that carry the gene LST.

Chart 57 gives the data on longevity of male mice for the two sublines under consideration. Males of Poly 1 are on the solid line; males of Poly 2 are on the dash line. Between $F_{16}-F_{25}$ and $F_{26}-F_{35}$ the two sublines are alike in this character. However, with further inbreeding between $F_{36}-F_{45}$ there is a very significant difference for longevity which is $10.7 \times P.E.$

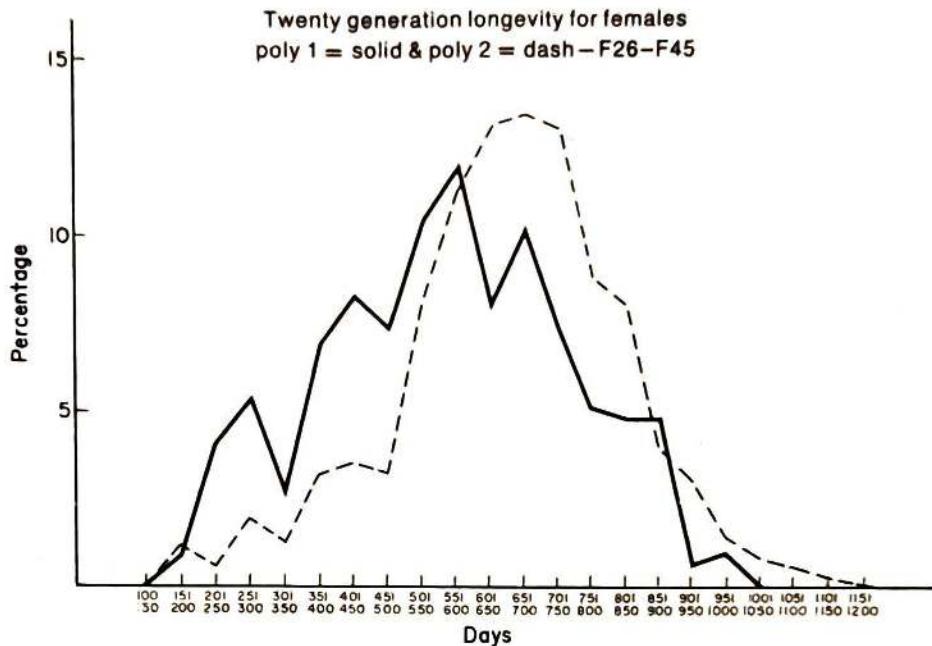


CHART 56. Twenty generation comparison for Poly 1 females and Poly 2 females, F₂₆-F₄₅.

Chart 58 presents the same data on longevity in male mice where it can be seen that between F₂₆-F₄₅ there is a significant difference in this character between male mice of the two sublines which had had a common origin.

Chart 59 shows frequency distributions on longevity for male mice of Poly 1 and Poly 2. Thus, male mice of Poly 1 outlived male mice of Poly 2 between F₂₆-F₄₅.

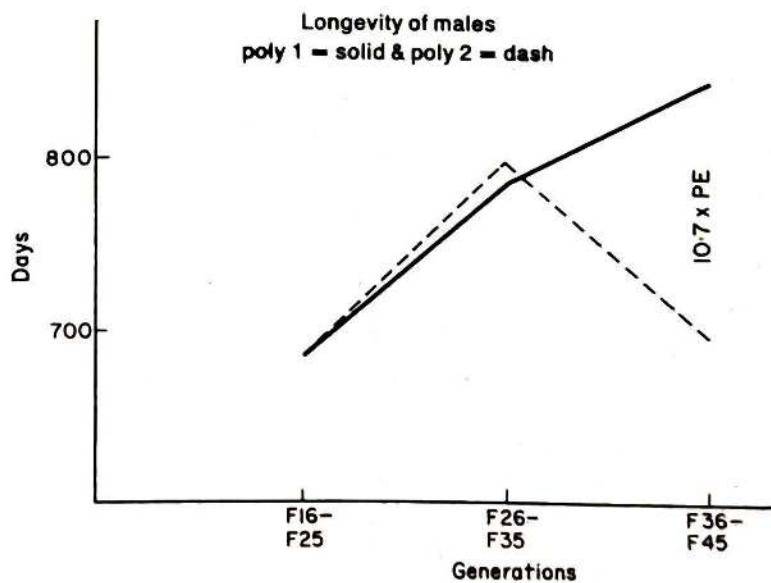


CHART 57. Comparison of Poly 1 males and Poly 2 males for twenty-nine generations, F₁₆-F₄₅.

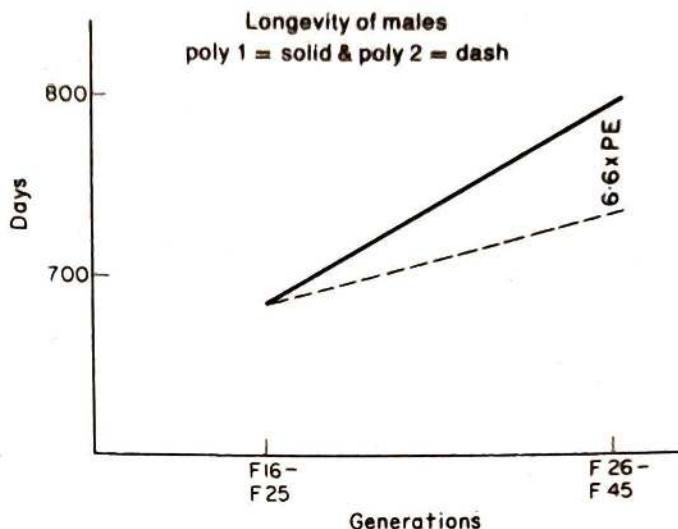


CHART 58. Comparison of Poly 1 males and Poly 2 males on a two group basis, F₁₆-F₂₅ and F₂₆-F₄₅.

Chart 60 shows the frequency of spontaneous mammary gland tumors in female mice of Poly 1 (solid line) and Poly 2 (dash line) in successive groups of ten generations during the process of inbreeding. Females of Poly 1 developed about 45 percent mammary gland tumors during this time, whereas female mice of Poly 2 with the gene 1st developed about 6 percent of similar tumors.

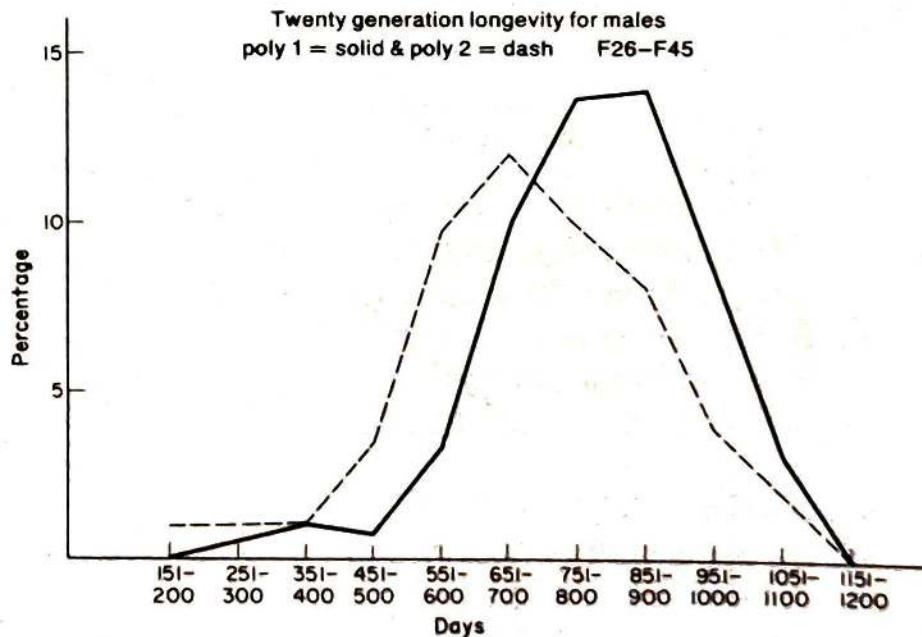


CHART 59. Twenty generation comparison for Poly 1 males and Poly 2 males, F₂₆-F₄₅.

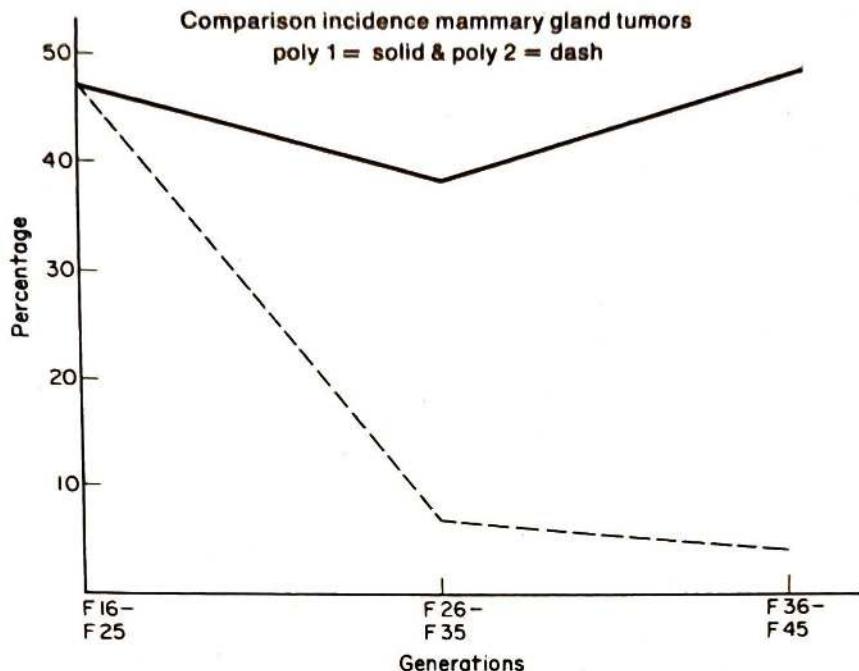


CHART 60. Comparison of Poly 1 and Poly 2 for twenty-nine generations, $F_{16}-F_{45}$.

Chart 61 combines the data on mammary gland tumors for $F_{26}-F_{35}$ and $F_{36}-F_{45}$ in order to emphasize the difference in this character between the female mice of these descents—one with

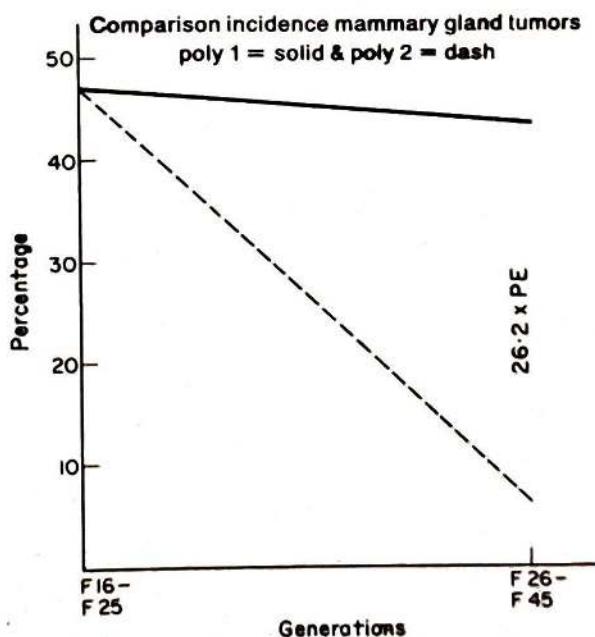


CHART 61. Comparison of Poly 1 and Poly 2 on a two group basis,
 $F_{16}-F_{25}$ and $F_{26}-F_{45}$.

LST and the other with 1st. The difference in the incidence of mammary gland tumors is $26.2 \times P.E.$

Chart 62 gives the data on the frequency distribution of the age of onset of mammary gland tumors in female mice of the two strains of mice. Data for Poly 1 are on the solid line; those for Poly 2 on the dash line. The data on Poly 1 appear to be normal with a high point between 601 and 650 days of age. The

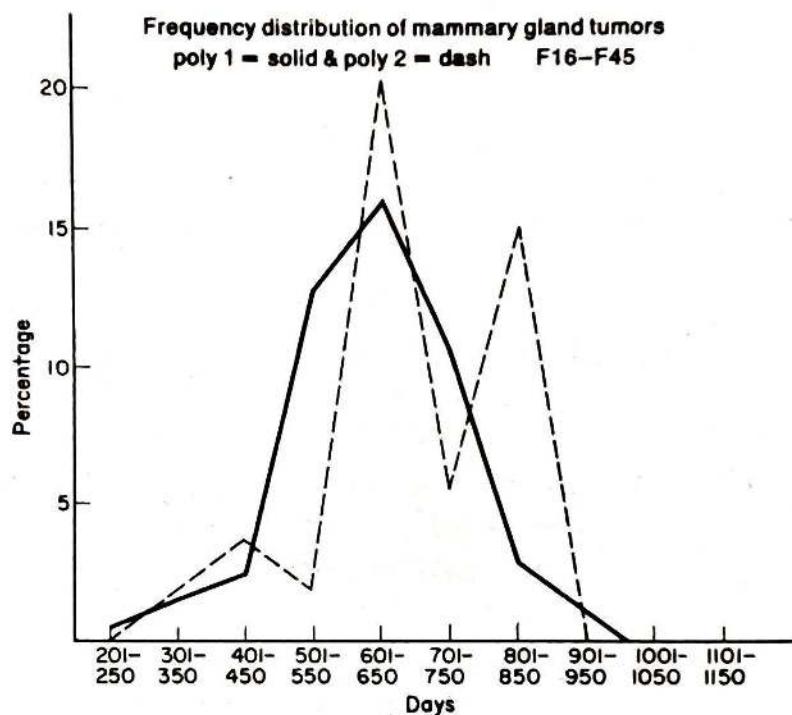


CHART 62. Frequency distribution on Poly 1 and Poly 2 for $F_{16}-F_{45}$.

data for female mice of Poly 2 are somewhat later in age of onset but the curve is quite irregular due, in part, to the small number of mice developing this type of neoplasia.

Chart 63 presents the data on age of onset of mammary gland tumors for female mice of Poly 1 and Poly 2. The data are tabulated into three classes: $F_{16}-F_{25}$ (common to both descents), $F_{26}-F_{35}$ and $F_{36}-F_{45}$.

Chart 64 compares the data on the age of onset of mammary gland tumors for Poly 1 and Poly 2 into two groups, $F_{16}-F_{25}$ (common to both) and $F_{26}-F_{45}$ after independent descent. In each case, mammary gland tumors are appearing later in life between $F_{26}-F_{45}$ than they did in $F_{16}-F_{25}$. The two curves are very similar with no obvious differences between them.

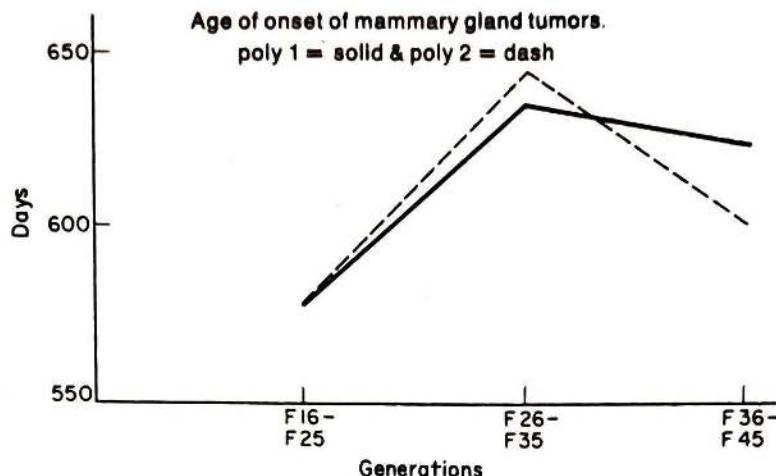
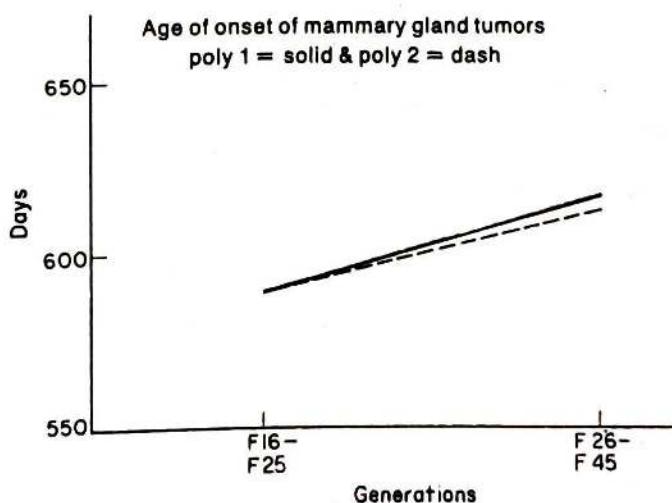
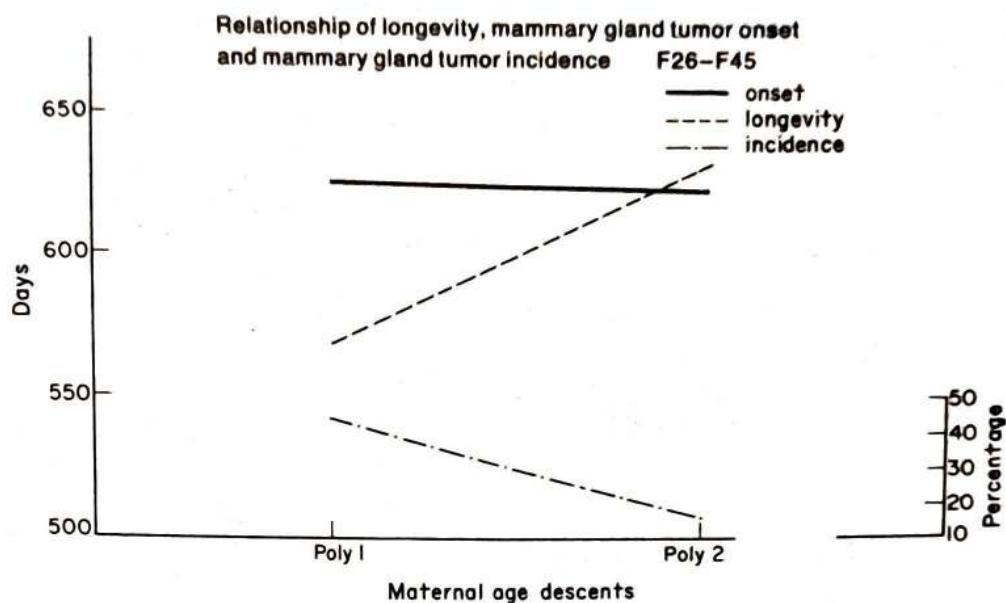
CHART 63. Age of onset of Poly 1 and Poly 2 for F₁₆-F₄₅.

Chart 65 shows the comparative analysis of the data bearing on the relationship between longevity (i.e. free of tumors), mammary gland tumor onset and mammary gland tumor incidence. The measures of longevity (dash line) and age of mammary gland tumor onset (solid line) are on the left scale whereas the measure of the percentage incidence of mammary gland tumors is on the right scale (dot and dash line). The three points for the data on these characters for Poly 1 are on the left, whereas the comparable points (three) for Poly 2 are on the right.

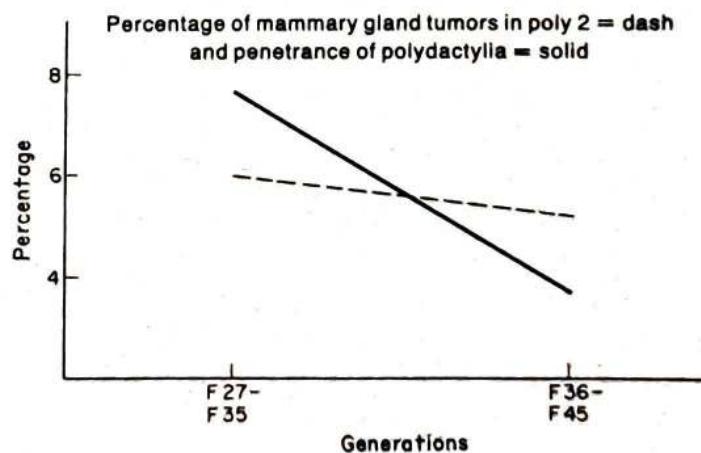
Chart 66 shows the percentage of mammary gland tumors and the penetrance of polydactylia in female mice of the Poly 2

CHART 64. Age of Poly 1 and Poly 2 using a two group basis, F₁₆-F₂₅ and F₂₆-F₄₅.

CHART 65. Comparison of Poly 1 and Poly 2 at F₂₆-F₄₅

descent at two levels of inbreeding (F₂₇-F₃₅ and F₃₆-F₄₅). Both values decreased between these two levels of inbreeding. However, it can be seen that the slope for the loss of polydactylia penetrance is more precipitous than that obtained for the percentage incidence of mammary gland tumors.

Chart 67 presents the data on the percentage incidence of lung tumors in female mice of the two descents, Poly 1, solid line, and Poly 2, dash line. From a common origin in the group F₁₆-F₂₅ where the incidence was about 7 percent, this lesion diminished in frequency in female mice of both descents until it reached a value of less than 0.5 percent by F₃₆-F₄₅.

CHART 66. Mammary gland tumor incidence and incidence of polydactylia. Percentage of mammary gland tumors in Poly 2, F₂₇-F₄₅.

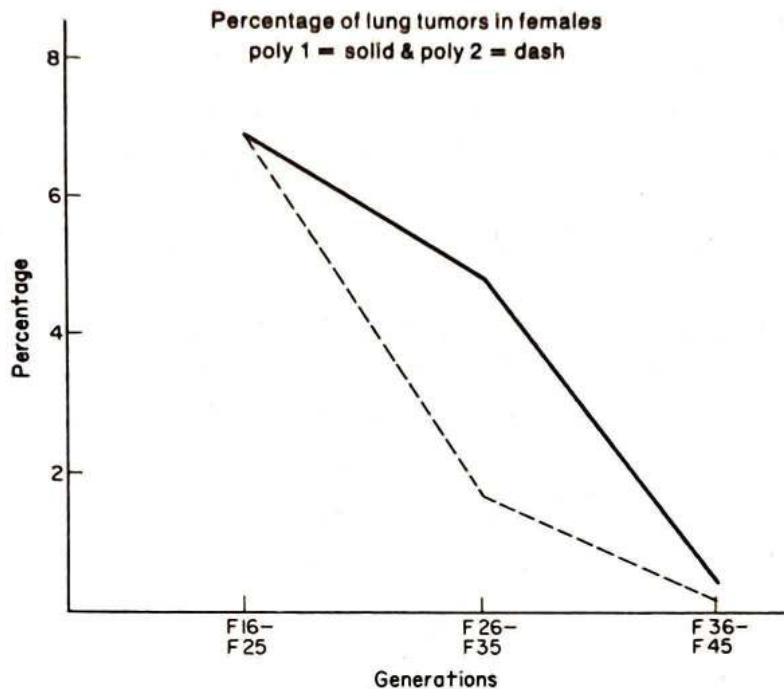


CHART 67. Percentage of lung tumors in females of Poly 1 and Poly 2 for F₁₆-F₄₅.

Chart 68 shows the data on lung tumors grouped together for two levels of inbreeding as follows: (1) F₁₆-F₂₅ which is the common point for both sublines and (2) F₂₆-F₄₅ where the two

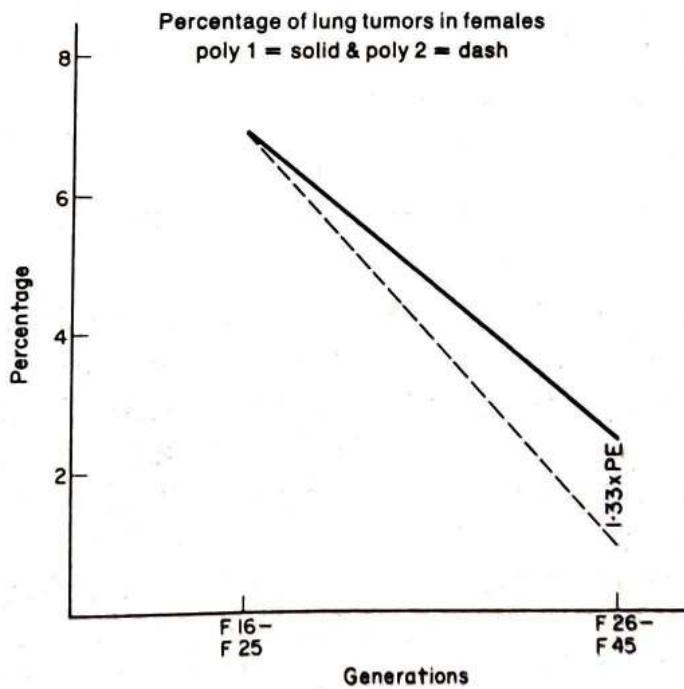


CHART 68. Penetrance of lung tumors in females of Poly 1 and Poly 2 on a two group basis, F₁₆-F₂₅ and F₂₆-F₄₅.

descents were separated (Poly 1 on solid line and Poly 2 on dash line). The difference between the incidence of lung tumors between the two descents is only $1.33 \times P.E.$ and is, therefore, not significant. Thus it can be concluded that this neoplastic lesion decreased in frequency during the process of inbreeding of approximately the rate of decline in female mice of both but independent descents.

Chart 69 shows the similar data on the incidence of lung tumors in male mice of the two descents (Poly 1 solid line and Poly 2 dash line). The results are very similar to those obtained with

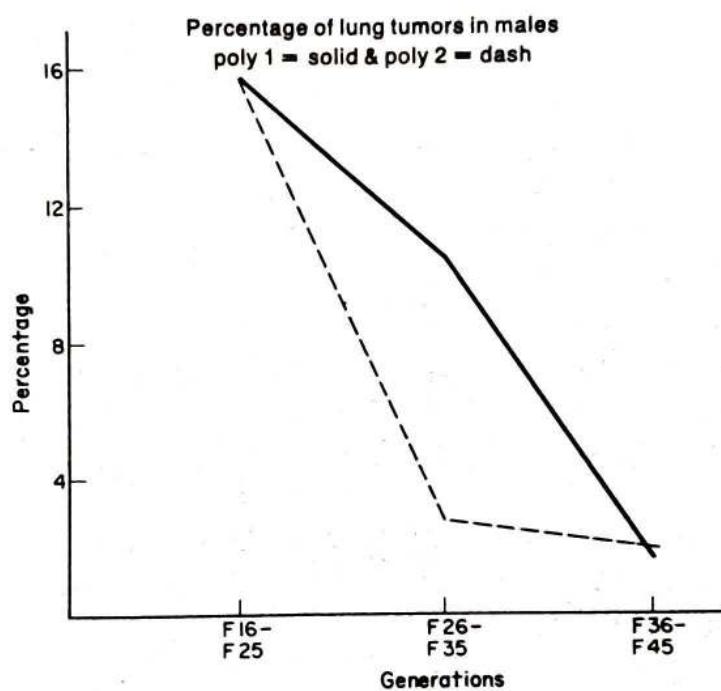


CHART 69. Percentage of lung tumors in males of Poly 1 and Poly 2 for F₁₆-F₄₅.

female mice. The only difference is the somewhat higher value of lung tumor incidence compared with females. (In F₁₆-F₂₅ about 16 percent males developed lung tumors and about 7 percent females.) The rate of decline in the incidence of lung tumors during the process of inbreeding is practically the same for males as it is for females.

Chart 70 presents the data on the incidence of lung tumors in male mice for the two descents (Poly 1 solid line and Poly 2 dash line). Here the difference in the penetrance of lung tumors between Poly 1 and Poly 2 in F₂₆-F₄₅ is $5.34 \times P.E.$ and, therefore, is significant.

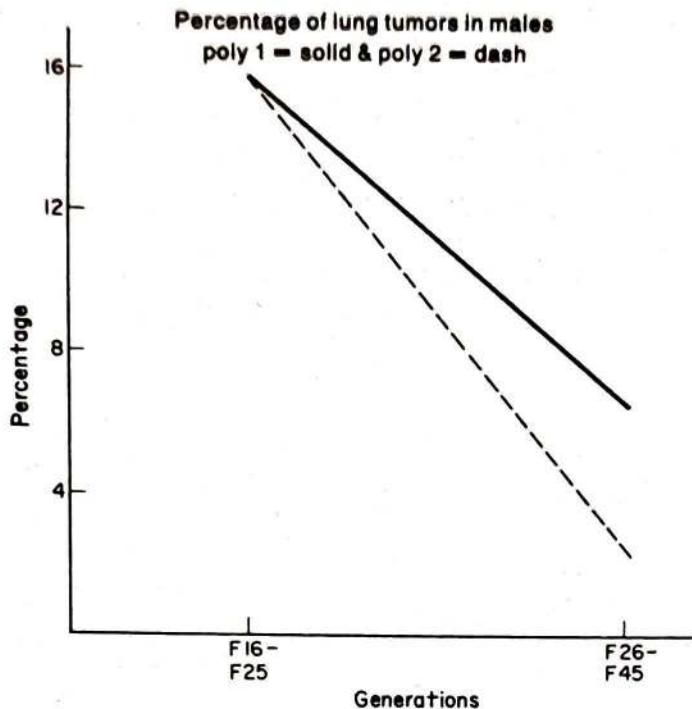


CHART 70. Percentage of lung tumors in males of Poly 1 and Poly 2 on a two group basis, $F_{16}-F_{25}$ and $F_{26}-F_{45}$.

Chart 71 shows the data on the age of onset of lung tumors in female mice of Poly 1 solid line and Poly 2 dash line. Lung tumors appear to come later during the process of inbreeding in female mice of the Poly 1 descent. The same trend is also present in female mice of Poly 2 but this cannot be finally concluded since there was only one lung tumor in female mice of Poly 2 between generations $F_{36}-F_{45}$.

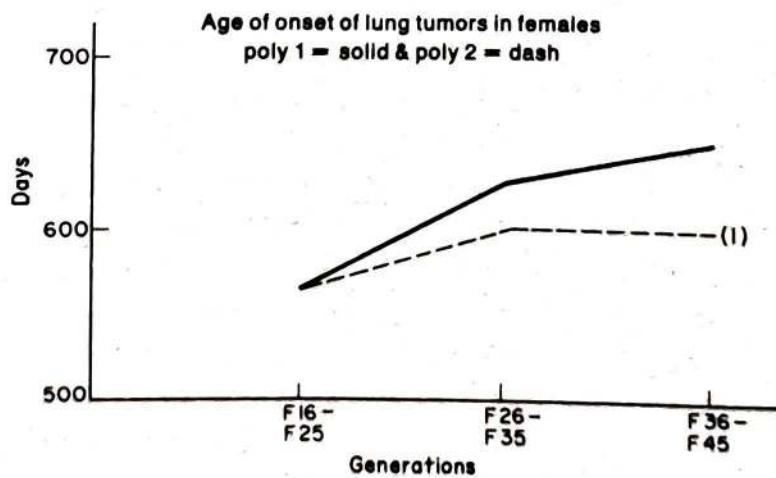


CHART 71. Age of onset in females of Poly 1 and Poly 2 for $F_{16}-F_{45}$.

Chart 72 shows the data on age of onset of lung tumors in female mice of Poly 1 (solid line) and Poly 2 (dash line) at two levels of inbreeding. The first point is at F_{16} - F_{25} when the two eventual groups were the same and the second at F_{26} - F_{45} when the two descents had been separated for several generations. The

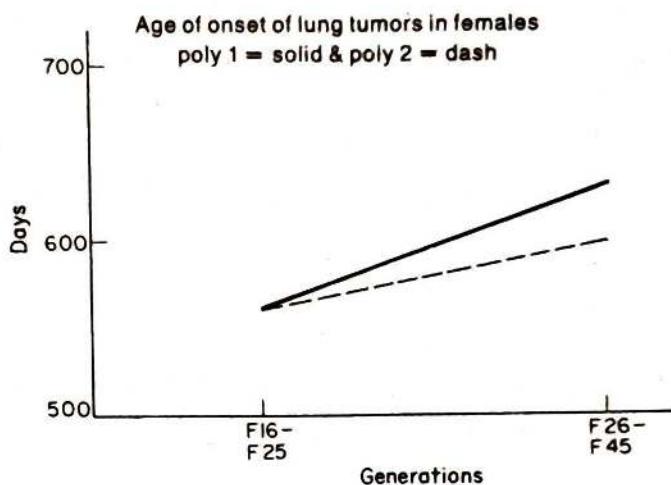


CHART 72. Age of onset in females of Poly 1 and Poly 2 on a two group basis, $F_{16}-F_{25}$ and $F_{26}-F_{45}$.

difference in age of onset of lung tumors between female mice of the two sublines is not significant.

Chart 73 gives the data on age of onset of lung tumors in male mice of Poly 1 (solid line) and Poly 2 (dash line). Here the age of

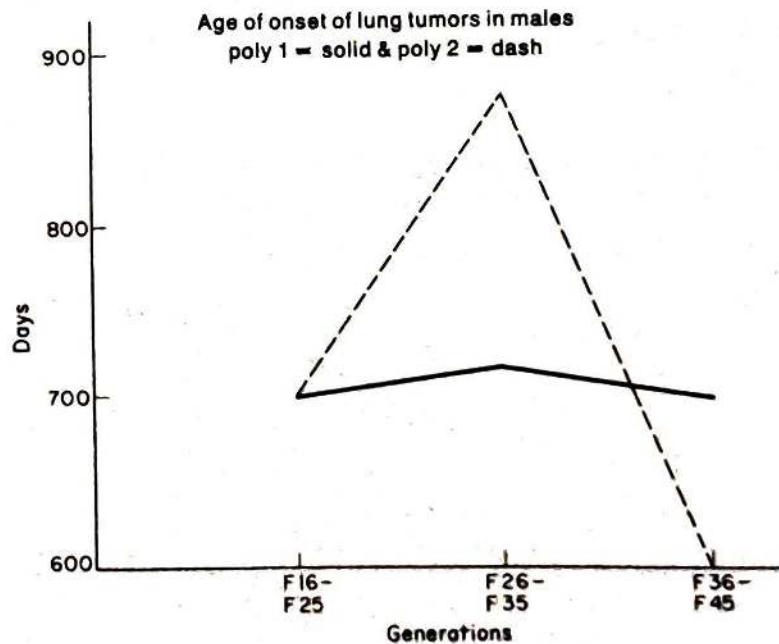


CHART 73. Age of onset in males of Poly 1 and Poly 2 for $F_{16}-F_{45}$.

onset of lung tumors appears to be greater in male mice of Poly 2 shortly after the separation of this subline than it is in male mice of Poly 1. However, the incidence of lung tumors was so low that this difference in age of lung tumors is not significant as indicated in Chart 74 where the data for $F_{26}-F_{35}$ and $F_{36}-F_{45}$ were combined together. The actual difference in age of lung tumors in Poly 1 and Poly 2 at $F_{26}-F_{45}$ is only $1.16 \times P.E.$ which is not significant.

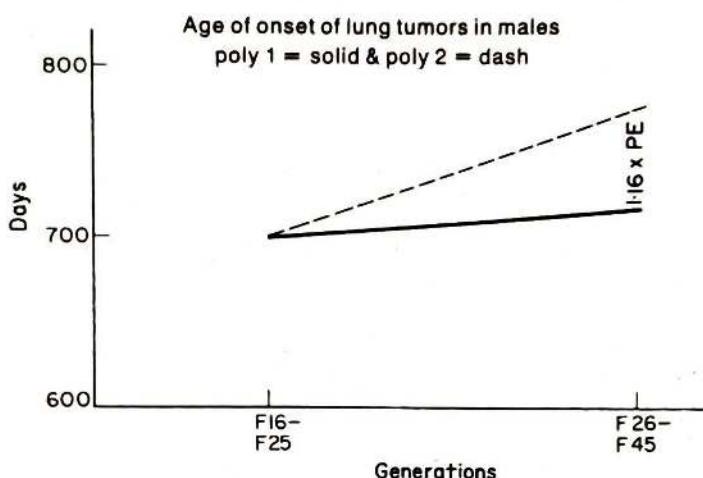


CHART 74. Age of onset in males of Poly 1 and Poly 2 on a two group basis, $F_{16}-F_{25}$ and $F_{26}-F_{45}$.

General Discussion

Already-published and the present data have produced evidence of biological variability and uniformity of several characters in the descendants of mice of two independent descents that have had a common origin of twenty-nine generations of restricted brother-to-sister matings. In some characteristics, the mice of the two descents show similarities. In others, there appear to be differences following the establishment of the second subline. Some of these differences such as fecundity, Chart 52, however, did not persist but seem to be lost in more advanced generations of inbreeding. But the most interesting fact was established that some characteristics such as age of first litters, Chart 43, longevity of females, Charts 54, 55, 56, and percentage incidence of mammary gland tumors, Charts 60, 61, 62, appear to be permanently altered following the separation of the two sublines.

The most obvious change that was encountered at the time of separation of Poly 2 from Poly 1 was the replacement of the gene LST by its recessive form 1st. There is always the possibility that some of these differences may be due to delayed segregation of some heterozygous alleles. But this seems unlikely since the mice were submitted to twenty-nine generations of restricted brother-to-sister matings before separation, and this procedure is supposed to produce a high degree of homozygosity.

Another factor must also be taken into consideration for the evaluation of the data. That is, that the mice of Poly 1 and Poly 2 descents were all the result of a continued selection toward young parents. All mice of these descents were from first or second litters only and thus all litters were born before the mothers were 100 days of age.

Data on many biological characteristics of the Poly 1 descent have shown that continued variability was encountered even in advanced generations of inbreeding following, however, a continuous selection toward early maturing parents. For example, as indicated in the introduction to this paper, the age of first litters was not established at a non-variable level even after fifty-four generations of restricted brother-to-sister matings. We quote from this earlier work, "after fifty-four generations of brother-to-sister matings, with selection for early maternal age and polydactylism, a relatively stable or predictable response with respect to age at first litter was not attained" (Strong, Johnson and Rimm, 1965).

There appear, therefore, to be at least two factors that need to be discussed in reference to these established facts. These are (1) a continued selection toward young parents and (2) the replacement of the pleomorphic gene LST by its recessive form 1st.

Of these two interpretations, it seems to be more evident that the replacement of LST by 1st may be more important, particularly with those characters which appear to arise suddenly at the time of a change from LST to 1st and continue to appear in mice of succeeding generations.

The pressure of continued selection toward an early parental age must have been fairly constant not only in the common descent of Poly 1 but also in Poly 1 and Poly 2 after the separation of the two descents, where the same method of mating was continuously employed.

We are, therefore, faced with the possibility that many of the biological characteristics are dependent upon the presence of either LST or *lst*. This conclusion does not seem to be too fantastic when one considers the pleomorphic effects of many morphological and physiological characters already implicated with LST.

Another set of data also need to be discussed. These data have a bearing upon uniformity of expression over a period of time or continued variability that is instability in the expressivity of a character. For example, Chart 44 shows the data obtained on litter spacing or time between subsequent litters when the sexes were kept continuously together. In Poly 1 (with LST) there was continued alteration or change in this character between F_{31} - F_{35} and F_{51} - F_{55} . Whereas mice of Poly 2 descent (with *lst*) had a very uniform expression of this character during the same period of inbreeding.

Litter size (Charts 48, 49, and 50) showed very constant values between F_{31} - F_{35} and F_{51} - F_{55} in Poly 2 (with *lst*) whereas this character showed continuous change in Poly 1, with LST, during the same period of inbreeding.

Fertility (Chart 51), on the other hand, showed a remarkable similarity (almost identical) between mice of the two sublines between F_{26} - F_{30} and F_{41} - F_{45} , but after this degree of inbreeding the two sublines appear then to deviate from each other.

Female mice of Poly 2 (with *lst*) outlived the female mice (with LST) with significant values. In F_{26} - F_{35} , this difference is $10.2 \times P.E.$ and in F_{36} - F_{45} it is $6.4 \times P.E.$ (Chart 54). This significant difference in longevity of mice bearing LST or *lst* is also illustrated in the frequency curves in Chart 56.

Longevity in males, however (Charts 57, 58, and 59), appear to be affected differently by the replacement of LST by *lst*. In F_{26} - F_{35} there was no difference in longevity of males between the two sublines whereas in F_{36} - F_{45} it would seem that male mice with LST outlived mice with *lst*. This observation opens up the possibility that the gene "LST" and its allele "*lst*" may have a differential effect upon the characteristics of the sexes.

The comparative analysis of the data on the appearance of spontaneous tumors of mammary gland origin in Charts 60, 61 and 62 also discloses some very significant facts. In F_{16} - F_{25} of Poly 1 (with LST) this neoplastic lesion appeared in 48 percent of all females; in F_{26} - F_{35} the penetrance was reduced to 41 percent;

in $F_{36}-F_{45}$ the incidence of mammary gland tumors was 51 percent. In female mice of Poly 2 (with *lst*) there was a precipitous drop in the appearance of mammary gland tumors to about 8 percent. This lesion then became less frequent in appearance and, in $F_{36}-F_{45}$, occurred in only about 5 percent of the female mice. As indicated earlier, the degree of difference in the incidence of mammary gland tumors between Poly 1 (with LST) and Poly 2 (with *lst*) was $26.2 \times P.E.$ Thus, there is a strong correlation between the appearance of the pleomorphic gene LST and a high susceptibility to spontaneous tumors of mammary gland origin. The low incidence of mammary gland tumors in Poly 2 is not associated with an earlier appearance of death since according to Charts 62 and 63 when mammary gland tumors did arise in female mice of Poly 2, they did so at approximately the same age as those of Poly 1, and the data of Chart 54 demonstrate that female mice of Poly 2 (with *lst*) which did not develop spontaneous mammary gland tumors actually outlived the female mice of Poly 1 (with LST).

The occurrence of spontaneous lung tumors appear to be quite similar in both female and male mice of Poly 1 and Poly 2. In both sublines the appearance of this lesion was more frequent in the males than in females. The lesion dropped out at approximately the same rate of decline during the process of inbreeding. By $F_{36}-F_{45}$ lung tumors became practically a rare lesion in both sublines.

In attempting to clarify what appears to be a very complex situation of biological facts, one can visualize one interpretation as follows. The facts briefly stated are these. Mice bearing the gene "*lst*" appear to have characteristics that do not vary much, in mice of successive generations, at least between F_{29} and F_{45} . The mice may thus be interpreted as being in biological equilibrium. On the other hand, mice bearing the gene "LST" are more variable in several characteristics during the same period of inbreeding. The mice may then be said to be unstable. As a result of this unstable state, with LST, mammary gland tumors are more frequent and female mice without tumors die younger than do female mice bearing the "*lst*" gene. There is also evidence that mice bearing "LST" have first litters earlier, and bear subsequent litters more frequently than female mice bearing the stabilizing gene *lst*.

The simplest interpretation, therefore, for the large number of data already obtained is that the LST gene increases the tempo or rate of biological activity by which more instability may be forthcoming. On the other hand, mice bearing "lst" have a lower threshold of biological activity and hence possess more stability or are in better equilibrium.

An earlier death or more frequent mammary gland tumors appear to be the resultant of this increased tempo. A longer life and fewer tumors are the resultant of biological equilibrium produced to some extent by a lower threshold of change conditioned by the presence of the "lst" gene.

Conclusions

1. In addition to the biological effects during embryological development and morphogenesis of the pleomorphic gene LST and its allele lst, these genes have other effects on the organism, particularly associated with the reproductive capacity (age of first litters, litter frequency, fecundity, etc.), longevity and the incidence of spontaneous tumors of mammary gland origin.
2. In almost all cases investigated the LST gene has a stimulatory effect (earlier first litters, more rapid production of successive litters, a shorter life span and higher incidence of spontaneous tumors) and the "lst" gene a lessened effect on these same characters.
3. The increased tempo or rate of biological activity produced by LST increases also the appearance of instability in the measure of several biological characteristics during successive generations of inbreeding. The lessened tempo or rate of biological activity by lst produces a more stable manifestation of several characters, as measured by continuous uniformity in successive generations, i.e. equilibrium.
4. A shortened life span and a higher incidence of spontaneous mammary gland tumors are manifestations of an unstable biological "constitution".
5. Conversely, a long life span and fewer tumors of mammary gland origin are manifestations of a more stable biological condition.
6. There may be a differential effect of LST and lst between the sexes. This conclusion is based upon the observations that

female mice with "Ist" outlived female mice with LST, but male mice with "Ist" apparently lived shorter times, compared to those male mice bearing "LST".

7. The evidence suggests that the LST gene is one that regulates the level of biological activity—not only during embryological development but also, perhaps, during the entire postnatal existence of the individual.

CHAPTER 5

Observations on the Control of Spontaneous Tumors in Mice*

ATTENTION was drawn to the effect of the aging process on biological characteristics in the preparation of a doctoral dissertation published in 1922 (Strong). This research was on tumor susceptibility and/or resistance. Since there were no inbred strains of mice in existence at that time, it was decided to inoculate transplantable tumors from one group of mice into wild mice caught in the vicinity of The Carnegie Institution of Washington at Cold Spring Harbor, Long Island, New York. It was assumed at that time that, due to the system of matings in wild mice with a single dominant male being the father of most of the offspring in a restricted area, a certain degree of inbreeding would be the result, thus leading to some degree of biological uniformity.

It was found that "when a non-susceptible race of mice is employed, susceptibility toward transplantable tissue decreases with age up to the period of maturity" (Strong, 1922), and further that after a constant level of tissue reactivity during sexual maturity, "the interesting point was discovered in our experiments that susceptibility to transplantable tumors (for a non-susceptibility race) increases with old age" (Strong, 1922).

Thus it was concluded that "with maturity the animal manifests its most pronounced biological characteristics". Further, that "The susceptibility curve toward transplantable tumors suggested the possibility of analyzing the factors which underlie tissue specificity more carefully by removing the gonads from individuals in the different age groups before inoculating them with the

*Lecture given the trainee program of The Roswell Park Memorial Institute, Biological Station, Springville, New York, July 1965, and presented, in part, in Heidelberg, Germany, for the 75th birthday celebration of Professor K. H. Bauer, 21 September 1965.

transplantable tumor". Finally, "The failure of old gonadectomized individuals to show any reactions to implants may be explained partly on the decreased physiological activity of old animals, and partly as a result of the genetic composition which they, in common with all adult animals of their race, possess" (Strong, 1922).

A possible clue to the resistant and/or susceptible state to cancer was discovered in 1936 (Strong, 1936; Strong and Werner, 1936a, 1936b) with precipitation tests employing trichloracetic acid on whole blood which later were changed to work on hemoglobin (Strong and Francis, 1937; Francis and Strong, 1938; Strong, 1938; Strong and Francis, 1940), Chart 78.

In a third paper dealing with trichloracetic acid precipitates on whole blood entitled, "A Disturbance Between Two-Hundred and Three-Hundred Days of Life", it was concluded that mice between 201-300 days of life (while they were in the prime of life) had a different type of curve in the final precipitate reading when compared to young mice between 101-200 days of age and old mice between 301-600 days (Chart 75).

Comparative analysis of precipitates also indicated a possible correlation between mice showing different degrees of susceptibility to spontaneous tumors (Charts 76 and 77) (Strong, 1940).

Later it was decided to test out the toxic effect of salycilaldehyde on mice of different ages and belonging to the various inbred strains which by then (1940) had been well established. One of the conclusions reached was "the tolerance of A/St male mice reaches the maximum value at that age when the hemoglobin level is highest, i.e. between 101-200 days of life". Following that period of tolerance of salycilaldehyde decreased (Chart 79). Another conclusion was that "the C₃H/St, A/St, and JK/St strains of mice can be classified in the same sequence according to (a) tolerance of the males to salicylic aldehyde, (b) shifts of hemoglobin per unit of time and (c) the tendency for breeder female mice to give rise to, or not to give rise to, spontaneous tumors of the mammary gland" (Strong, 1940).

Evidence has recently been obtained that biological variability in several characteristics, such as age of first litters, reproductive capacity as measured by litter frequency, fertility, fecundity, etc., is altered through the process of inbreeding, as expected by the laws of inbreeding. This rate of stabilization is, however, neither

uniform nor random in several independent descents, even from a common origin, and appears to be influenced by maternal age selection. Part of this divergence of sublines is due, no doubt, to mendelian segregation and recombination. What else that could be involved is challenging.

For example, even after sixty generations of restricted brother-to-sister matings in an early maternal age selection descent (< 100 days), the age of first litters has not been stabilized. Again, in a descent in which the parents were between 201–300 days at the time the offspring were born, reproductive capacity as measured by litter frequency and litter size appears to be quite uniform even through several successive generations of inbreeding, i.e. only partially inbred between F_{15} and F_{25} .

All, except one, of the various maternal age selected sublines have lost reproductive capacity as measured by fertility (total number of offspring produced when the two parents were continuously together from birth to death of one) at different rates through the process of inbreeding. For example, between F_1 – F_5 and F_{21} – F_{25} fertility has changed from 76.5 to 40.5 mice per female, a difference of –36.0 young in the < 100 maternal age class; from 66.0 to 47.2 or a loss of 18.8 mice in the 101–200 maternal age class; and in the 301–400 maternal age class fertility changed from 65.5 mice to 56.8 or a loss of 8.7 mice between F_1 – F_5 and F_{16} – F_{20} . Mice of this last descent have not reached F_{21} – F_{25} generations of inbreeding yet.

In contrast, however, fertility changed from 60.1 mice to 60.8 mice or a gain of 0.7 mice between F_1 – F_5 and F_{21} – F_{25} in the 201–300 maternal age class.

As a matter of fact, the descent from parents of the 201–300 maternal age class are more uniform in numerous biological characteristics than the descents of very early or very late maternal age classes—providing, of course, that the data are compared for the different descents at the same level of inbreeding. In addition, mice of this 201–300 maternal age selected descent will live longer relatively free of tumors and, when tumors arise, these mice develop fewer of them and at later ages of onset than the offspring of parents on either side of this 201–300 maternal age class.

Thus the concept must be entertained, although considerably more work should be done, that biological stability and/or instability may be involved in the origin of cancer since cancer is

less frequent in descents of mice where a greater degree of biological stability has been demonstrated by the analysis of a various number of biological characteristics — and more frequent where biological instability is apparent.

Throughout this extended maternal age selection experiment, the morphological character of polydactylia (LSTLST) has occurred in the vast majority of individuals (80–100 percent penetrance). Since this gene has several pleomorphic effects, it seemed reasonable to conclude that perhaps this gene may also be involved in or have some effect on biological stability and/or instability involving other characteristics than the obvious morphological components of the polydactylia syndrome.

Consequently, several attempts have been made to free a highly inbred (a relatively high state of homozygosity) descent of this gene. In all cases where this procedure has been successful, the descent loses some vigor (smaller litters and at more advanced ages, etc.) and become more stable than mice of the strain of the origin with LST. Here the original descent and the subline freed of polydactylia are at the same level of inbreeding, in one case at F_{25} and in another at F_{45} .

Thus it appears that in addition to genetic recombination there are two biological influences on biological stability and/or instability. These are (1) maternal age and (2) polydactylia. Just how these influences function through the genetic phenomena of heterozygosity and/or homozygosity is not yet clear.

In order to test out the possibility that a "stabilizing" factor may be present in mice of certain descents, tissue extracts have been prepared and some of them have been injected into mice bearing spontaneous tumors of mammary gland origin (C_3H/St and a subline characterized by microphthalmic anemia C_3HB/St) as test animals.

It is not the present intention of recording all these attempts — most of them being entirely ineffectual on the growth rate of tumors and survival time of mice bearing these spontaneous tumors.

One series alone has shown some promising leads or a favorable effect on spontaneous tumors and this series will be partially discussed now. Following several modifications of techniques of preparation of liver, extractions and separations of components by various means, the following techniques have been developed.

Modifications of technique of preparation are constantly being considered.

Mice belonging to the 201-300 maternal age descent, where great biological stability has been demonstrated, have been killed at different ages and their livers ground in a Virtis homogenizer in 80 percent alcohol.

Starting with Experiment 7D on page 89 and continued to date, the ground material was washed several times with 80 percent alcohol. The collected washings were then evaporated under negative pressure using dry ice (lyophilized). The desiccated residue was washed several times with 100 percent alcohol and the washings collected. The residue not soluble or removed by 100 percent alcohol was then taken into a standard amount of distilled water, depending upon the number of livers used (2 cm^3 distilled water to one liver). This procedure formed a whitish or yellowish opaque solution and was given the symbol W (water soluble) although it is obvious that this was not a true solution. There was a clearing of the solution by standing in a refrigerator. The 100 percent alcohol moiety was again evaporated by reduced pressure with dry ice and then shaken with a measured quantity of distilled water depending upon the number of livers used, 2cm^3 to one liver. These mixtures varied in appearance but usually were pearly white and were given the symbol A (alcohol solution). Here again the mixture is obviously emulsoid, which thickened upon standing in the refrigerator.

Thus we had extracts of liver which belonged to the W and the A series at several ages of mice (from both parents and offspring).

Procedures for Liver Experiment

Experiment No. 1

Livers pulverized by placing in freezer (later carbon dioxide), with hammer and steel block, and placing frozen liver on cold block and striking with hammer.

Pulverized liver material then placed in water.

Then rinsed with water.

Water decanted and "filtrate" placed in alcohol.

Water solution ready for injection.

Experiment No. 2

Livers frozen and pulverized as above (No. 1).

Materials placed in alcohol then rinsed with alcohol.

Alcohol decanted.

"Filtrate" placed in water and thymol.

Alcohol evaporated.

Alcohol soluble residue taken in sesame oil.

This oil solution ready for injection.

Experiment No. 3

201-300 livers (from mice 201-300 days of age), frozen in carbon dioxide, ground with mortar and pestle.

Washed three times with 20 cm³ of alcohol (each wash).

Alcohol decanted.

Liver residue left in refrigerator to dry.

To dried liver added 100 cm³ of water and thymol.

10 cm³ ether added to water liver solution.

Separated ether and water with separatory funnel. Repeated three times.

Cloudy ether emulsion left in refrigerator to dry.

Water solution left to evaporate excess ether.

Experiment No. 3 started, injection of 1 cm³ of water solution.

Experiments No. 4 and No. 5

Prepared as in Experiment No. 3.

Experiment No. 6

Eight livers frozen with carbon dioxide.

Livers ground with mortar and pestle.

Ground liver rinsed three times with alcohol.

Residue placed in refrigerator to dry overnight.

100 cm³ water added to dry liver residue and refrigerated.

Decanted water solution made ready for injection of 1 cm³.

New solutions made periodically (2 weeks).

Experiment No. 7 Series A

Ten (201-300 class) livers homogenized by Virtis in distilled water.

Centrifuged under refrigeration 40-minutes at 5000 rev/min.

Supernate diluted with 75 cm³ water and thymol.

Residue collected and stored in 100 percent alcohol.

Experiment No. 7 Series B

Prepared as above.

Residue added to residue of 7A and stored in 100 percent alcohol.

Experiment No. 7. Series C

Homogenized ten livers in water, then added water to make 100 cm³ and let stand in refrigerator overnight.

Solution then centrifuged at 5000 rev/min.

Supernate decanted as 7C.

Residue added to residue 7A and B in 100 percent alcohol.

Color of above solutions dark mauve.

Note. From these solutions mice received masses in peritoneal cavity, thus turned to subcutaneous injections.

Experiment No. 7. Series D

Homogenized ten livers in 80 percent alcohol (100 cm³ total).

Left in refrigerator overnight.

Alcohol and liver material separated overnight.

Alcohol decanted as 7DA.

Washed two more times with 50 cm³. 80 percent alcohol each time.

Both washings added to 7DA.

Precipitate centrifuges and supernate added to 7DA.

100 cm³ distilled water added to precipitate after allowing alcohol to evaporate from precipitate.

Water precipitate solution mixed and placed in refrigerator.

7D solution in water settled leaving water solution.

This is centrifuged at 5000 rev/min for 30 minutes.

This supernate becomes 7D.

Experiment No. 7. Series E

Ten (201-300 class) livers removed and homogenized in 80 percent alcohol, 100 cm³ total.

Left in refrigerator overnight.

Decanted supernate, washed twice with 50 cm² 80 percent alcohol each wash.

Collected three supernates of 80 percent alcohol as 7EA.

Residue left to dry until all alcohol is gone.

Then 100 cm³ water added to residue.

The residue and water mixed and let stand overnight.

Then 7E solution centrifuged at 5000 rev/min for 30 minutes.

Supernate (water) decanted, thymol added, used as solution 7E.
Residue left in refrigerator.

Experiment No. 7. Series F

Solution 7F prepared as in 7E.

Eight 201-300 class livers used.

Total alcohol for experiments 7D, 7E, 7F, 20 cm³ per liver used.

10 cm³ water per liver used.

80 percent alcohol added to alcohol supernate alcohol solution
in Experiment 7E.: Solution 7EA + 7FA = solution 7EFA.

Experiment No. 7. Series G

Same as F.

Alcohol solution equals 20 cm³ per liver added to solution
7EFA making solution 7EFG-A.

Water supernate makes solution 7G.

Thymol added to ALL water solutions.

Note. It is not noted here that evidently the 80 percent alcohol
solution (now 7EFG-A) is being evaporated daily under
negative pressure.

Experiment No. 7. Series H

Prepared like 7F.

20 cm³, 80 percent alcohol per liver.

10 cm³ water per liver.

Alcohol washes (7H) added to 7EFG-A, making solution
7EFGH-A.

Water solution used as 7H.

Experiment No. 7. Series I

Prepared as 7F.

20 cm³ 80 percent alcohol per liver.

10 cm³ water per liver.

Alcohol (7I) added to 7EFGH-A making solution 7EFGHI-A.

Water solution used as 7I.

Experiment No. 7. Series J

Prepared like 7F.

20 cm³ 80 percent alcohol per liver.

10 cm³ water per liver.

Alcohol washes (7J) added to 7EFGHI-A, making solution
7EFGHIJ-A.

Water solution used as 7J.

Experiment No. 7. Series K

Prepared like 7E.

Alcohol solution (7K) added to 7EFGHIJ-A, making solution
7EFGHIJK-A.

Water solution used as 7K.

Experiment No. 8

Solution 7EFGHIJK-A split into two fractions.

First fraction evaporated to dryness.

Water added to residue putting entire material into one
solution.

This is the combined series.

Injections were subcutaneous.

Ulcerations forced injections back to intra-peritoneal.

Note. 201-300 class livers used but mice < 200 days when
sacrificed.

Experiment No. 9

Second fraction from solution 7EFGHIJK-A evaporated to
dryness. Residue weighed 0.3144 grams.

30 cm³ 100 percent alcohol added to remove all alcohol soluble
material.

Remaining material weighed 0.1896 grams taken up in 30 cm³
distilled water gave us solution 9W.

Experiment Nos. 9A², 9W², 9A³, 9W³

These experiments were prepared as in 9A and 9W.

The only major difference was in the washing.

Instead of washing only once with a given amount of alcohol a
series of washes (3) with an unspecified amount of alcohol
was used.

9W² was not used for experiments.

Experiment Nos. 10AW and 10W

The 10 series solutions were prepared as were 9A², 9W², 9A³ and 9W³.

One difference existed—9 series were of 201–300 females of the 201–300 age group but the animals had not reached 200 days when sacrificed.

The 10 series mice were sacrificed after the mice were 200 days of age.

Experiment No. 11

Due to observations from the previous experiments this experiment was formulated.

It was observed that first No. 8 was quite effective. This was a "combined" series so first an experiment was designed to test again the "combined" effects of the two components AW + W. Therefore, 10AW and 10W were injected on alternate days.

This is experiment 11¹.

Further observations indicated 9W³ to be more effective than 10W and that 10AW (the alcohol component of No. 10) to be more "potent" than the alcohol component of No. 9 (9A¹, 9A², 9A³).

Therefore, alternate injections of 9W³ and 10AW were started in 11².

The thinking behind this is that there may be two components in liver for regulation.

The first is effective when the mouse is young (< 200 days, for example, in accordance with the design of the 9 series) and soluble in water. The second is effective in animals > 200 days (as in the 10 series) and is soluble in alcohol.

The 11 series is a series of alternate injections of water and alcohol soluble components.

Note. Dilution series 11³.

Experiment No. 12

This experiment in order to follow up No. 11 was designed for the preparation of two solutions to be injected alternately.

The first used offspring of the 201-300 class mothers which were more than 300 days of age when the offspring were born (320).

These mice were aged between 250-300 days and then sacrificed.

The preparation of the solution is the same as in 9 and 10 except the washes were 300 cm³ of 80 percent alcohol per wash, seven times.

Experiment No. 13

The second solution will be prepared the same but using mice 120-150 days old from 201-300 class mothers (201-300 days old).

The mice used for the injections belonged to the C₃H/St or C₃HB/St inbreds, as indicated previously, and they all had spontaneous tumors. The tumors were found as early as possible by examining the mice weekly. Even with extreme caution the size of the original tumors varied considerably. However, the very large ones were discarded from use.

The tumor-bearing mice were examined periodically (three times weekly) and the size of the tumors carefully measured with verniers in the two larger diameters. It is realized that this measure is only an approximation of the actual size of the tumors since several variable factors are always present in the growth and survival of a spontaneous tumor, such as, variable thicknesses of overlying skin, pools of blood which have a tendency to combine to form large cysts, necrotic materials usually centrally located, ulcerations, etc. When one also requires the survival time of mice bearing spontaneous tumors the system of measurement can only be considered an approximation.

It has always been the policy of adding mice with tumors to a given series until a constant average growth rate has been obtained for the combined series. Thus a fair evaluation of the growth rate of a tumor can be realized, even to the point of reproducibility.

Results

The results are presented in a series of charts.

Chart 75 presents the data obtained by a trichloracetic acid precipitate with whole blood samples from mice of the A/St

strain at different ages. The first readings were obtained at 2.5 hours of separation from food and the last at 6.5 hours. Since the curves for mice between 101-200, 301-400 and 401-500 were quite similar, the data, for these classes, were added together. The shape of the curve for mice between 501-600 days was similar to but somewhat lower than the combined curve for mice between 101-200 and 401-500 days. It is obvious that the curve for mice between 201-300 days is different from those obtained

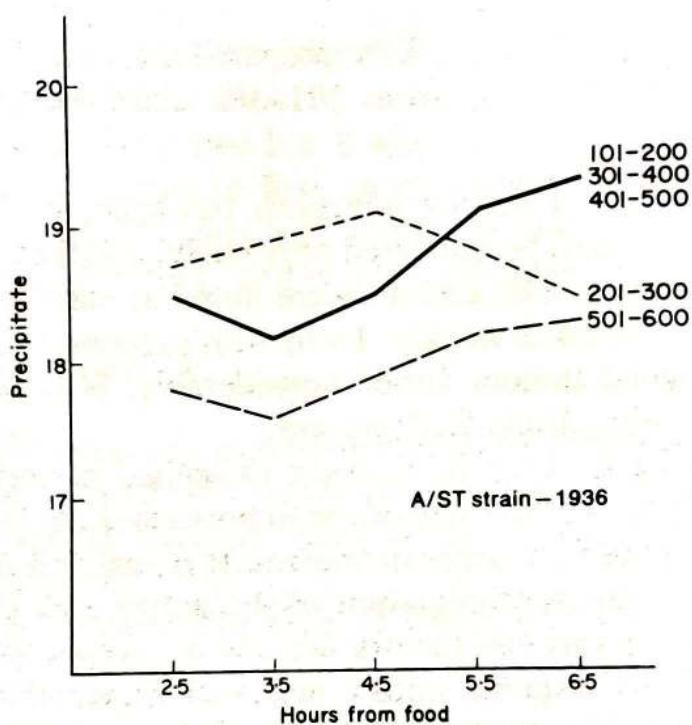


CHART 75. This chart presents data on the amount of a precipitate obtained by trichloracetic acid on the blood of A/St mice. The reading of the precipitate is on the vertical line; hours of separation from food on the base line. Ages of mice are also presented, as follows: (1) 501-600 days on heavy dash line; 201-300 days on short dash line; 101-200, 301-400 and 401-500 days on solid line (i.e. combined data).

at 4.5 hours of separation from food rather than at 6.5 hours as in the other age classes. These observations, therefore, indicated a "uniqueness" of a character in mice between 201-300 days of age.

Chart 76 presents data on trichloracetic acid precipitates in mice separated from food from 1 to 6 hours for mice of the D/St, C₃H/St, A/St, CBA/St and N/St strains. Mice of these strains show progressively increased amounts of the precipitate begin-

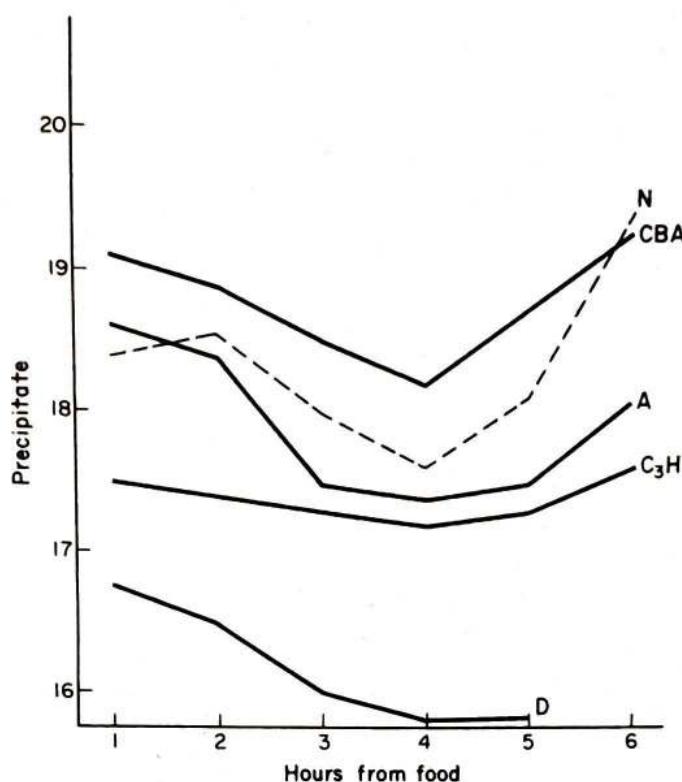


CHART 76. This chart gives the data on the trichloracetic acid precipitate on the bloods of several strains of inbred mice. Hours of separation from food is on the base line; the reading of the precipitate on the vertical line. All strains used, N/St, CBA/St, A/St, C₃H/St and D/St, were well-established inbreds.

ning with mice of the D/St strain. It is of more than passing interest that mice of these strains can also be listed in the same sequence when susceptibility to spontaneous tumors of mammary gland origin are measured, beginning with D/St and ending with CBA/St and N/St.

Chart 77 represents comparative data on trichloracetic acid precipitates obtained with mice of the A/St and CBA/St, but, in this case, chronological ages of the mice were taken into consideration. It can be observed that the two curves deviate somewhat after 200 days of life—or at that time just previous to the appearance of spontaneous mammary gland in mice of the A/St strain. CBA/St strain mice, at the time the experiment was performed, showed only a very low incidence of spontaneous tumors.

Chart 78 gives the data obtained by the determinations of hemoglobin on mice of the A/St and CBA/St strains on a chronological age basis. Here, as in trichloracetic acid precipitate determinations, the two curves deviate from each other shortly

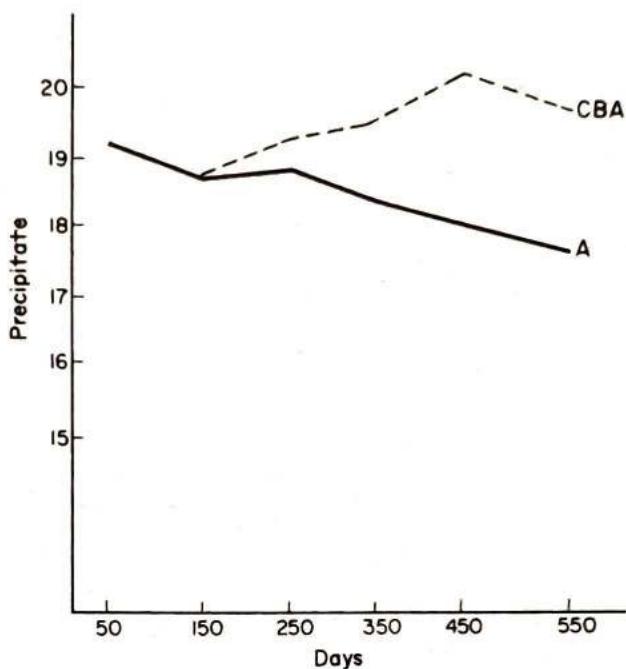


CHART 77. This chart shows the reading of the trichloracetic acid precipitate on the vertical line; age in days on the base line. Resistant-to-cancer (CBA/St) mice are on the short dash line; the cancer susceptible (A/St) are on the solid line.

after 200 days of life — thus indicating a difference between mice that develop spontaneous mammary gland tumors (A/St) and those that do not (CBA/St), at the time the appearance of spontaneous tumors is becoming imminent.

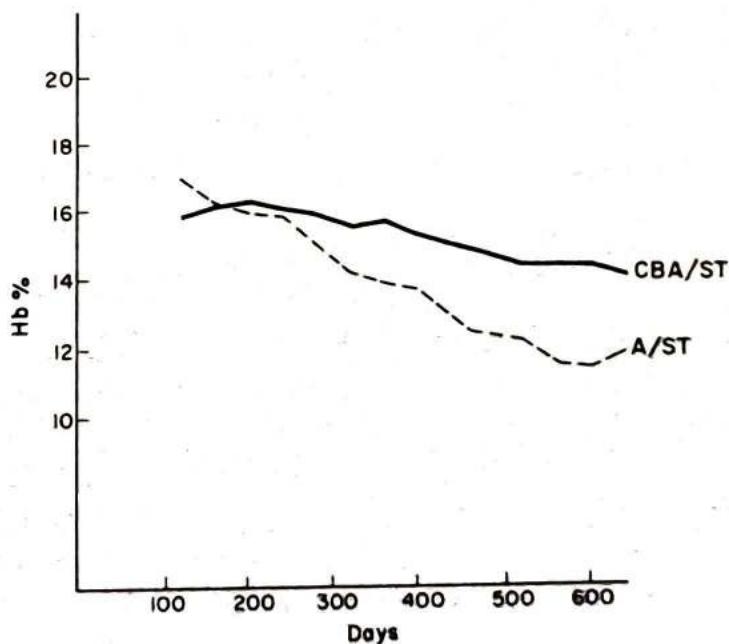


CHART 78. Data on hemoglobin determinations are on the vertical line; age in days on the base line. CBA/St mice are on the solid line; the A/St on the short dash line.

Chart 79 gives the data on the number of minutes required to kill an A/St mouse of 21 grams weight with a single dose of salicylic aldehyde dissolved in olive oil on a chronological age basis. It may be seen that the maximum tolerance to the salicylic aldehyde-olive oil mixture is reached between 101-200 days of life. From this point on, with advancing age, there is a gradual loss of tolerance to salicylic aldehyde.

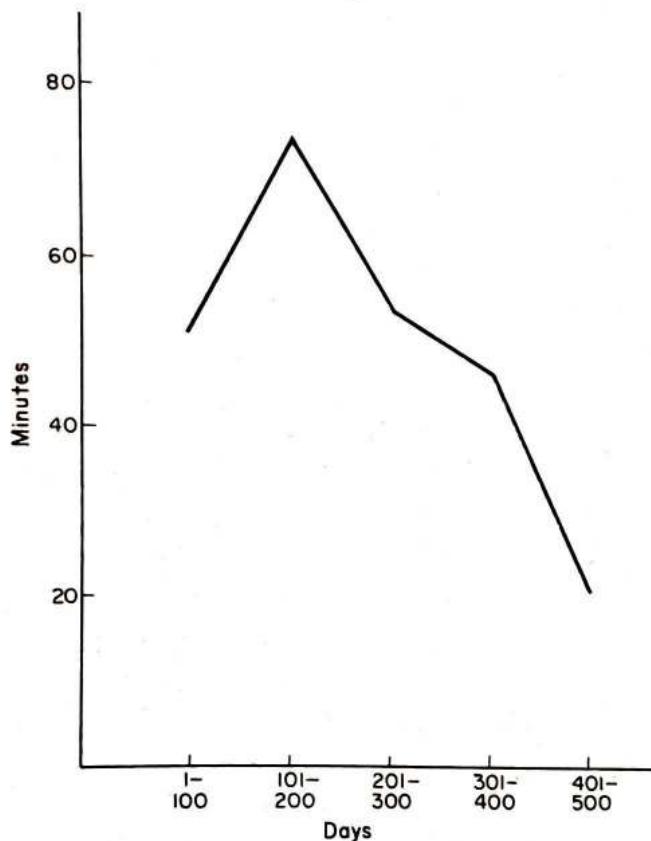


CHART 79. This chart shows the number of minutes it takes to kill a mouse with a standard amount of salicylic aldehyde. Time in minutes is given on the vertical line; age in days on the base line.

In summary, then, it is clear that the C₃H/St, A/St and JK/St strains of mice can be classified in the same sequence according to (a) tolerance to salicylic aldehyde, (b) shifts of hemoglobin per unit of time and (c) the tendency for breeder female mice to give rise to spontaneous tumors of the mammary gland. This is an early use of the comparative method of analysis that has become so useful in biological research.

Chart 80 presents the data on the loss of biological vigor following the loss of polydactylyia from a given descent.

This chart shows the age at which the first and subsequent

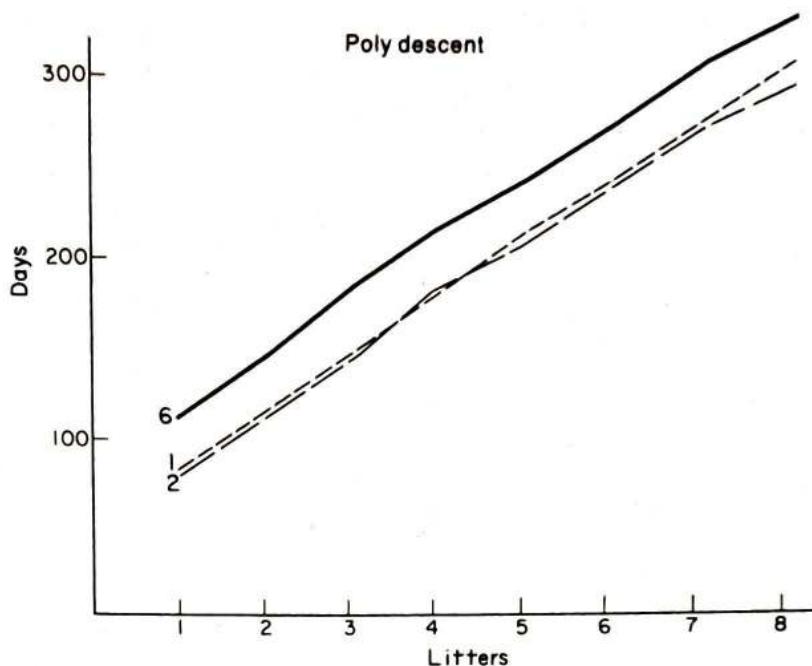


CHART 80. This chart gives the comparative data on age of first and subsequent litters obtained on Poly 1 mice showing a very high penetrance of polydactylia on the short dash line, Poly 2 with intermediate penetrance of polydactylia is on the long dash line and Poly 6 with an extremely low penetrance of polydactylia on the solid line. The chief difference is in the age at which first litters were born. After this point reproductive capacity as measured by litter frequency is relatively constant in the three descents.

litters were born to mother's when the sexes were kept continuously together. Subsequent litters are plotted on the base line and age of mothers on the vertical line. Poly 1 descent (with a very high penetrance of polydactylia) and Poly 2 (with a moderate degree) produced subsequent litters at practically the same rate. Female mice of the Poly 6 descent with extremely low penetrance of polydactylia (<1.0 percent) had their first litters more than 30 days later than mice of Poly 1 or Poly 2 but then developed subsequent litters at approximately the same rate.

Chart 81 gives data on the growth rate of spontaneous tumors in female mice of the C₃H/St strain at three periods of time and the employment of two methods of measurement by three independent observers. The NH series (New Haven, Connecticut) was determined in 1938 using only the measure of the longest linear diameter of the tumor. The DM series (Del Mar, California) was done in 1965 using the two longest diameters (multiplied together). Except in the earlier part of the two curves it can be seen that the growth rates of the tumors were practically

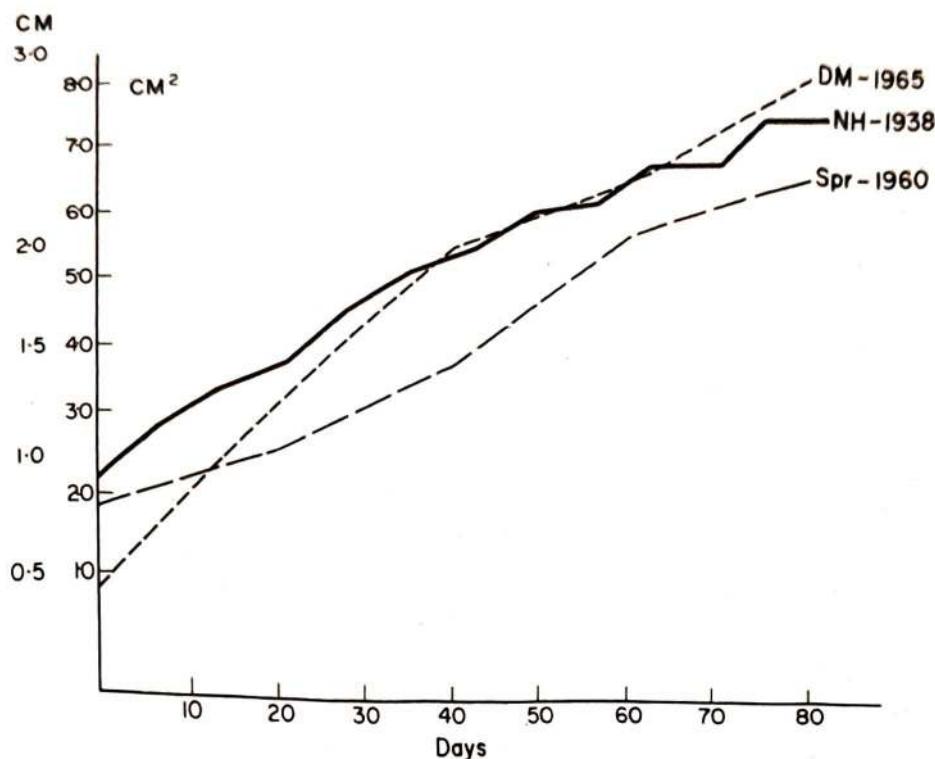


CHART 81. This chart presents the data on the growth rate of spontaneous tumors of C₃H/St origin in three separate series and spanning some 27 years in time. Days of growth are plotted on the base line and size of tumor (multiplication of two longest diameters expressed as CCM₂) on the vertical. NH—a series that was done in New Haven Yale University School of Medicine in 1938; Spr—a second series in Springville, New York (Roswell Park Memorial Institute, 1960); and DM—a third series done at Del Mar, California (The Salk Institute for Biological Studies, 1965).

identical, even though spaced 27 years apart. The third series Spr (Springville, New York) was done in 1960 and consisted of the measurement of the two longest diameters, multiplied together.

The growth rates of these tumors were very similar in spite of the fact that several years separated the series and the measurements were done by three individuals.

Chart 82 shows the same data of growth rate of spontaneous tumors on the Springville, New York, and the Del Mar, California, series of controls as were given in Chart 81. All data are based upon the determination of the two longest diameters multiplied together. Since two individuals were involved in the actual measurements, the apparent differences between the two average growth rates of spontaneous tumors may not be significant. However, the average survival times of mice of the two control series (Spr and DM) were identical (66.4 days in each series).

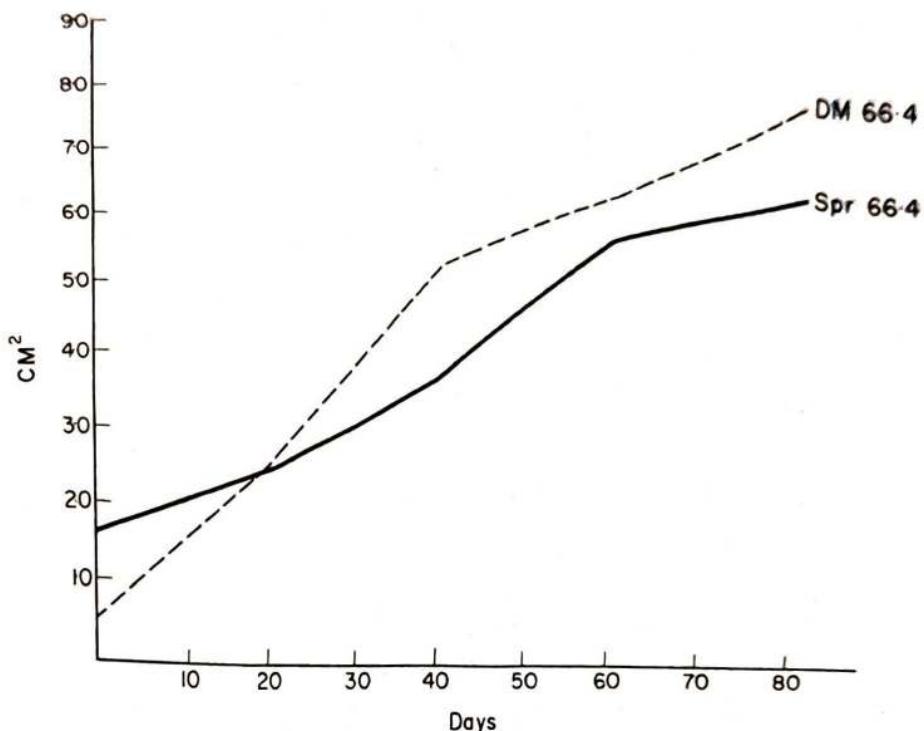


CHART 82. This chart presents the growth rate of the last two series of spontaneous tumors (Spr and DM) together with the survival time of the mice expressed in days (66.4 days in each case).

Chart 83 presents the data on growth rates of tumors in the Del Mar Controls (solid line) and in the liver extract injection series that gave the maximal effect of inhibition of growth rate of tumors by a single water soluble solution (Experiment No. 8, preparation page 91, short dash line). The controls lived 66.4 days, on an average, following the detection of their tumors, while the experimentals with a "water-soluble" liver extract lived 81.6 days.

Chart 84 gives the data on growth rates of tumors in mice which had been injected periodically with liver extracts of the W series. The 9W³ injected mice had slower growth rates for tumors than either 10W or the controls and lived on an average 60.8 days compared to 66.4 days for the controls.

Chart 85 presents the data on growth rate of tumors in mice receiving extracts 9A and 10A origin. Here the maximal inhibition of growth rate was obtained with the extracts of the older livers and these mice showed the best survival time (92.3 days).

Chart 86 shows the data on growth rate of tumors in those mice that were periodically injected at alternate times with extracts of

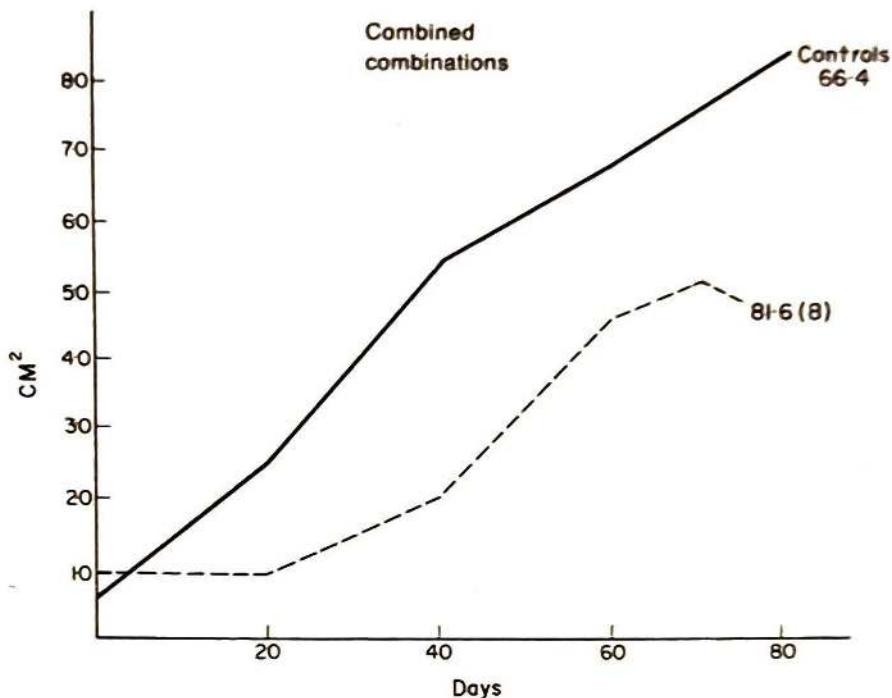


CHART 83. The data on growth rate of spontaneous tumors are presented in this chart. The controls with a survival time of 66.4 days are on the solid line; the experimental mice receiving injections of solution No. 8 are on the short dash line. The experimental mice showed a survival time of 81.6 days, bearing spontaneous tumors.

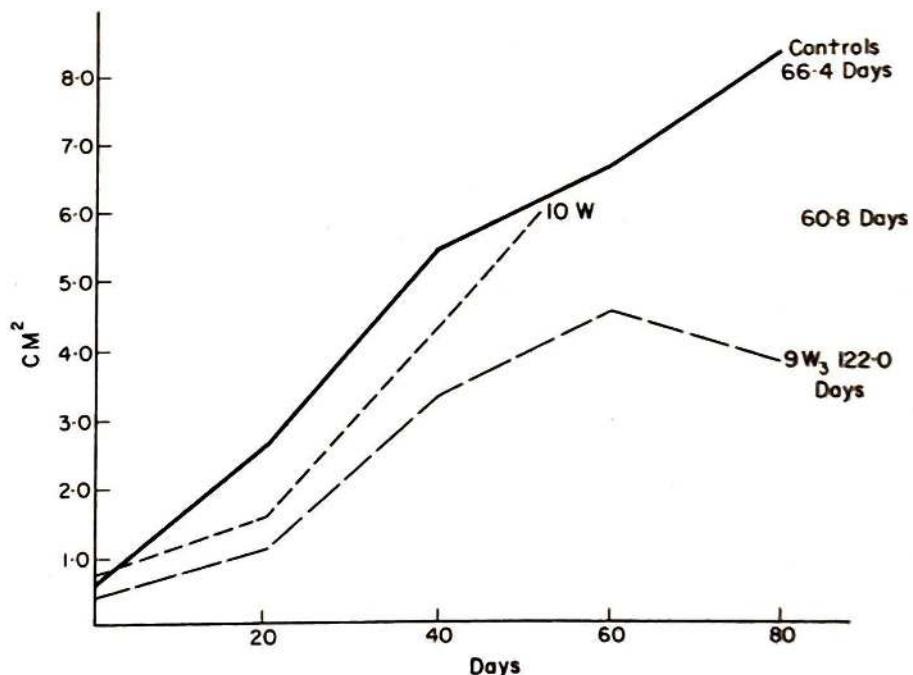


CHART 84. This chart presents the growth rate of spontaneous tumors on two series of experimental mice receiving injections of $9W_3$ on the long dash line with a survival time of 122.0 days, compared with 10W with a survival time of 60.8 days short dash line. The controls are on the solid line with a survival time of 66.4 days.

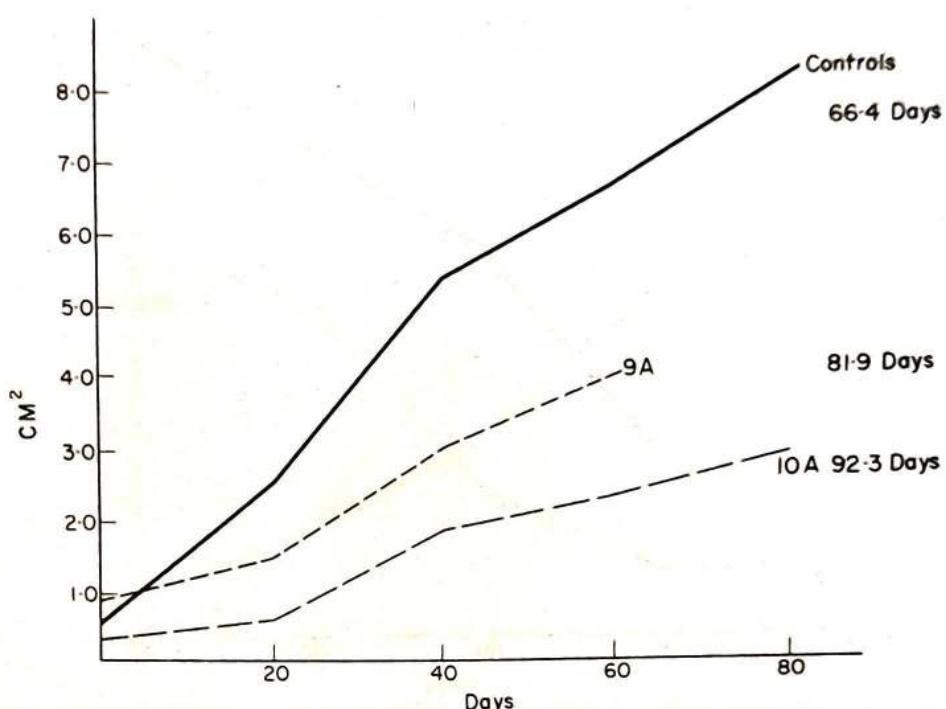


CHART 85. This chart compares the data on the growth rate of two series of tumors in mice receiving injections of "A" fraction of the 9A and 10A series. Survival time in 9A was 81.9 days; in 10A 92.3 days.

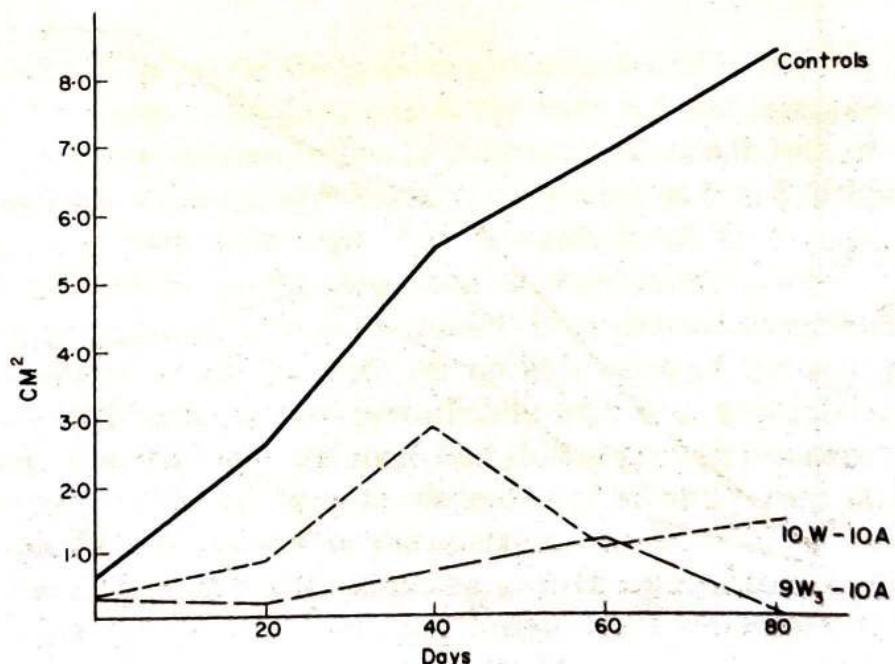


CHART 86. This chart shows the growth rate of tumors in two series receiving combinations of two injections each. The maximal inhibition of growth rates of tumors was obtained when 9W₃ (young livers) extract was alternated with 10A (old livers) extract.

the W series and extracts of the A series. Thus it can be seen that a tremendous inhibition of tumor growth was obtained by using a W solution from young livers with an A solution from old livers.

Discussion

Following the development of the inbred strains of experimental mice with controlled uniformity of biological characteristics, they were used primarily for two purposes. In the first place, it was soon found that reproducible results could be obtained by their use in many experimental fields of cancer research, such as, virology, endocrinology, toxicology, chemical carcinogenesis, etc. This contribution of the use of controlled inbred experimental animals and the results obtained by innumerable scientists throughout the world belong to the history of biological science and the controlled, by continuous selection, descendants of these mice, no doubt, will continue to serve in this capacity for many years to come.

In the second place, however, which, historically, was the first reason for their production, was the opportunity they afforded in determining differences between biological states, particularly in possible relationships to cancer and not cancer susceptibility or resistance. It was soon found that mice of the different inbred strains differed considerably in their capacities to give rise to, or not to give rise to, spontaneous tumors particularly of mammary gland origin. The increased incidence of spontaneous lung tumors, hepatomas, lymphatic and myelogenous leukemia were soon added.

The present author attempted to determine any obvious differences in the biological characteristics of mice that differed in cancer susceptibility or cancer resistance. The numerous attempts that led only to negative observations need not be discussed. They will probably never be published. However, there were a few studies that seemed promising. These were on a trichloracetic acid precipitate on the blood of mice which later developed into standard procedures of hemoglobin determinations. Another series was on the toxic effect of salicylic aldehyde—where it was found that a cancer-resistant mouse was more tolerant to this chemical on a body-weight and age basis than a cancer-susceptible one. Another observation, which was never published, was

on the toxic effect of carbon tetrachloride. Here a significant observation was made which was almost the end of all cancer-susceptible mice, since, at that time, they were largely confined to one small laboratory, in New Haven, Connecticut. One episode of clearing an armature in an air-conditioning unit killed more than 2000 mice in the laboratory within 36 hours — and the cancer-susceptible mice died at a tremendously higher rate than the cancer-resistant mice.

With the very significant observations in the fields of endocrinology, chemical carcinogenesis and more recently virology, these results have, it seems to me, been overlooked. The ideas, however, have been retained, in the mind of the present author, particularly in the observations that disturbances occur between 201–300 days of life — and that a cancer-susceptible mouse and a cancer-resistant one begin to deviate in some biological readings at about 200 days of life or just before the mouse becomes susceptible to the imminent appearance of a spontaneous tumor.

The concepts of biological stability and instability have also had an extensive investigation in a series of studies on maternal age effects and the bizarre effects of the pleomorphic gene LST, but the discussion of these findings would necessarily lengthen this present presentation. They have been discussed elsewhere.

Hence the discussion should be concerned with the present attempts to influence the growth rate of spontaneous tumors and the survival time of tumor-bearing mice by the injection of tissue extracts, based upon this early research on biological states and/or variations in these states as to stability and instability.

Here again the discussion will center only on the results obtained by the injection of extracts of liver derived at different ages of mice from parents at different chronological ages, where according to a concept built up on extensive data a possible positive or active principle affecting tumors was looked for.

There appears to be two liver extracts that have an inhibitory effect on the growth rate of spontaneous tumors of mammary gland origin in mice of the C₃H/St and C₃HB/St strains. The first inhibitor is water "soluble" and decreased in effectiveness with age of the livers from which the extracts were prepared. "Water soluble" is used only to express that part of the liver extracted with 80 percent alcohol from homogenized liver dried to solid

form under reduced pressure, washed with 100 percent alcohol several times and then taken up in distilled water. The resulting material is yellowish or whitish in color and either opaque or clear and is, no doubt, emulsoid. It loses some of its opaqueness by standing in the refrigerator.

The second inhibitor of the growth of spontaneous tumors is alcohol soluble but forms a pearly white emulsion, when freed of alcohol and taken up in distilled water. Its effective inhibition of the growth rate of tumors and increased survival time of mice bearing spontaneous tumors increases with advancing age of the livers used, at least, between 120 and 250 days of age.

A very good inhibitor of growth rates of tumors was obtained with the combined presence of the two inhibitors before any attempt was made to separate them (Experiment No. 8, preparation page 91).

The injection of 10A and 10W with both fractions from old livers on alternate days of treatment (three times weekly) had some inhibitory effect on the growth rate of tumors but certainly nothing very striking.

A very good effect of inhibition was obtained on the growth of tumors by alternating 9W³ (young liver extract) with 10A (old liver extract) solution. In fact, by some measures, this combination of solutions has shown the best inhibitory effect on the growth of spontaneous tumors to date. However, at present there has been an increased toxic effect of the injected material since four mice (three starting on 9W³ and one on 10A) have died shortly after the first or second injections. This result had never been seen in any of the previous experiments. The solutions, now several months old, have always been kept in the refrigerator with a small amount of thymol added, as a preservative, and they are gradually increasing in viscosity, especially the A "solution". The nature of death has not been determined. Cultured pearls on agar have proven that no infective organism is in the liver extracts used. This result of death can be avoided by diluting the injected materials, by using smaller doses per injection, or even by avoiding, occasionally, the injection of any material.

Thus it can be concluded that the inhibition of growth rates of spontaneous tumors and increased survival time of tumor-bearing mice have been obtained.

The further development of the experiment as to the nature of the inhibition and its possible relation to the biological concepts of biological stability and/or instability and others that made possible the development of the experiments remain for the future to determine. It is obvious that the extracts processed and used may contain several components so that the problem of purification and identification of the active component or components remain, at present, unsolved.

CHAPTER 6

The Effect of Liver Extracts on Spontaneous Tumors in Mice*

IN A recent chapter (5) the conclusions were reported that "there appears to be two liver extracts that have an inhibitory effect on the growth rate of spontaneous tumors of mammary gland origin in mice. . . . The first inhibitor is water 'soluble' and decreases in effectiveness with age of the livers from which the extracts are prepared. . . . The second inhibitor of the growth of spontaneous tumors is alcohol 'soluble' but forms a pearly white emulsion, when freed from alcohol and taken up in distilled water. Its effective inhibition of the growth rate of tumors and increased survival time of mice bearing spontaneous tumors increases with advancing age of the livers used, at least, between 120 and 250 days of age" (Chapter 5).

It was further concluded that "a very good effect of inhibition was obtained on the growth of tumors by alternating the injection of a 'W' fraction from the liver of young mice ($9W_3$) with an 'A' fraction from livers of old mice (10A)" (Chapter 5).

The present paper deals (1) with additional information on the conclusions on tumor inhibition contained in Chapter 5, and (2) observations on new series of tumor-bearing mice injected with new liver preparations.

New solutions of liver extracts have been prepared following the outline previously given, as follows:

Briefly stated, the techniques consist of grinding livers in 80 percent alcohol at speeds of 4000 rev/min in a Virtis apparatus, and then washing the ground material several times in 80 percent alcohol under refrigeration. The washings are collected and then reduced to dryness under reduced pressure by means of a vacuum pump attached to a Virtis cylinder with dry ice. The resulting dry material is then washed several times with absolute alcohol, thus

*Based upon a lecture given at the Springville Laboratories of Roswell Park Memorial Institute, Springville, N.Y., 7 June 1966.

separating the original lyophilized liver material into two parts. The alcohol solution part when reduced to dryness is taken up in a measured amount of distilled water (2 cm³ per one mouse liver). A small amount of thymol is added as a preservative. The residue that was not soluble in absolute alcohol is dissolved in distilled water (2 cm³ per one mouse liver). A small amount of thymol is added to this solution also. Thus there are produced two extracts from mouse livers which are given the symbols A and W.

There has been only one change of technique in preparation of the W solution from the preceding experiments reported in Chapter 5. This technique was as follows: it was found that from the "W" or water-soluble solution a small amount of a fine sticky precipitate formed. In some of the earlier experiments this precipitate was included in the injections of mice since it was found to be readily suspended by slight shaking of the W extract before use. However, beginning with all materials in the sixteenth series and continuing up to the present time the "W" moiety was divided into two preparations, both of which were suspended or dissolved in distilled water. These were given the symbols (1) 16W supernate (the clear pale-yellowish solution) and (2) 16W precipitate. The preparation of the A "solution" or emulsion has not been changed from the earlier work reported in Chapter 5.

In the present report, observations on series 8, 9A, 9W, 10A, 10W, 12A, 12W, 13A, 13W, 16A, 16W, 17A and 17W supernate and precipitate are reported. All mice belonged to the 201-300 maternal age descent of Strong's Polydactylia origin in which a selection of offspring born to parents between 201-300 days of age had been practiced for several generations. Series 8 contained both "moieties" of liver material whereas in all other series the A material was separated from the W by the method outlined previously. The parents of offspring were in all cases, except 12A and 12W, between 201-300 days of life when their offspring used in these experiments were born. In preparations 12A and 12W, the parents were more than 301 days of age when the liver donors were born.

The ages of the offspring that were used as a source of liver material differed as follows: 9A 9W, 150-200 days; 10A 10W, 201-300 days; 12A 12W, 250-300 days; 13A 13W, 120-150 days; 16A 16W, 250-300 days and 17A 17W supernate and precipitate, 125-150 days.

Only mice of the C₃H/St and C₃HB/St inbreds were used for a supply of spontaneous tumors. This source of spontaneous tumors was selected since it has been clearly indicated (1) that these mice have a fairly uniform growth pattern as indicated by periodic measurements over a period of many years, (2) that these mice have a very high incidence of spontaneous tumors of mammary gland origin, (3) that the mice have a very high incidence of multiple tumors when compared to other inbred strains, (4) that in the controls, the mice will live on an average of 65 days from the appearance of the spontaneous tumor and (5) that these tumors never regress spontaneously but continue to grow progressively until the death of the mouse. Occasionally, however, for a few days before death the spontaneous tumor will actually decrease somewhat in size which in all cases is evidence that death is imminent.

In old C₃H/St and C₃HB/St an occasional regression of a spontaneous tumor occurs but these mice are never included in an experiment such as the present one. One complication of the use of these spontaneous tumors is the appearance of hemorrhagic areas but this difficulty is always taken into consideration when a comparison between a control tumor and an experimental one is being made.

Each experiment consists usually of observations of four mice bearing spontaneous tumors of mammary gland origin. As in previous experiments, measurements of the two largest diameters of the tumors were taken and the relative size determined by the multiplication of these two diameters.

Again it may be stated that when it is desirable to study both the growth rate or behavior of a tumor and the survival time of the tumor-bearing mouse, some method of estimation of tumor size must be designed. The ideal method of killing a number of the mice at periodic times and weighing their tumors is out of the question, and, in fact, due to central necrosis, varying amounts of connective tissue and blood, etc., this method of weighing is also not too accurate. It has been found by extensive experimentation that by multiplying the two longest diameters of a tumor together, a reproducible result of growth rate or behavior can be obtained and this procedure of estimation of tumor size also permits the eventual determination of survival time. One further complication is always evident. In order to determine survival time some

of the mice die during the night and thus undergo postmortem changes. Bearing this in mind it is, therefore, desirable to select the tumors and organs for pathological analysis from those mice which have died recently and their tissues fixed before postmortem changes have occurred.

All experimental mice were injected with the materials, in varying amounts due to an evaluation of the physical wellbeing of the mouse, intraperitoneally—care being executed not to perforate the intestines. All solutions were warmed to room temperature before use, thus avoiding spasms which were produced by injecting mice directly from the refrigerator.

Subcutaneous injections were not used in the present experiments since it had been determined that by this method of administration, skin ulcerations were produced.

Periodically a new series of four mice were injected with the same solutions, either alternating an old liver A solution or emulsion with a young liver W extract or vice versa—a young liver A material with an old W solution.

Thus in one group of experiments six series (twenty-four spontaneous tumor-bearing mice) were injected with the same solutions, the only variable being that the solutions or emulsions (estimated from the time of adding the distilled water and thymol) were older. Thus in one experiment with six series when 16A was alternated with 17W, these successive times of "aging" solutions were 28, 32, 61, 74, 104 and 137 days.

In another series when 16W was alternated with 17A, five series of four tumor-bearing mice were done. These were spaced according to the age of the solution as in 16A and 17W at 104, 121, 160, 190 and 223 days for the 17W series and 70, 87, 126, 156 and 189 for the 16A series.

Two methods of presenting the growth pattern of tumors were also used. In the first method the actual value for the estimated tumor size was used; in the second the measure of successive increments of tumor size was plotted. The only difference between the analysis by the two methods is that by the use of the second method the initial size of the tumor at the time of its discovery, which is a variable quantity, is reduced to zero, thus all growth curves start at a single point.

Results

The results obtained in this experiment of treating spontaneous tumor-bearing mice with extracts of liver are presented in a series of charts and one table. These charts are numbered in sequence following those already presented in Chapters 1-5.

Chart 87 contains the data obtained by the injection of various solutions in the W series. Here it can be seen that there was obtained a variable degree of inhibition of tumor growth when

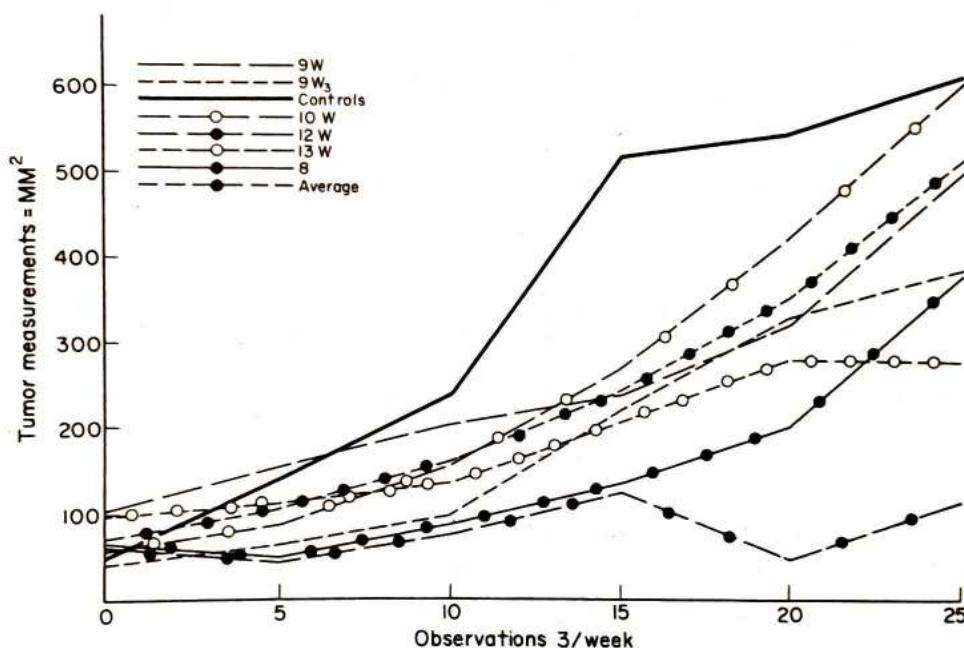


CHART 87. This chart shows the data on the growth rates of tumors in mice injected with (1) different solutions in the W ("water-soluble") series (lines 9W, 10W, 12W and 13W), (2) the combination of the W moiety with the A moiety before separation (line 8), and (3) the average growth rate of tumors in the controls (solid line). Successive observations (three per week) are on the base line and tumor measurements (multiplication of the two longest diameters) are on the vertical.

compared to the control growth rate (solid line) by the injection of the W material. The solid dot and solid line (series 8) contain the data on the original liver extract before the separation into the A and W moieties. The only variables in the W series were (1) the age of the mice used for the source of the liver and (2) the age of the solution between the time of the addition of distilled water and the start of the actual injection of the materials into tumor-bearing mice. It is obvious that there has been obtained an inhibition of tumor growth by the experimental procedure.

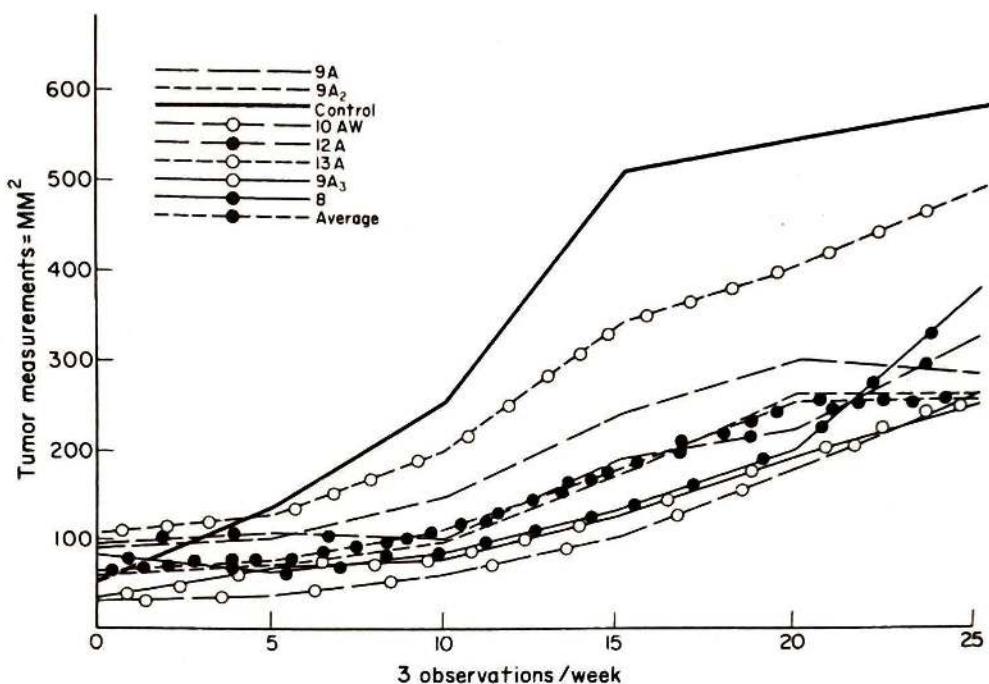


CHART 88. This chart shows the data obtained on the growth rate of tumors in (1) mice injected with solutions in the A series (9, 10, 12 and 13), (2) the injection of a combined liver extract line 8 (solid line and open circle) and (3) the controls (solid line). Successive observations (three per week) are on the base line and tumor measurements (multiplication of the two longest diameters) are on the vertical.

Chart 88 shows the data on the inhibition of growth rate of tumors by the injections of the A material. Here again as in the W series, the only variables were (1) the age of the mice used for liver extracts and (2) the age of the A solutions or emulsions.

Chart 89 simplifies the presentation of the same data of the W series as presented in Chart 87 by reducing the number of series. An additional comparative point at thirty periods of observation is also included.

Chart 90 presents the data of Charts 87 and 89 in still another manner. Here the size of tumors obtained with the injection of the W material are compared to the size of the controls at 50 days of growth. It can be seen that the maximal amount of inhibition of tumor growth was obtained by the W material prepared from mice of 135 days of age. The minimal deviation, or perhaps no deviation, from the controls was obtained by the injection of the W material prepared from mice of 225 days of age. Thus it may be concluded that the inhibitory effect of the W material upon tumor growth is decreased with the advancing age of the mice giving the liver preparations.

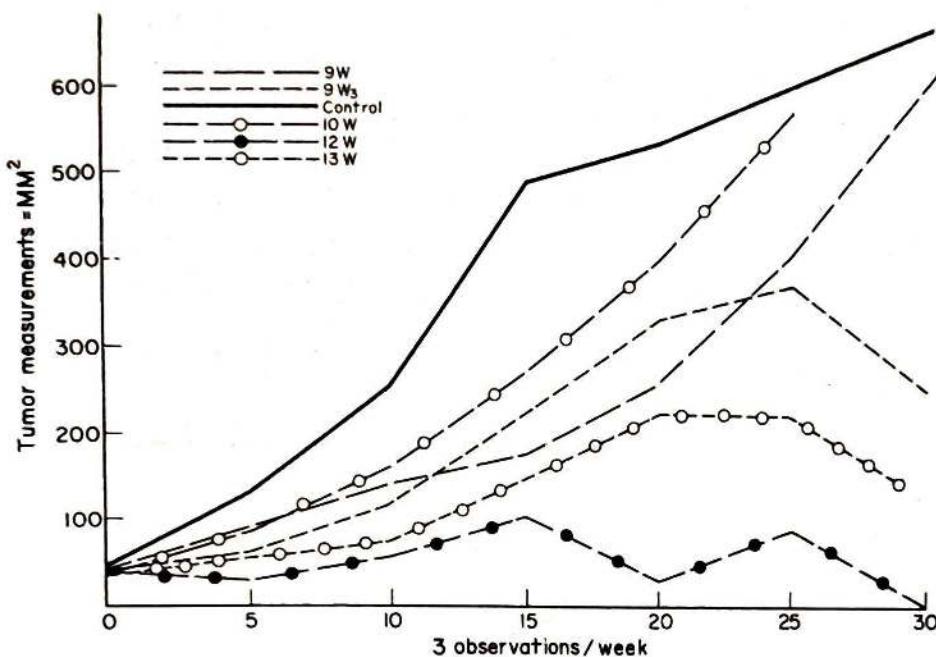


CHART 89. The same data are included in this chart as shown in Chart 87 but with the experiment extended to the thirtieth observation period. The same trends are obvious.

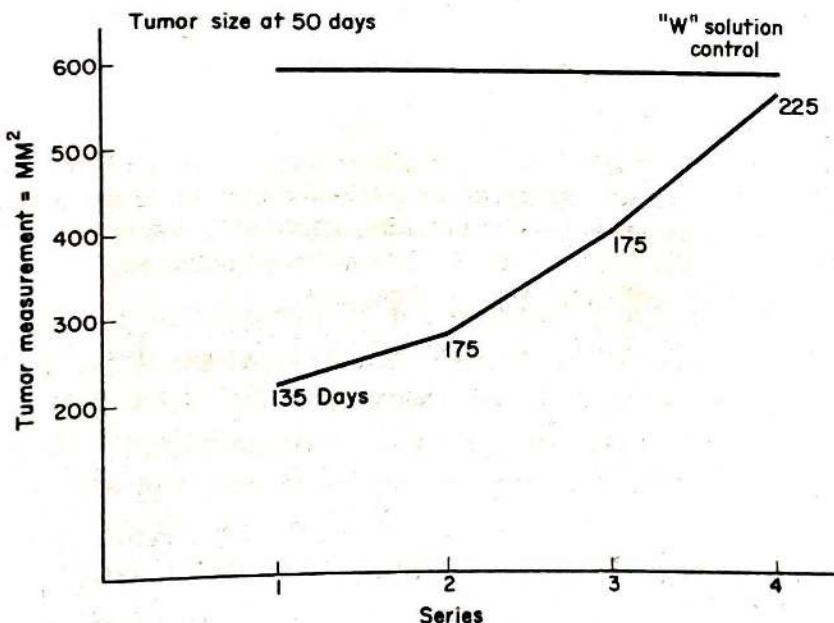


CHART 90. This chart shows the data on size of tumors at 50 days of growth for (1) controls, horizontal straight line, at about 600 mm² and (2) four series of experimental animals injected with the "W" solution. Ages of mice which were used in the preparation of the "W" solutions were, in successive series, 135 days, 175 days, 175 days, and 225 days. There is thus a loss of inhibition of the growth size of tumors with the advancing age of mice from which the "W" solutions were obtained.

Chart 91 presents comparable data to Charts 88 and 90 but obtained with the A preparations and separated into groups of successive ages of mice used for the liver preparations. Here the maximal effect of inhibition of tumor growth was obtained when the A material was obtained from the livers of mice of 225 days of age. Thus the inhibitory effect of the A material increases with the advancing age of the mice for the liver preparations.

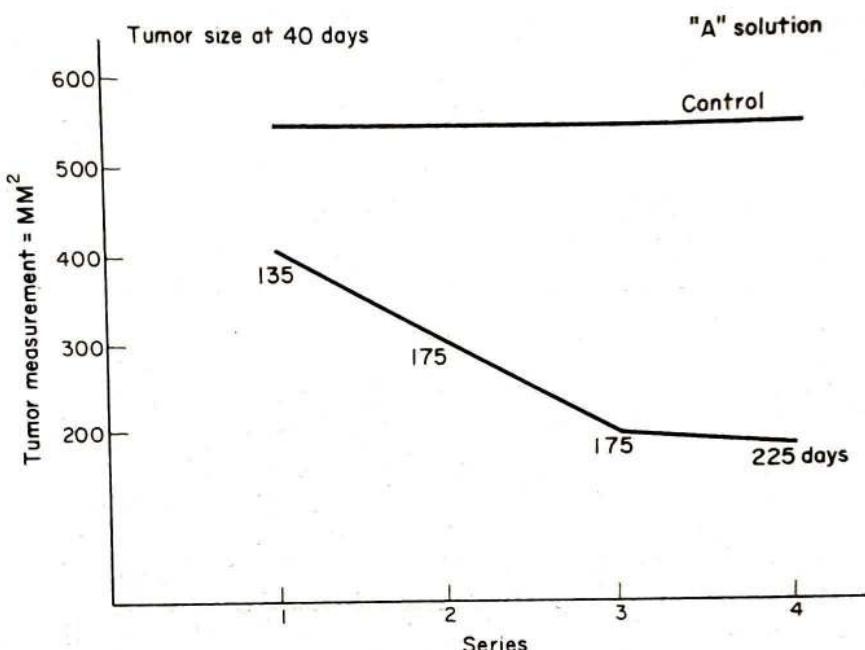


CHART 91. This chart shows comparable data to Chart 90 but they were obtained by the injection of the "A" solutions. Here there was a progressive increase of tumor inhibitory action with the aging of the mice from which the "A solutions" were obtained.

Chart 92 gives the data obtained by the alternate injections of solutions 9W (175 days) and 10A (225 days) on the growth rate of tumors. The solutions used were (1) 9W-10A full strength (2 cm^3 of distilled water per one liver extract moiety (W and A)) (solid line and solid dot). (2) the injected material diluted to 50 percent of full strength with the addition of an equal amount of distilled water (short dash line) and (3) the 9W-10A materials diluted to 25 percent of original strength (long dash and solid circle curve). One mouse left in the series injected with 50 percent diluted material had completely regressed its tumor by the thirteenth observation period. Except for this one point there is no overlapping of data and the provisional conclusion may be entertained that there is a correlation or dose relationship between the amount of injected material and the inhibition of tumor

growth—the greater the amount of injected material the greater inhibition of tumor growth. For example, the average increments of growth in the three series up to the twenty-fifth period was (1) in full-strength material 0.049 cm^2 per observation period, (2) for 50 percent diluted material 0.116 cm^2 and (3) for 75 percent diluted 0.152 cm^2 . The ratio of dose of injected material was as

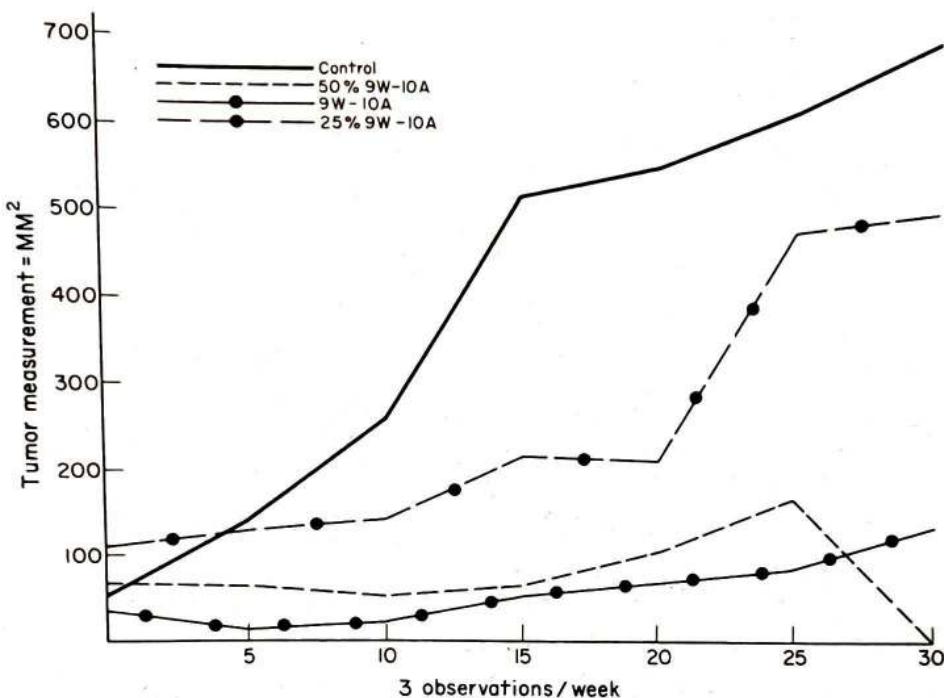


CHART 92. This chart presents the data on growth rate of tumors in (1) controls, solid line, and (2), (3) and (4) obtained with the alternate injections of solutions 9W and 10A. In each case there was an injection on alternate days with these two solutions, (2) full strength 9W-10A on solid line and solid dot curve, (3) 9W-10A diluted 50 percent, short dash line, and (4) 25 percent strength of 9W-10A. There is thus a dose relationship between concentration of tumor inhibitor or inhibitors in "solutions" and the amount of inhibition on growth rate of tumor growth.

1:2:4 and the average increments of growth 0.049:0.116:0.152. For a 1:2:4 ratio of increments the values should be 0.045:0.090: 0.180.

A similar dose relationship was also obtained in two series where the original 9A solution was diluted to two-thirds concentration. In this case, the amount of inhibition of tumor growth was decreased by the use of the two-thirds diluted material. The average increments for twenty-eight periods of observations were (1) for the undiluted 9A solution 0.24 cm^2 whereas for the two-thirds strength of 9A it was 0.10 cm^2 .

Chart 93 presents data on the effect of inhibition of tumor growth by the injection of various combinations of the W and A

materials. It is obvious that there is inhibition of tumor growth except when the combination 9W-10A was alternately injected into mice bearing initially large tumors (with an average size of 1.50 cm^2 at the start of the experiment). By the twentieth observa-

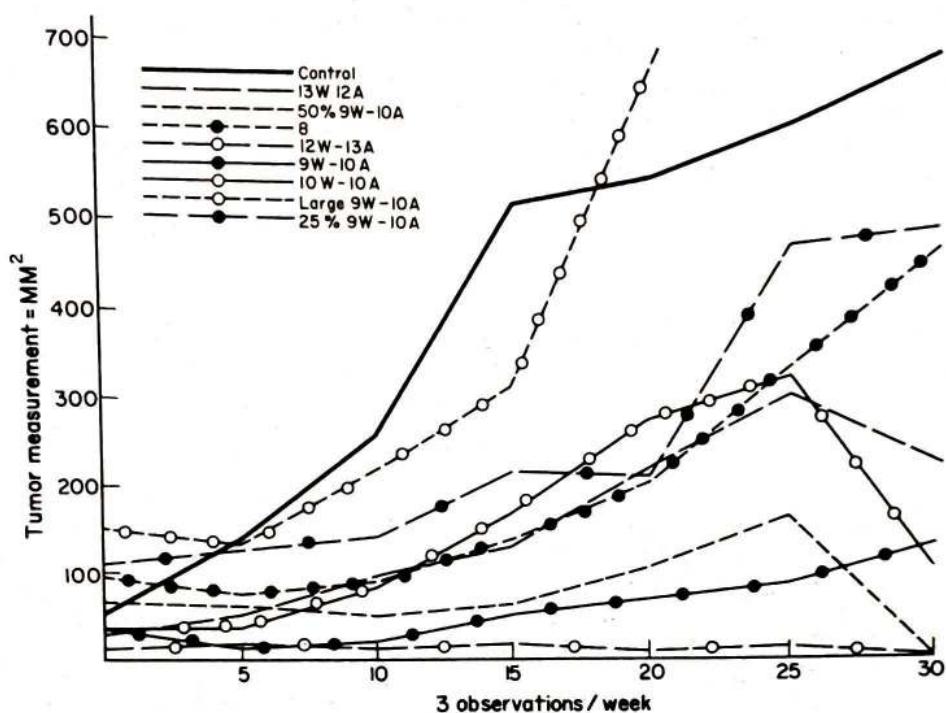


CHART 93. This chart continues the data on the use of combined "A" and "W" solutions on alternate days of injection on the growth rates of tumors. The growth of initially large tumors (average original size at start of injections about 150 mm^2) is on the short dash open circle curve. These mice had received injections of 9W and 10A solutions and the growth rate of tumors did not deviate from the growth rate of tumors for the controls, solid line. The smaller tumors (average original size at the beginning of injections between 10 and 110 mm^2) in all experimental series were inhibited in growth rate. The maximal inhibition of tumor growth was obtained with the combination 12W-13A solution (large dash and open circle curve).

tion period those mice receiving 9W and 10A had larger tumors than the controls although this difference is probably not significant.

Chart 94 presents the data on growth rates of tumors in mice receiving combinations of 12A and 13W. Here there were five series of four mice each. In order to simplify the presentation of data, series 1, 2 and 3 (twelve mice), where there had been overlapping of data, were combined into one curve (long dash line). All three series were very similar in tumor size at the start of the experiment and even up to the fifteenth observation period.

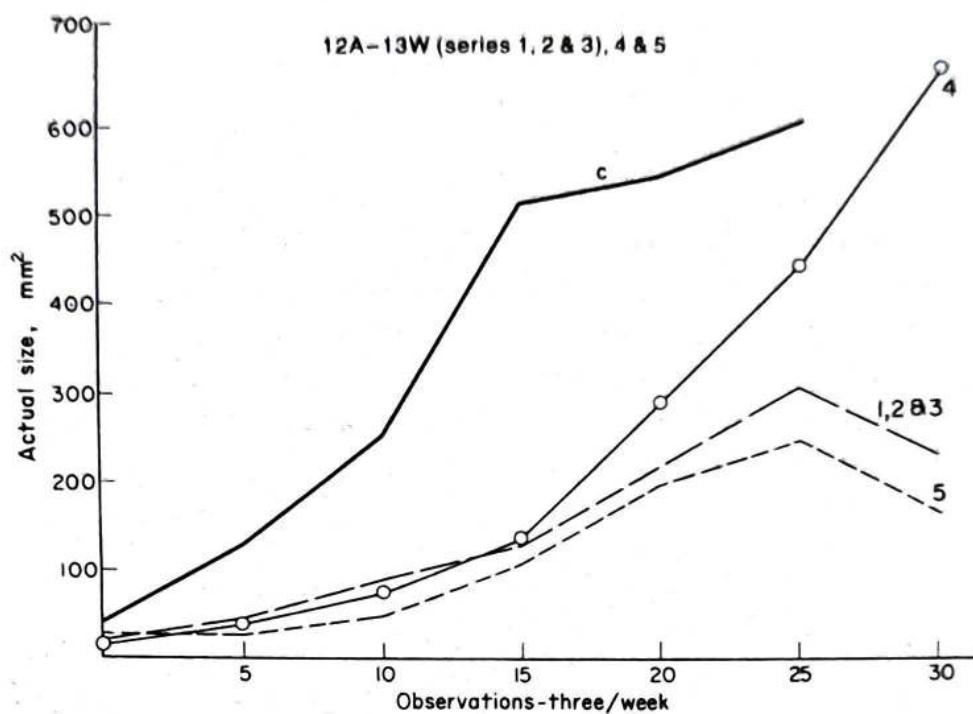


CHART 94. This chart shows the inhibition of growth rate of spontaneous tumors with a combination of solutions 12A and 13W in five successive series spaced approximately 30 days apart. Series 1, 2, 3 are combined on the long dash line; series 4 on the solid and dotted circle line and series 5 on the short dash line.

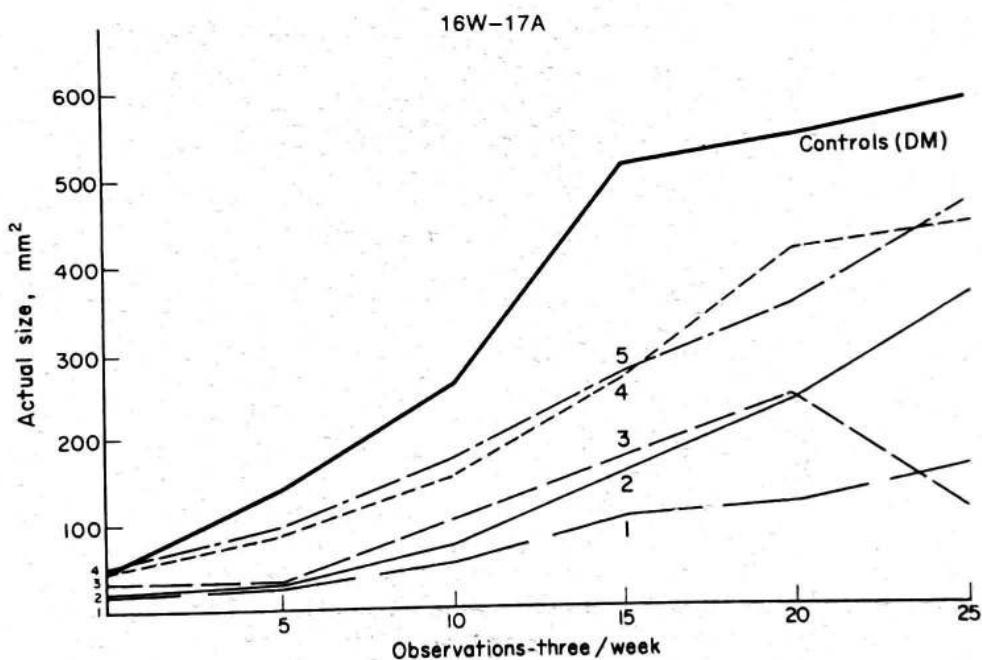


CHART 95. This chart shows the inhibition of growth rates of spontaneous tumors in five successive series of mice injected with combinations of 16W and 17A. Here it can be seen that there was an increased loss inhibition of tumor growth with the aging of the solutions.

Beyond this point, series 1, 2, 3 and 5 were very similar, whereas, series 4 apparently deviated from these by showing less inhibition of tumors. One explanation of this difference may be in the fact that tumors are mixed populations of cells, and a few with very rapid growth capacities may have been fortuitously placed in series 4, but this concept must not weigh too heavily in the interpretation of established facts, and consequently, the deviation of series 4 and from 1, 2, 3 and 5 must, for the present, be unresolved.

Chart 95 presents the data on growth rate of tumors in mice of five successive series receiving alternate injections of 16W and 17A. Here it can be seen that there appears to be a successive

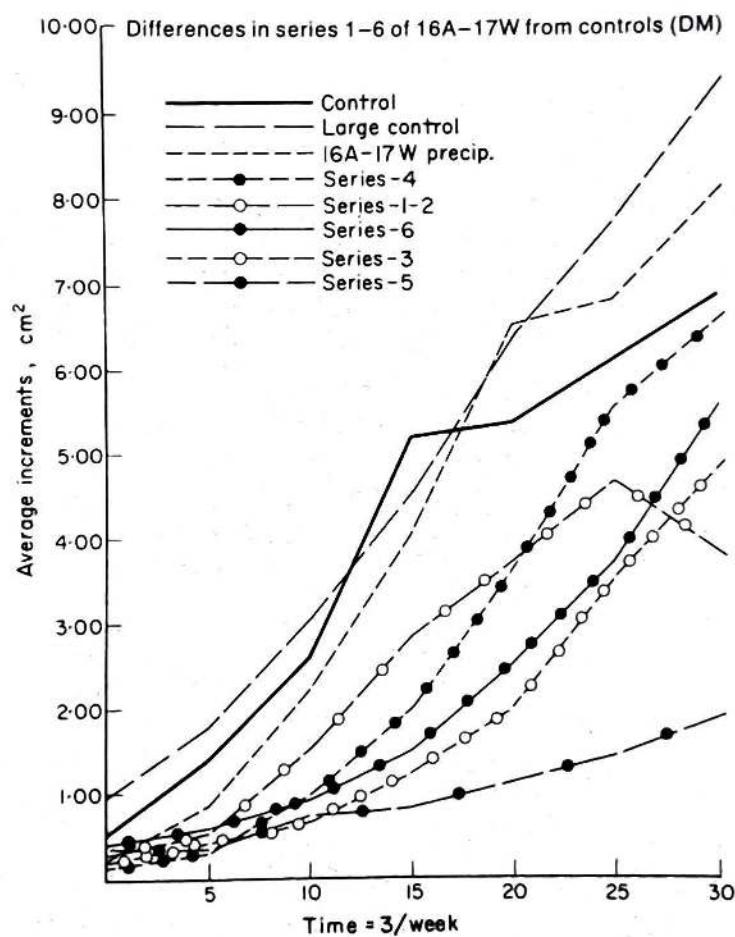


CHART 96. This chart shows a series of mice injected with combinations of solutions 16A and 17W. It can be seen that there was inhibition of growth rates of tumors in all series except one which was injected with 16A and the precipitate obtained from 17W (two mice only, short dash line). The maximal effect of inhibition of tumor growth was obtained in series 5 when the injected solutions were 104 days old (long dash and solid circle line).

series, but this difference is small although apparently linear in sequence 1, 2, 3, 4, and 5 (at the fifteenth observation period).

Charts 96 through 108 present the data obtained by alternate injections of 16A and 17W into tumor-bearing mice. The data are analyzed and presented by a series of different methods. Twenty-four mice bearing spontaneous tumors were divided into successive groups of four mice each. Successive experiments were started when the solutions had aged from the time of addition of distilled water and thymol to first injections for 28, 32, 61, 74, 104 and 137 days.

Chart 96 presents the data on the growth rate of two sets of controls, as follows: (1) with original "average size" (0.56 cm^2) on solid line and (2) with initial large size average 1.18 cm^2 on the long dash line. A third group of tumor-bearing mice which received alternate injections of 16A and 17W precipitate is plotted on the short dash line. Here it can be seen that in this experiment there was no inhibition of tumor growth and hence it can be assumed that the precipitate formed from the 17W solution

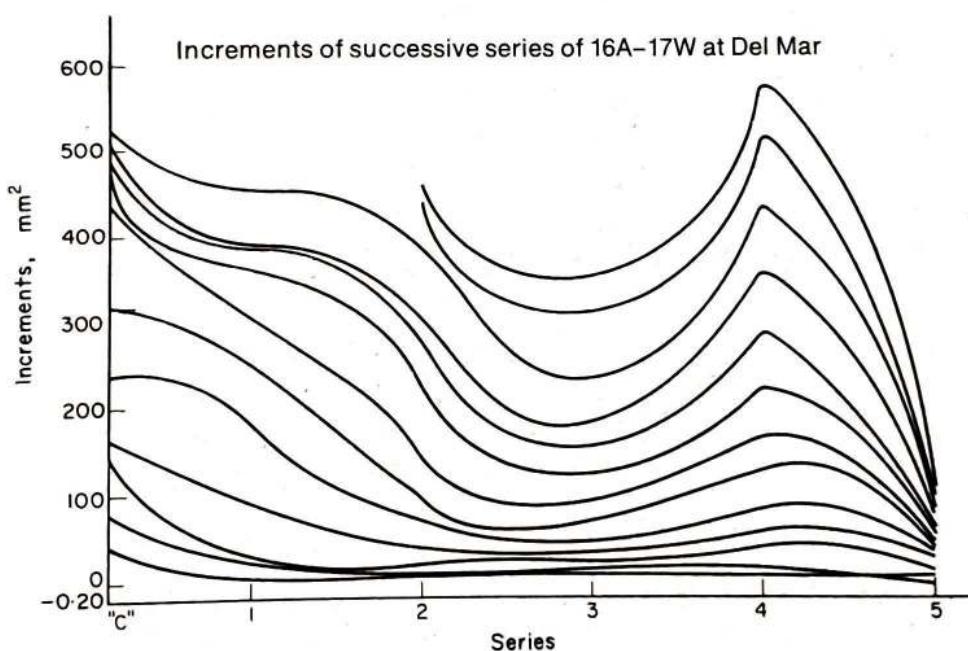


CHART 97. This chart shows the increased inhibition of tumor growth following alternate injections of 16A and 17W in the five successive series of mice. Measurements of tumors are of the 2 to 26 periods of injections and comparable points of the successive series are connected by smooth curves. The maximal amount of inhibition of tumor growth (less spread in tumor size) was obtained in mice of series 5 when the injected solutions had been aged 104 days. Successive series are presented on base line and tumor size on the vertical line.

under refrigeration had no inhibitory action on tumor growth. The growth rate of tumors in mice belonging to series 1, 2, 3, 4 and 5 apparently were all inhibited to some extent by this treatment. The maximal effect of inhibition was obtained in series 5 when the solutions were 104 days old at the start of that series.

Chart 97 presents an attempt to draw curves between the points indicating the size of the tumors in the successive series 1-5 receiving alternate injections of 16A and 17W supernate. Except for mice in series 4 there appears to be a downtrend in tumor increments of growth from the controls (C) and series 1 to 5.

Chart 98 presents the same data by spacing the series apart according to age of solutions at the start of successive periods and using straight lines between the controls (C) and the suc-

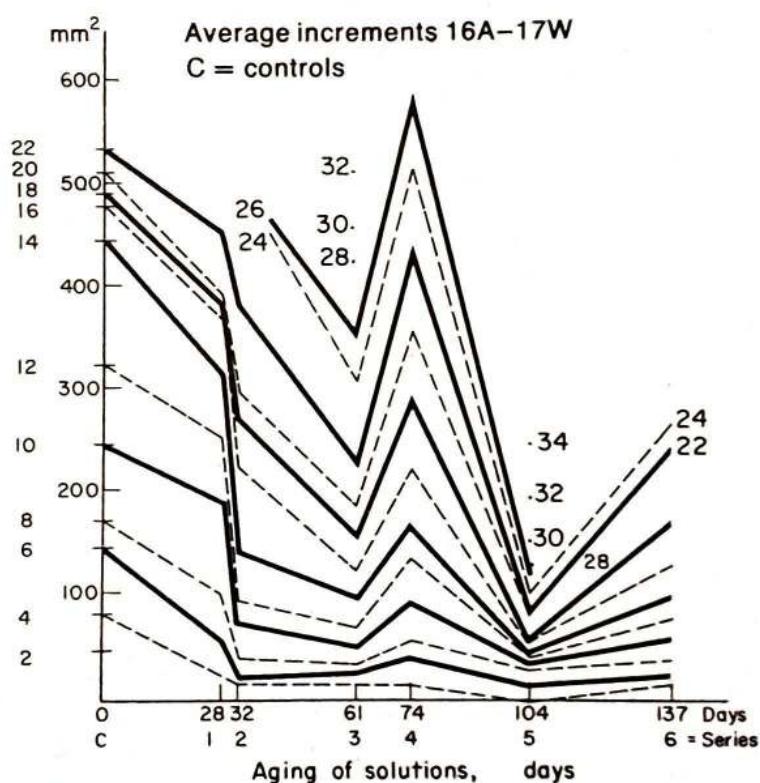


CHART 98. This chart is based upon the same data as presented in Chart 97 except series 6 is also included. Straight lines are drawn between the comparable data for six series of mice. The successive ages of the solutions used were 28, 32, 61, 74, 104 and 137 days. The data are presented as successive increments of growth—thus reducing the variable in tumor size at the start of each experiment. The controls are on the vertical scale indicated C and the successive series 1, 2, 3, 4, 5 and 6 spaced along the base line according to the age of the solutions at the start of each series. Only the data for every second period of measurement of tumors and injections of materials as 2, 4, 6 up to 26 are presented.

sive series. Here again it appears that except for series 4, when the "aging" solution was at 74 days, there is a downtrend in inhibition reaching a maximal result in series 5 at 104 days of aging solutions.

Chart 99 combines the data on successive series 1-2, 3-4 and 5-6. The size of tumors expressed as increments are for 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 and 26 periods of observations. Thus there is demonstrated an increased inhibition of tumor growth between the successive series. The maximal amount of inhibition was obtained in the combined 5 and 6 series.

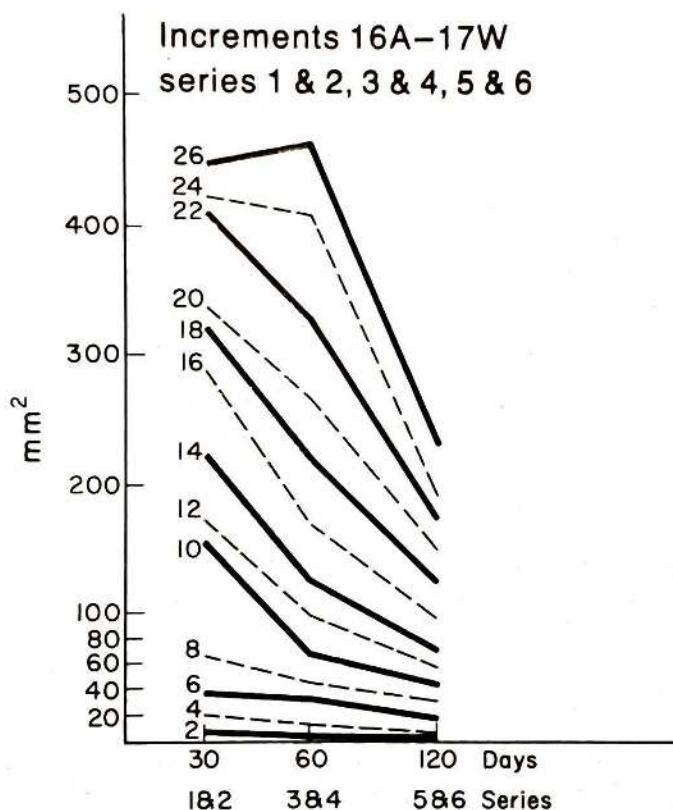


CHART 99. This chart simplifies the data presented in Chart 98 by combining successive series together. Thus the age of the solutions of series 1 and 2 was 30 days; 3 and 4, 60 days and 5 and 6, 120 days. The inhibitory action of the solutions increases with the aging of the solutions.

Chart 100 presents the data on average increments of growth rate of the controls at the fourth, eighth, twelfth, sixteenth, twentieth and twenty-fourth periods of observation (C). Combined series 1-2, 3-4 and 5-6 are also included. Thus there is a clear indication of increased inhibition of tumor growth by the alternate injections of 16A-17W at 30, 60 and 120 days of aging. The maximal amount of inhibition is reached in series 5-6.

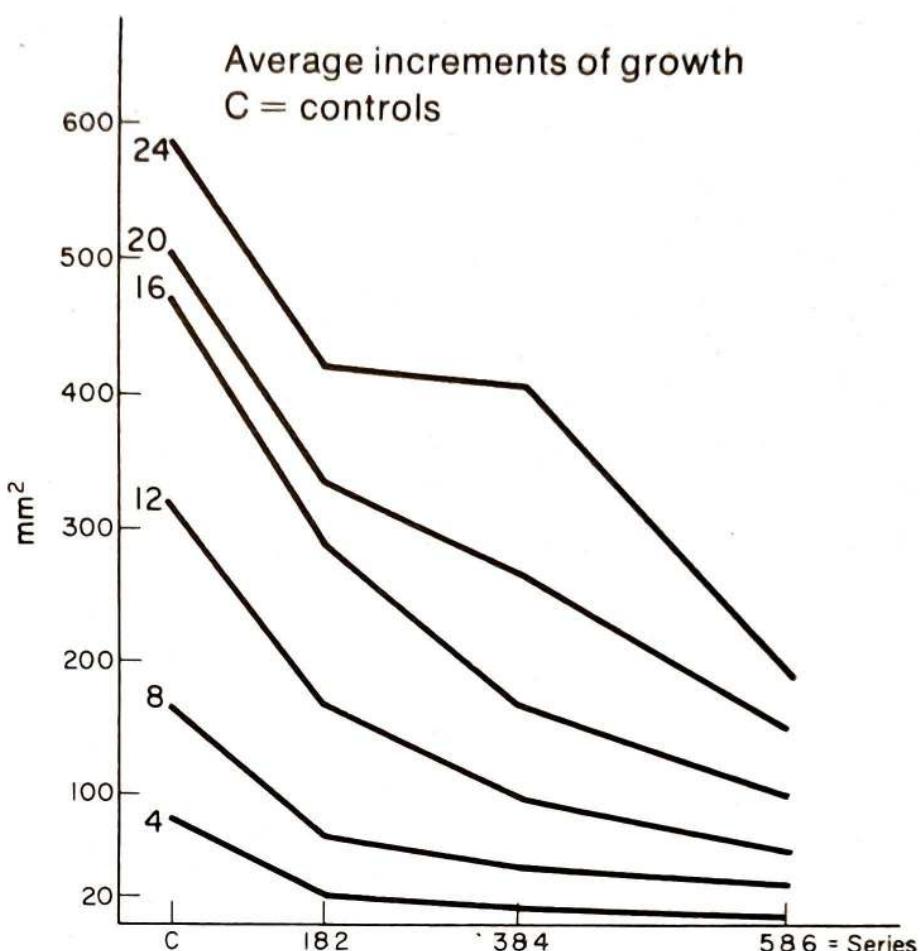


CHART 100. This chart reduces the presentation of data to every fourth period of observation and injection of solutions, i.e., 4, 8, 12, etc., for the six successive series of mice injected with solutions 16A-17W combined into three groups. The controls are indicated as C. It is obvious that there was obtained a greater increase of inhibition of tumor growth with the aging of solutions.

Chart 101 presents the data on average increments of progressive growth of tumors in the controls (C) as well as in series 1, 2, 3 and 5. Here again it is evident that the average increments of growth per unit of time is less in series 5 than in series 1, 2, 3 and the controls. In series 4 (for some unknown reason) there was a trend counter to expectation and was therefore not included in this analysis. Its inclusion in the data on growth rate inhibition is amply covered in Charts 98-100.

Chart 102 gives the data on average successive increments of tumor growth in series 1-2, solid line, series 3-4, short dash line and series 5-6 on long dash line. Between observation periods 2 and 24 there is thus evidence showing greater inhibitory action of the solutions in series 5-6 than in either 1-2 or 3-4. At

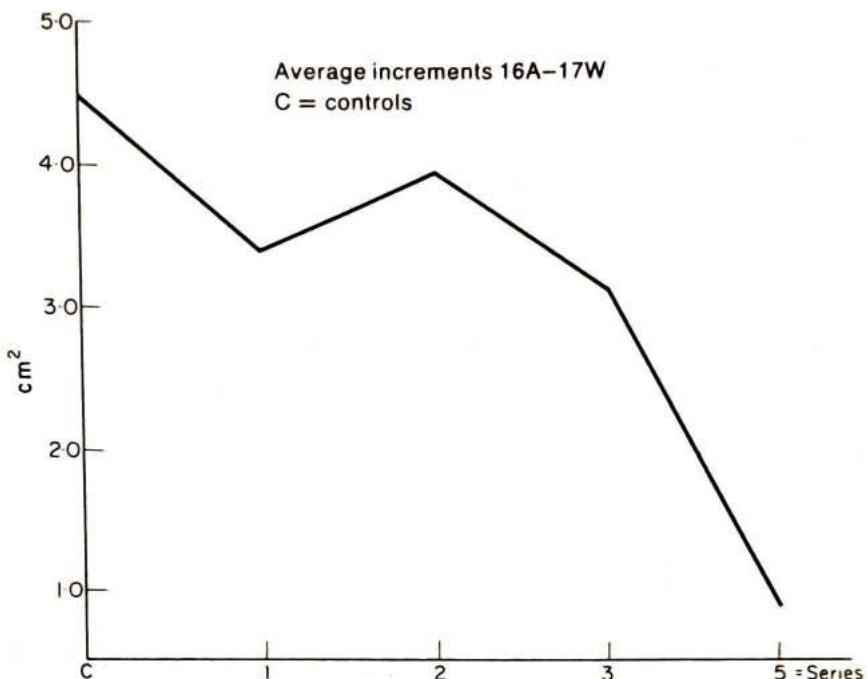


CHART 101. This chart presents the data on inhibition of tumor growth by the use of solutions 16A-17W in still another manner. Here the data are presented as average increments of tumor growth in series 1, 2, 3 and 5 compared to the average increments of tumor growth in the controls, C.

the twenty-sixth period of observation series 1-2 drops between series 3-4 as a result of the death of some mice with large tumors. It is a clearly demonstrated fact that a mouse with a large rapidly growing tumor will die earlier than one with a slower growing smaller one. All mice in series 5-6 which survived the fiftieth period of observation had completely regressed their tumors under treatment with alternate injections of 16A-17W.

Chart 103 presents the data on inhibition of tumor growth by still another manner. Here the successive differences in average increments of tumor growth between mice of series 1-2 and 3-4 are given. In all cases between observation periods 52 and 22 the increments were greater in series 1-2 than in series 3-4. The maximal difference is obtained at observation period 16 but is still significant up to observation period 22.

Chart 104 gives the successive differences in average growth rate increments between series 1-2 and 5-6 for observation periods 2 through 22.

Chart 105 is a combination of Charts 103 and 104 in order to show the differences in average tumor-growth increments

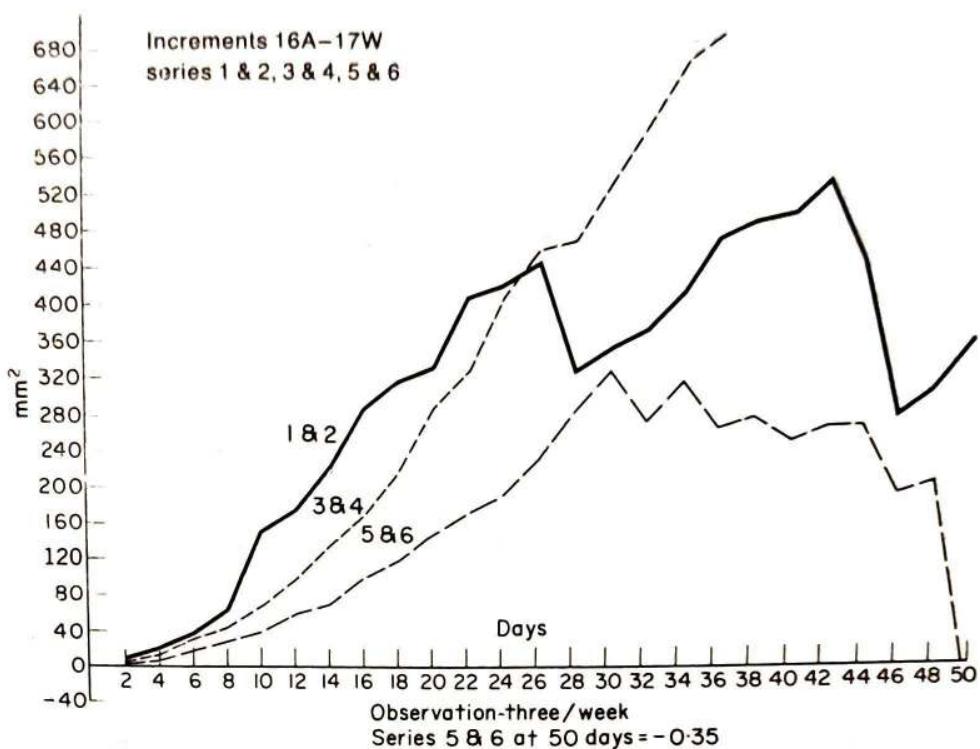


CHART 102. This chart presents the data on successive increments of tumor growth in series 1 and 2, solid line; 3 and 4, short dash line, and 5 and 6, long dash line, obtained with tumor-bearing mice injected with solutions 16A-17W. It can be seen that there is thus progressive inhibition of tumor growth in mice of the successive combined series. The one discrepancy is in series 1 and 2 beginning with observation period 28 and this is due to earlier death of mice growing their tumors faster and thus dying earlier. (A mouse with a large tumor usually dies earlier.)

between series 1-2 and 3-4 (solid line) and between series 1-2 and 5-6 (short dash line). It is apparent that the mice in series 5-6 have had the growth rate of their tumors inhibited greater than in series 3-4.

Chart 106 shows the data of the average deviations from the controls for tumor-growth increments for series 1, 2, 3, 5 and 6 for mice receiving alternate injections of 16A and 17W. There can be seen that there was obtained a progressive degree of inhibition of tumor growth in series 1, 2, 3 and 5, but there was a loss from the maximal value of inhibition in series 6.

Chart 107 shows the differences in average deviations between series 1-2 and 3-4 (solid line) and between series 1-2 and 5-6 (short dash line). All deviations from series 1-2 are minus except at observation period 26 between 1 and 2 and 3 and 4 where the one deviation is +12.

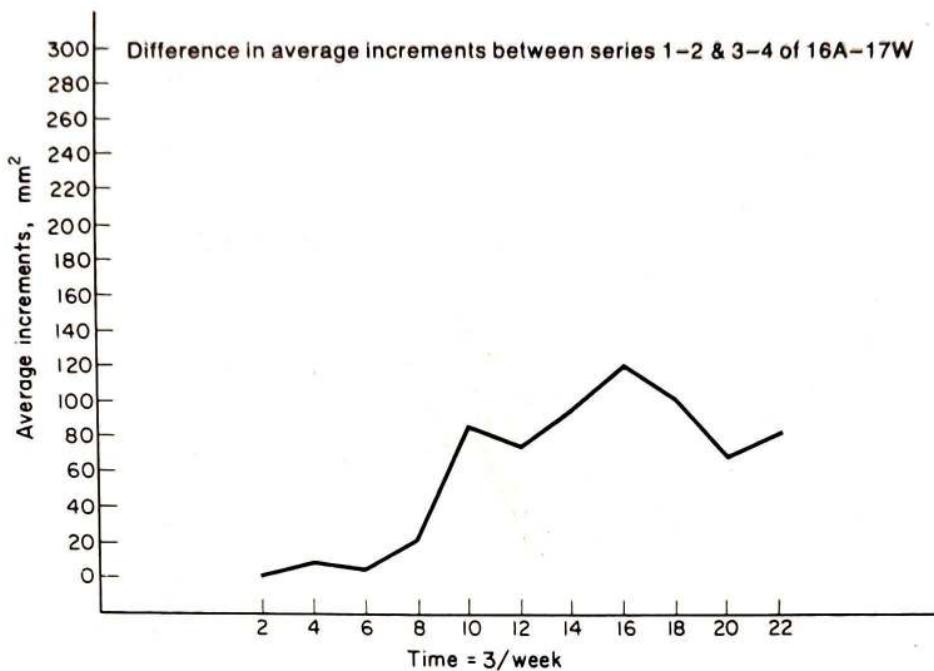


CHART 103

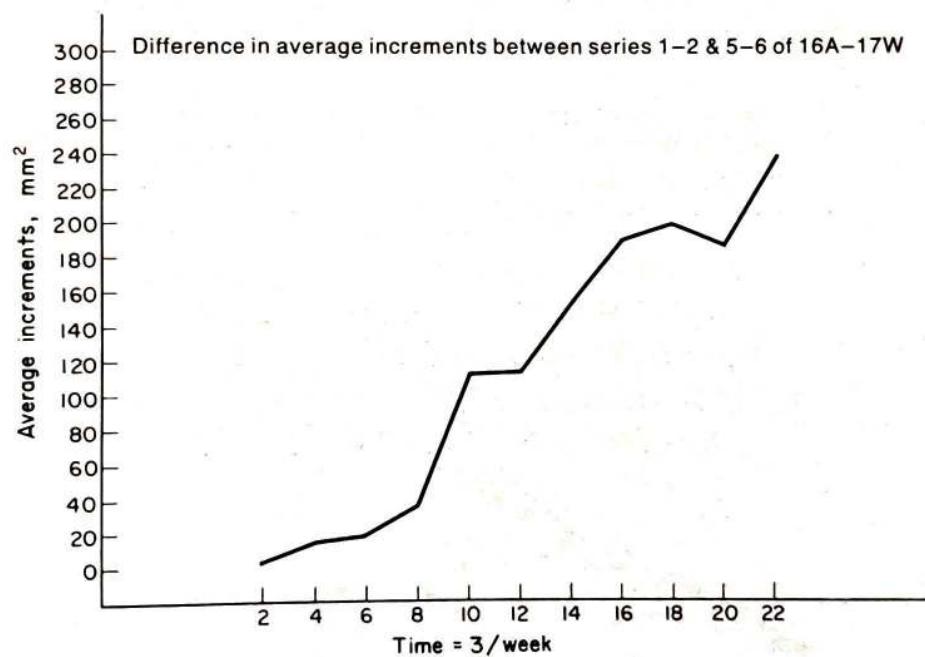


CHART 104

CHARTS 103, 104 and 105. These charts show the data on successive increased inhibition of tumor growth in combined series 1 and 2, 3 and 4, and 5 and 6 in still another manner. These charts show the difference in average increments between 1 and 2, 3 and 4, and 1 and 2, 5 and 6. There is thus indicated successive increases in the amount of inhibition during the extent of the experiment—the maximal differences in each case being in the twenty-second period of observation and injection of either the solutions 16A or 17W.

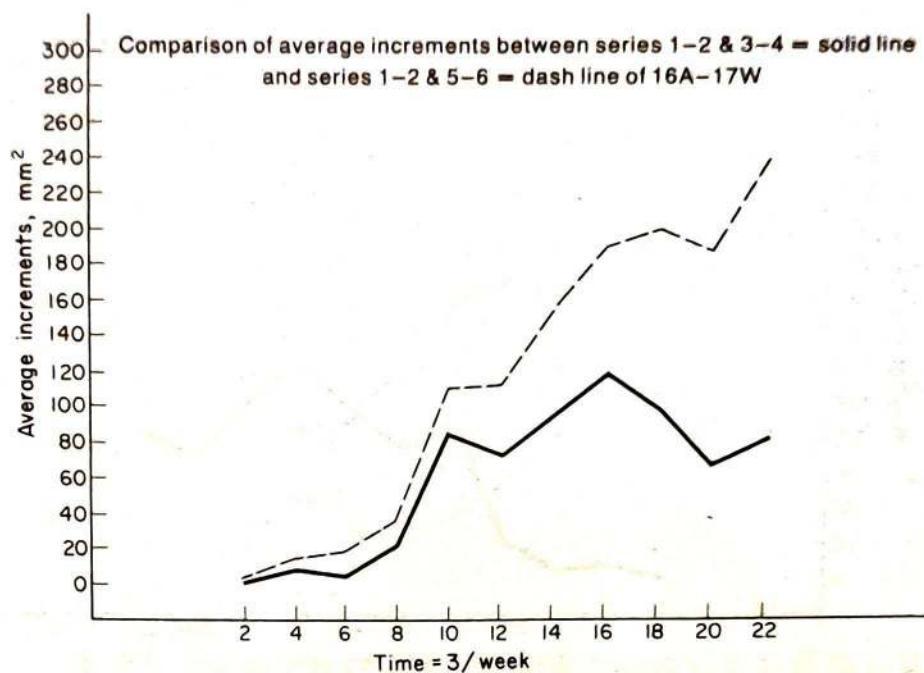


CHART 105

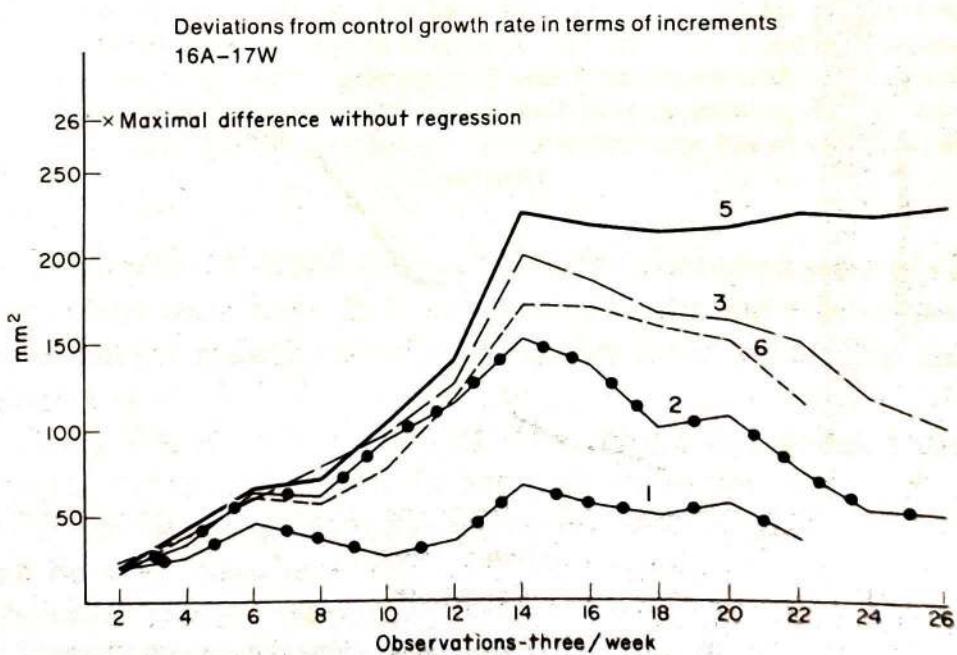


CHART 106. This chart presents the data on the successive deviations of the size of tumors from the controls in the successive series 1, 2, 3, 5 and 6 receiving solutions 16A-17W. The smallest deviation from the controls is in series 1, the maximal in series 5. Thus there was a progressive inhibition of tumor growth in the successive series which can only be ascribed to the injections of the "aging" solutions.

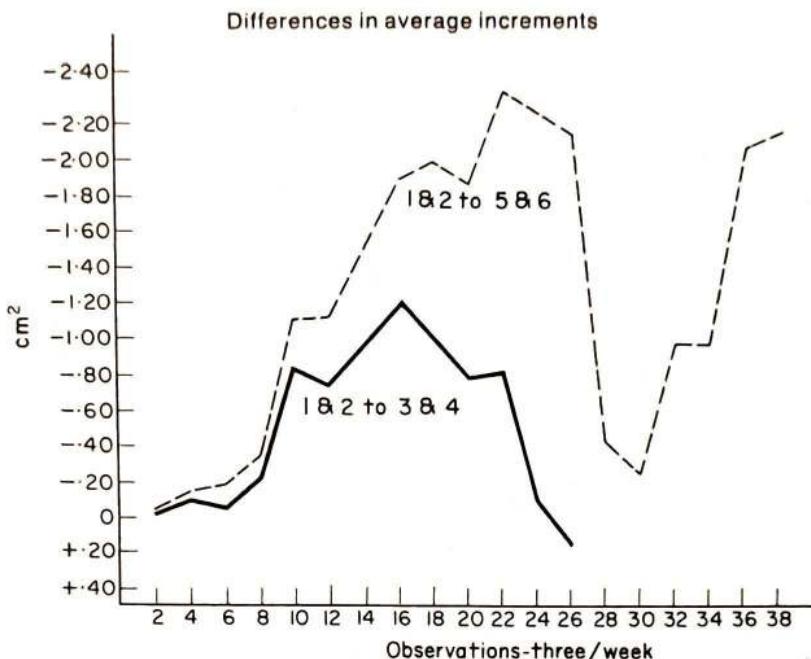


CHART 107. The data on differences in average increments between 1 and 2 and 3 and 4 and 1 and 2 and 5 and 6 are presented in this chart. It can be seen that series 5 and 6 deviate more from 1 and 2 than does 3 and 4, thus indicating a greater inhibition of tumor growth.

Charts 108 through 111 show the deviations of average increments of tumor growth between the controls and series 1 and 2, Chart 108; between the controls and series 3 and 4, long dash line and between the controls and series 5 and 6 on the short dash line, Chart 111. Thus it appears to be reasonable to conclude that there have been progressive increases of average increments of tumor growth in mice of the successive series treated with alternate injections of solutions 16A-17W.

General Discussion

In the final evaluation of research dealing with the inhibition of the growth rate of spontaneous tumors and the survival time of mice, or any other species bearing tumors, it would be desirable to ascertain whether the results obtained were consistent with the commonly accepted facts and ideas on the nature of cancer itself. It would also be desirable to discover whether the obtaining of positive evidence on inhibition was dependent upon a well-established theory or concept as to the nature of cancer and the actual cancer process itself.

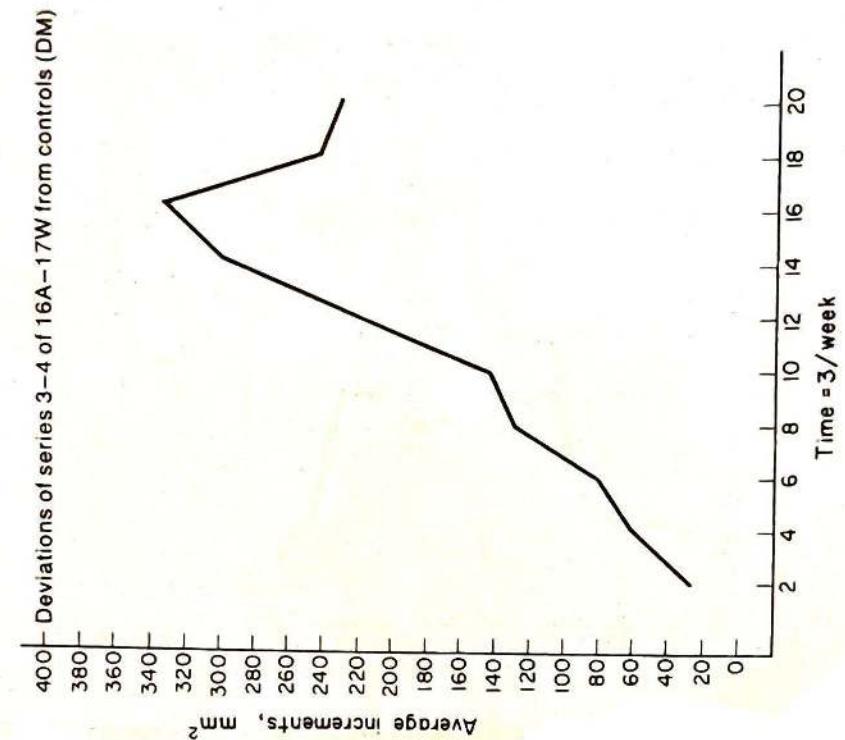


CHART 109

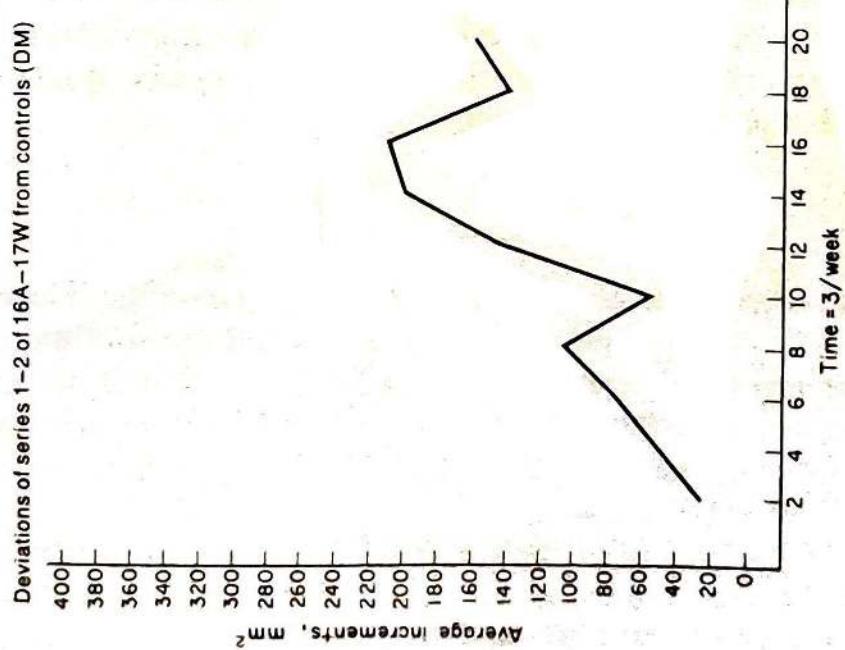
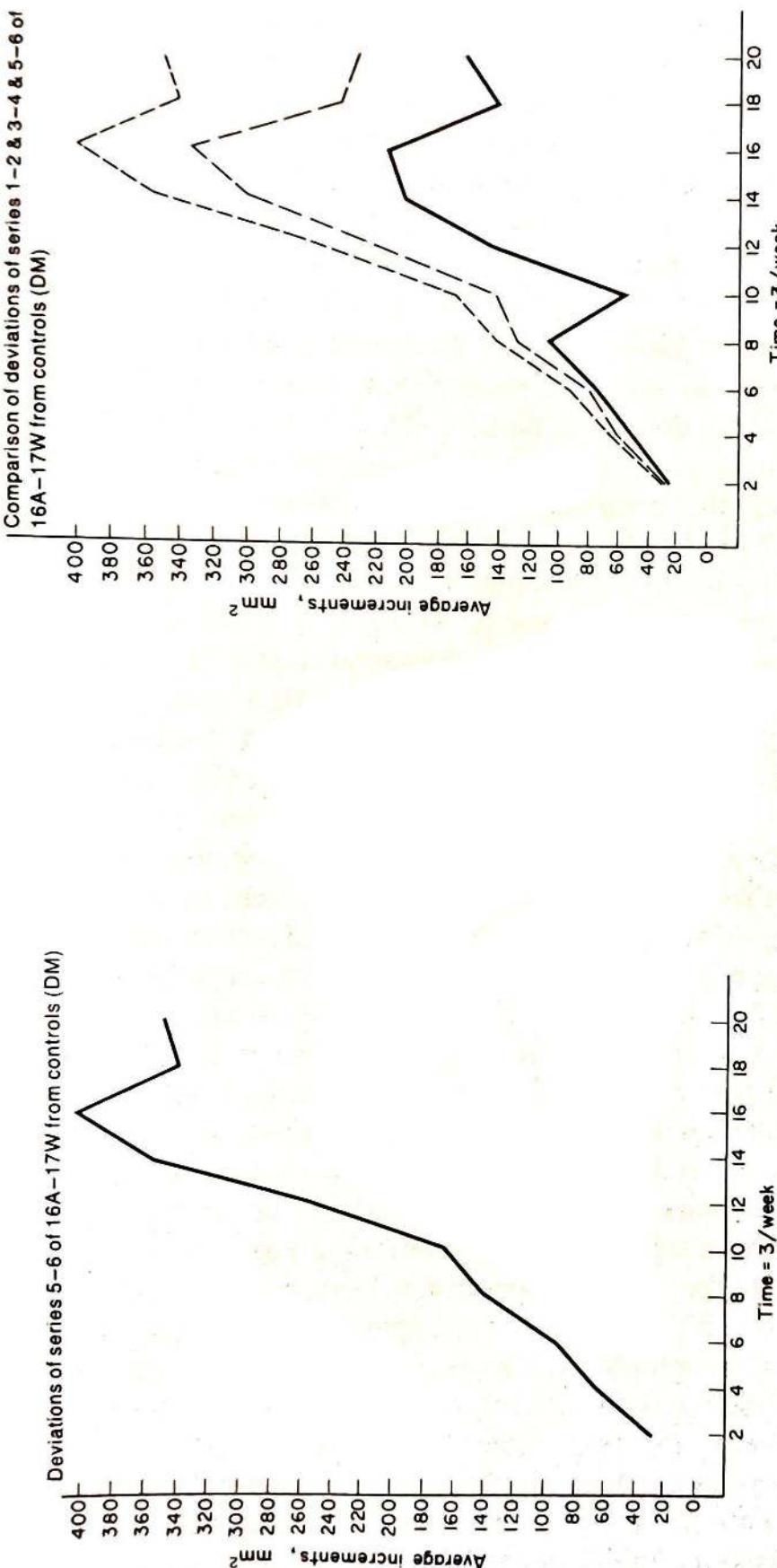


CHART 108



CHARTS 108, 109, 110 and 111. These charts present the data on deviations from the controls for experimental series 1 and 2, 3 and 4, and 5 and 6 receiving 16A-17W solutions for the average increments in growth size of spontaneous tumors. Chart 108 compares the data between the controls and series 1 and 2; Chart 109 between the controls and series 3 and 4; Chart 110 between the controls and series 5 and 6; and Chart 111 between the controls and all three combined series, 1 and 2 on the solid line; 3 and 4 on the long dash line; 5 and 6 on the short dash line. It is obvious that there was a progressive increase of deviations from the controls in the measure of successive average increments of growth of tumors in series 1 and 2, 3 and 4, and 5 and 6, thus indicating an increased inhibitory action of the solutions with time.

However desirable these discussions would be in the final evaluation of obtained results, it does not seem to be appropriate for this experiment at the present time. The discussion now will hinge, primarily, upon the variables involved in attempting to bring about the regression of spontaneous tumors in mice by tissue extracts. In this presentation, liver extracts alone have been used. It is considered highly probable that, with the identification of most, if not all, of the variables involved in the inhibition of spontaneous tumor growth by single entities and their practical combination into one experiment, this approach may lead to further, if not complete, control. Then it would be time to indicate the bearing of the fundamental nature of cancer or of an individual concept this nature has upon tumor control.

The present experiment consists of the injection of liver extracts prepared by relatively few methods, injected intraperitoneally periodically into spontaneous tumor-bearing mice always at intervals of three per week (Monday, Wednesday and Friday).

The new data verifies the conclusions drawn in a previous report (Chapter 5) that there are apparently, at least, two inhibitors of tumor growth in liver extracts. One of these (W) is water soluble and decreases in effective inhibitory action of tumor growth with advancing age of the mice from which the liver is derived. The other inhibitor is alcohol soluble (A) but forms a pearly white emulsion in water. It increases in effectiveness with the advancing age of mice from which the livers are derived at least between 120 and 250 days of age. There is no attempt now to resolve the two inhibitory materials into purer form. It is probably true that the solutions or emulsions contain many entities. Thymol, which had been used as a preservative for the liver extracts in water has been shown to have no effect upon the growth of spontaneous tumors in mice. This is true, at least, for the concentrations of thymol used as a preservative.

Again, as in the preceding experiment, both components, the A and the W, inhibit the growth rate of tumors when used separately. They also apparently have a synergistic effect on inhibition when used for injections at alternate periods. Again, as in the former experiment, the best combination for inhibition is an alcohol-soluble portion (A) from an old mouse alternated with a water soluble (W) from a young animal although the reverse combination has some inhibitory effect.

In one experiment where serial dilutions of the fractions were employed (9W and 10A) it was found that apparently there was a relationship between tumor inhibition and concentration of materials (Chart 92). Here it can be seen that when the solutions were diluted in the ratio of 1, $\frac{1}{2}$ and $\frac{1}{4}$ the measure of tumor size was approximately in the ratio of 1, 2 and 4. However, since there were only four tumor-bearing mice in each series, the positive degree of difference between the three series can only be accepted on a provisional basis. In all cases, however, in the experimentally injected mice, there was obtained some inhibitory action on tumors, since, in all cases the tumors in the serially injected mice were smaller per unit of time than in the controls.

The most significant new finding was that it was discovered that the "aging" of the solutions determined from the time of the addition of the distilled water and thymol had an altered effect on the inhibition of tumor growth. This finding applies only to the series of mice that had received alternate injections of both the A and the W materials. The experiment has not been done separately with either A or W so it is impossible, at present, to determine whether one or the other or both solutions or emulsions are changing with age. It is clear, however, that by gross inspection the W solution does not change in appearance of color or opacity with age but remains as a clear pale-yellow solution. On the other hand, the A material in distilled water increases in opacity with age and apparently improves as an emulsion since less material settles out with standing when the A material is kept under refrigeration and not being used for injection purposes. Before the W solution and A emulsion are injected into mice they are brought to room temperature in order to avoid spastic responses of the animals.

In the six series of tumor-bearing mice receiving alternate injections of 16A (old livers) and 17W (young livers) there appears to be a progressive increase of inhibition of tumor growth with time, at least, between 28 and 104 days (Charts 96-111). On the other hand, in the five series of tumor-bearing mice receiving alternate injections of 17A (young livers) and 16W (old livers) there appears to be a reversed situation although the differences between successive series are not great (Chart 95). It may be of some significance that the solutions in the 16W and 17A series were older at the time of injection into mice than when the 16A

INCREMENTS—DEVIATIONS EXPERIMENTAL 16A-17W FROM CONTROLS (DM)

All subsequent increments of controls are positive.

All deviations from controls are negative.

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Control Increments	0.30	0.59	0.71	0.88	1.10	1.24	1.71	1.93	2.07	2.75	3.15	3.47	4.24	4.67	5.00	5.27	4.60	4.67	4.97
Deviations between controls and 1 and 2	0.26	0.51	0.51	0.62	0.75	0.76	1.06	0.95	0.56	1.22	1.43	1.46	2.00	2.14	2.10	2.46	1.39	1.33	1.59
Deviations between controls and 3 and 4	0.27	0.51	0.60	0.67	0.80	0.88	1.28	1.33	1.42	1.94	2.18	2.35	2.97	3.21	3.30	3.33	2.40	2.43	2.28
Deviations between controls and 5 and 6	0.29	0.58	0.65	0.77	0.93	1.02	1.41	1.53	1.68	2.21	2.56	2.82	3.54	3.79	4.00	4.16	3.39	3.31	3.47

CHART 112. This table presents the data on average deviations of the increments in tumor growth from the controls of the Del Mar series for the three combined series, 1 and 2, 3 and 4 and 5 and 6 receiving 16A and 17W. Observations on twenty successive observations are given. It will be noted that all successive increments of the growth rate of the controls are positive. All deviations of the experimental series from the controls are negative, thus indicating progressive inhibition of growth in the successive series.

and 17W materials were used (28-137 days compared to 104-223 days).

However, since several variables have already been indicated as having an influence on tumor inhibition by liver extracts and perhaps there may be others not, as yet, indicated, these changing effects must not be considered for final analysis. They do, however, lead to the conclusion, although perhaps to be considered in a preliminary manner, that a positive effect of aging solutions and emulsions has been obtained. What the final analysis will be can only be determined by further work.

CHAPTER 7

An Historical Perspective for the Control of Spontaneous Tumors in Mice

SECTION I

Genetic Concept of the Nature of Cancer

In 1920 the author published his first observations on cancer research! It was reported then "that the two histologically identical tumors possess different physiological reactions". This observation led to the provisional conclusion that "since normal tissues are, to a large degree, dependent upon the genetic complex of the individual, may we not also look for the causes underlying susceptibility to transplantable tumor as being similarly correlated with genetic factors?" Further observations since that time have completely vindicated this early conclusion.

This 1920 paper is not commonly referred to in the literature of cancer research. The findings and derived conclusion reported then have, however, been retained by the author and were the original bases for the development of the genetic concept for the origin of cancer (Strong and Little, 1920).

The expansion of a genetic concept of cancer was extremely slow at first but has received an ever-increasing support from many sources as time goes on.

The observations of the 1920's and 1930's particularly those of the trilogy of the author and his graduate students, Bittner (1931-1932) and Cloudman (1932a), laid a sound foundation not only for the genetic nature of the inheritance of susceptibility and resistance to the transplantable tumor (foreseen by the previous observations of Tyzzer (1916) and Little (1920) but also provided presumptive evidence for the genetic nature of the cancer cell itself.

The first "suggestion" that a mutation may presumably be involved in the origin of cancer was made by Murray (1908) and

later amplified by Tyzzer (1916). The author basing his conclusion upon experimental evidence arrived at a conclusion of a somatic mutation concept in 1922 (Strong). At that time, he stated "sudden fluctuations in growth activity of a tumor may sporadically occur, due possibly to a process analogous to mutation".

In 1924 Little and Strong arrived at a conclusion as to the nature of the cancer cell itself as follows; "the fate of the implanted tumor tissue when placed in a given individual (host) is brought about by a reaction between the host, determined to a large extent by its genetic constitution and the transplanted tumor cell, controlled to some extent by its genetic constitution" (Little and Strong, 1924).

Additional evidence for a mutation concept in relation to the origin of cancer was published in 1926 and the following years. Thus in a general survey published in *The American Naturalist* (Strong, 1926d) the conclusion was reported that

genetic changes in the internal constitution of the tumor cell occur sporadically during the process of transplantation. These genetic changes result in different physiological characteristics of the tumor mass—they change the transplantability of the tumor mass as well as changing the proliferative energy of the tissue. The genetic constitution of the tumor cell has deviated from the genetic constitution of the normal tissue from which it arose, presumably through the process of mutation (Strong, 1926a).

The experimental evidence for the above conclusion on mutation and the origin of cancer was published in two papers in 1926 (Strong, 1926b; 1926c). The first paper was "On the Occurrence of Mutations within Transplantable Neoplasms," and the second, "Changes in the Reaction Potential of a Transplantable Tumor". In these papers evidence was presented that demonstrated that the genetic requirement for the transplantation of neoplastic tissue was serially reduced with time—not gradually but sporadically and suddenly.

These changes in "tissue specificity" for cancerous tissue growth have been verified many times by several authors. As far as the author knows, the reverse of this situation for an increased genic requirement has only been reported twice; once by Hauschka (1958a) and secondly by the author.

These sudden appearances of fewer gene requirements for the successful propagation of transplantable tumors were always

accompanied by a sudden increase of their growth capacity. As a matter of fact, by measuring the growth rate of a series of transplantable tumors, the observation of any growth rate far beyond the mean for the entire population of cancer cells should always lead to a suspicion that a new "mutation" had occurred. The concept of genic change can readily be verified by the use of suitable genetically controlled experimental animals, especially mice.

An early work that was published in 1929 (Strong) again served in the development of the genetic ideas of the author as to the nature of cancer. In this paper studies on two of the spontaneous tumors that arose in Mouse No. F₁ S79 were genetically analyzed. This mouse (No. F₁ S79) could have served as a whole arsenal of materials for cancer research but unfortunately not all of this material could be utilized. The contributions to cancer and other biological sciences that this mouse made partially possible were legion and could serve for a special lecture as did one of her descendants, Mouse No. S46801 (Strong, 1962). Mouse F₁ S79 had been produced by a cross between mice of two "strains" in order to produce animals for a Ph.D. thesis (Strong, 1922). She became the progenitor of the so-called "C" family of inbred mice. These were given the symbols C, C₃H, CBA, C₁₂I and CHI. All except C₁₂I are still in existence after 46 years of independent descent together with many derived sublines set up for a number of reasons and are being used for a vast amount of research in many fields. The C₃H/St and CBA/St are still some of the most desirable mice for several aspects of cancer research.

But in addition to being the progenitor of this valuable descent, Mouse No. F₁ S79 was the source of fourteen spontaneous tumors all of which were probably genetically different. However valuable the complete genetic analysis of these spontaneous tumors would have been it was impossible, due to restricted budgets for cancer research in the 1920's, to analyze completely no more than two with any degree of finality. In 1929 (Strong) a discussion on the presumptive nature of the cancer cell was based partly upon the analysis of these two tumors. In this paper the problem was discussed as follows;

The two tumors employed in this experiment, even in spite of the fact that they are derived from the same individual and histologically indistinguishable, are indeed quite different. They are physiologically different and presumably

genetically different. If one assumes, for the sake of argument, that one of these tumors possesses the same genetic constitution as the mouse tissue from which it arose, then the other tumor tissue cannot have it. This assumption is a valid one, since the recent interpretations of histogenesis would lead us to the conclusion that quantitative cell-divisions do not occur in animate forms.

Every cell of the adult body is supposed to be endowed with the same genetic potentialities. Since, therefore, one of the tumors must have a different genetic constitution from the mouse tissues from which it was originated, it must have deviated, presumably by some such a process as mutation, from the somatic tissue from which it arose (Strong, 1929).

There has been some further research in analyzing the genetic characteristics of several tumors arising from the same animal, although the concept that they may deviate from each has not been exploited enough. This should be possible now especially since larger funds are available to support adequate numbers of essential genetic hybrid mice. It is also true that not enough research has been done on primary tumors through the first few transplant generations, where greater gene requirements are to be expected thus leading to possibly more bases for differences in biological and genetic characteristics. It is an established fact that the serial changes in genic requirements for transplantation of cancer is usually a simplification process—and thus there are less specific differences to be expected between long-established tumors of an original or similar origin, as is usually the actual case.

Again the contributions of the trilogy Strong (1920), Bittner (1931-1932), and Cloudman (1932a) on genetic analysis of the transplantation of cancer could very well be expanded into a separate lecture, but now only a brief discussion can be given. The research centers around the transplantation of tumors arising in mice of two original ancestral stocks A/St and D/St together with their derived F₁ offspring. Strong (1926a) had transplanted the tumors that arose in mice of the D stock and found that they would grow in all mice of the D stock but not in the A. They would grow in all F₁'s and in some but not all F₂'s. The ratio in the F₂'s was consistent, in all cases, with expected Mendelian frequency. Thus the dBrB tumor would grow when only two independent (non-linked) genes were present; the dBrA tumor, however, required the presence of three genes for its continued

growth. He found that, in the F_2 's, some mice would grow both tumors; some would not grow either; some mice would grow the B tumor without growing the A; and no mouse was found that would grow the A but not the B tumor. Thus it is clear that two of the genes responsible for the inheritance of susceptibility to the transplantable tumor were common to both tumors but that a third gene was necessary for the successful growth of the A tumor (Little and Strong, 1924).

Cloudman (1932a) investigated several tumors that arose in mice of the A/St strain. It was found that these tumors would all grow in mice of the A/St strain but not in the D/St. They would grow in all mice of the F_1 and showed various ratios of susceptibility to resistance to tumors in the F_2 .

Bittner (1931) analyzed the spontaneous tumors derived from F_1 mice. It was found that these tumors from F_1 individuals would grow in all F_1 mice but would not grow in mice of either ancestral stock. Segregation again occurred in the F_2 . In one case, Bittner determined that eight independently segregating genes were responsible for the continued growth of a tumor derived spontaneously from an F_1 . By the use of the two backcross generations he was able to prove that three of these genes were derived from one ancestral stock and five from the other. Thus there was clear evidence obtained that an F_1 mouse is of a mosaic nature deriving its characteristics from both parents, as had already been demonstrated by innumerable investigations starting with Mendel. The significant conclusion from this program of cancer research was the demonstration that a tumor derived from an F_1 is also of a mosaic nature, thus again emphasizing the genetic nature of the cancer cell.

Cloudman (1932b) also reported the finding similar to the case of mouse No. F₁S79 (Strong, 1929), that two tumors derived from the same gland of a single mouse were genetically different. Strong (1958), in his review in the Symposium at the New York Academy of Sciences, commented briefly as follows:

From this early work it was determined that no two tumors, even derived from the same mouse, ever gave the same Mendelian ratio of susceptible or resistant mice in the F_2 . In all early transfer generations this is evidence of populations of cancer cells rather than pure cultures. With later transfer generations, after the process of serial or progressive "breakdowns" of susceptibility have occurred, some tumors have similar and perhaps identical genetic backgrounds.

That a cancerous growth is a mixed population of genetically determined different cells and not a pure culture of a single type will be taken up later in Section IV.

These observations (1920-35) of a pioneer nature must be retained in order to evaluate adequately the historical development of the genetic nature of cancer.

The great expansion of this early genetic work on cancer can be briefly indicated by a few comments on three topics. According to D. B. Amos (1956), one of the pioneers in the development of one of these topics, that of histocompatibility, progress has been so rapid that by the time a new observation or conclusion has been published, advances beyond this point have already been discovered and made the published article obsolete. However, a few points of an historical nature may be mentioned.

(A) The first development, pioneered by Gorer (1942) in England and greatly expanded in this country by D. B. Amos (working in Gorer's laboratory in London, England, then in Hauschka's laboratory in Buffalo, New York, and more recently in his own in Durham, North Carolina), by G. Snell of Bar Harbor, Maine, and by several others is in the further analysis of the genes involved in the transplantation of cancerous tissue, that are, at present, referred to as being histocompatible. It has been repeatedly shown that there are several genes involved in the successful transplantation of cancerous tissue, inherited on an independent (non-linked) basis. Some of the histocompatible genes (such as H_2) are "strong reactors" while others are weak. They can be identified by various serological tests. Improvement of the techniques of indicating the presence or absence of these genes has aided considerably in their investigation since, by using serological techniques, it is no longer necessary to wait long periods of time for the growth of a cancerous tissue. Several cases of linkage between histocompatible genes and known markers have already been indicated (Hauschka, 1958a). According to Hauschka (1958a) "there is no conflict between the cytological and genetic data and the validity of transplantation genetics, as established through the classic experiments of Tyzzer (1916), Little (1920) and Strong (1922, 1926b), the serologic work of Gorer (1942), and the elaboration of histocompatibility loci and linkages by Snell".

The expansion of the work on histocompatibility is so rapid

that it is difficult to report the present status. However, just to quote one statement by Amos (1958):

the experiments showing that some well-established tumors can be made to increase the apparent number of factors required for transplantation may mean that the ability to form antigens other than H_2 may be "waived" in serial passage without alteration in strain specificity. This may be associated with an increase in growth rate of the tumor, and is adaptive (it may be a parasitic type of phenomenon in which the necessity for some metabolic processes is not required in a favorable environment). The change in H_2 is possibly a different process involving complete reorganization of the economy of the cell, and may be associated with morphologic and growth-rate changes, in which case the H_2 locus could be considered to elaborate some essential process for normal cellular economy, which is, incidently, strongly antigenic.

Just what the relation of the action of these histocompatible genes to the origin of cancer itself is, is still in doubt since it is known that at least some of these same genes that are involved in the successful grafting of cancerous tissue are also involved in the grafting of normal tissues, especially skin.

(B) The second great development in the analysis of the genetic factors involved in the grafting of cancerous (and normal) tissues was the introduction of the use of isogenic strains of mice where comparative analysis of "single genes" and their physiologic or morphologic effect can be more precisely made on a common genic background. This work was greatly developed by Snell (1953). Perhaps the greatest use of isogenic strains of mice has been in the more precise analysis of histocompatible genes but they do have many other applications.

(C) The third development of the genetic concept for the nature of cancer and its probable origin from an altered genome is in the interpretation of genetic phenomena in terms of molecular biology with all its potential bearing on the biochemistry of DNA, the coding mechanism and the other aspects of genetic influence on physiological behavior at the cellular level. The newer work also has had the great opportunity of incorporating into a concept the recent contributions of virology, chemical carcinogenesis, etc.

For example, the polyoma virus which has been the source of considerable research on cancer for a decade has been shown by Di Mayorca *et al.* (1959) to be DNA. The application of the new techniques of molecular biology initiated by many scientists including Nobelist Lederberg (1958, 1959) has permitted Dulbecco to analyze, to a certain extent, the gene "pool" of viral particles.

These techniques of molecular biological analysis were reviewed in Lederberg's Nobel Prize Lecture at the Royal Caroline Medico-Surgical Institute in Stockholm on 29 May 1959. But in addition to the development of new techniques, Lederberg has been thinking on the cancer problem and presented some interesting ideas that are of importance in the development of the analysis of the origin of the cancer cell. In 1946 Lederberg stated:

If one correlates normal tissue cells with culture of leucineless *Neurospora*, both regulated by their environment, a simple analogy for cancer is evident in the newly found capacity of a cell to synthesize an essential metabolite otherwise available only in limiting and regulatory amounts. While the *Neurospora* experiments suggest a mutational origin for this capacity, virus infection, by providing a missing link for a blocked enzyme system, could play a corresponding part.

Another significant comment by Lederberg (1946) was as follows:

Similarly, the origin of viruses from normal constituents of the cell is likely to receive a more considered hearing from virologists and geneticists if the concept is not overburdened with dogmatic but unsupported generalizations as to the specific organelles from which they come. The generality of the subject demands imaginative speculation checked by the most cautious criticism.

But to return to the polyoma analysis as revealed by Dulbecco *et al.* In 1960 Dulbecco *et al.* (Smith, Freeman, Vogt and Dulbecco) by using special techniques (isolation of P^{32} labeled deoxyribonucleotides from purified P^{32} labeled P Y virus by aminopterin and bromodeoxyuridine (5BDU)) "support the conclusion the P Y virus contains DNA as the essential nucleic acid".

In 1965 Hartwell, Vogt and Dulbecco stated:

as regards the possible role of viral genes in the specification of the enzymes, it is, however, important to remember that the polyoma viral genome contains only about 5000 nucleotide pairs. Assuming a coding ratio of three, this would allow for the coding of about 1700 amino acids by the viral genome. It is likely that the viral capsid protein requires a large percentage of this coding capacity. In addition, some of the coding capacity is probably needed for the polyoma-specific transplantation antigen (Habel, 1961; Sjögren *et al.*, 1961) and for the complement-fixing antigen (Habel, 1965) found in polyoma-induced tumor cells. It is evident that the number of proteins which can be coded for by the viral genome is limited. The possibility must therefore be entertained that the viral genome is not capable of specifying all the enzymes which are evaluated in the infected cells. Rather it may act by inducing the synthesis of enzymes which are specified by cellular genes. This does not seem too unlikely since the virus has also been shown to induce the synthesis of cellular DNA.

The recent work, therefore, on the nature of polyoma virus and its role in altering or changing cellular physiology not only in contributing its own genic material but also its capacity to induce changes in cellular genes appears to be supporting evidence for the concept of "action and reaction systems of spatially related genes" in determining cellular behavior even to a possible role in the origin of cancer.

The possibility of having other viral particles changing cellular physiology by blocking transfer RNA, i.e. the information being transported from DNA to the cellular RNA, has been considered by many investigators.

In 1959 Luria arrived at the conclusion that gene control and virus control over cellular functions are two aspects of the same genetic mechanism. This concept removes any *a priori* incompatibility between a viral and a genetic theory of cancer etiology.

Recently there has developed a voluminous literature on the combining power of the carcinogenic hydrocarbons with DNA. The problem was particularly called to the attention of cancerologists by a Symposium on the Interaction between Chemical Carcinogenesis and the Living Cell at the recent meeting of the American Association for Cancer Research in Denver. In addition to the preliminary and concluding remarks by Heidelberger as chairman of the meeting, two papers are of particular interest. The first paper was by P. Brookes on "Quantitative Studies of the Reaction of some Carcinogens with Nucleic Acids" and the second by M. B. Sporn, "Carcinogenesis and Gene Action". Unfortunately, these papers are not to be published in time to be included in the present report. Consequently, it will be necessary to refer briefly to a few significant references.

In 1964 Brookes and Lawley published a short paper in *Nature* on the relation between carcinogenic power of hydrocarbons and their binding to deoxyribonucleic acid (1964b).

In this 1964 paper, the authors report the binding ability of several hydrocarbons to cellular components. The binding of naphthalene to DNA, RNA or protein was insignificantly small. For all other hydrocarbons and for all three cellular constituents isolated, namely DNA, RNA and protein, a low but significant extent of binding was found. It was clear that the binding to total cellular protein or to RNA showed no correlation with carcinogenesis. However, DNA binding whether expressed as the ratio

of specific radioactivity of DNA to that of RNA or to that of protein shows a significant positive correlation with carcinogenic potency. In conclusion, Brookes and Lawley state: "however, the unexpectedly consistent correlation observed throughout the present series of experiments would support the view that DNA is the significant cellular receptor of carcinogenic hydrocarbons, as implied by the mutational theory of carcinogenesis".

Similar results, including the alkylating agents, were reported by Brookes and Lawley (1964a) at a Symposium on Molecular Action of Mutagenic and Carcinogenic Agents, under the auspices of the Biology Division of Oak Ridge National Laboratory and published in the Supplement of that year by the *Journal of Cellular and Comparative Physiology*.

The present development of the relation of the carcinogenic compounds to the problem of cancer is of more than passing interest to the author.

In 1940 (Strong, 1940a) it had occurred to the author that the oncogenic viruses, provided, of course, that they were of the DNA variety, could very well enter into the "action and reaction system of the spatially related cellular genes" and thus change physiological activity leading, in some cases, perhaps to cancer. Later, in 1949 (Strong, 1949c) the author expressed the idea that an oncogenic chemical may be able to combine at some reactive point, to DNA, be a source of energy and thus change gene action.

The exact quotation from this 1949 paper was:

Another concept to explain mutations by chemical means as well as the origin of cancer from a somatic cell attempts to show the direct effect of the chemical upon the genic material. The concept is a composite contribution of many investigators and is as follows: The carcinogenic hydrocarbons have at least two k regions at which electronic vibrations are maximal. It has been suggested that it is perhaps at these k regions, if not at other regions, that the carcinogens or some of their metabolites are capable of combining with a nucleoprotein of the cell. Boyland has suggested that the above combination of a gene with a foreign molecule would interfere with cell division thus leading to chromosomal aberrations. However, all data obtained with methylcholanthrene on mutagenesis in mice indicate that we are dealing not with chromosomal aberrations but with point mutations. A further consideration of Boyland's concept is thus indicated. After the carcinogen or one of its metabolites has combined with a unit of nucleoprotein within the cell (that is, a gene) then it can be eliminated by oxidation, by demethylation, or by some other chemical means by which energy is involved. If this energy be released or absorbed at the electronic level it could not be dissipated in the form of heat. Thus a source of available energy at close proximity to a gene may very

well combine with such a gene or nucleoprotein and change the energy content of a derived nucleoprotein. The derived nucleoprotein would thus be a mutant (a derived or altered biochemical unit). This biochemical concept would explain reverse mutations as well as a series of multiple alleles, and is thus capable of explaining more phenomena than would the old concept of "hit" mechanics (see H. J. Muller's extensive bibliography).

This early work on the development of the Genetic Concept for the Origin of Cancer, together with the collateral contributions of investigators in many laboratories, was published by the New York Academy of Sciences in 1958 (Strong) and translated into Russian, *TeHeTnKa paKa* (genetica raka), in 1961 (Strong).

I cannot leave this section on the genetic concept for the origin of cancer without referring briefly to the pioneer researches of J. and K. H. Bauer. In 1925 J. Bauer maintained that cancer susceptibility was due to the inheritance of two genes, one a general cancer character, the other a specific determiner for each special type of tumor. His actual words were: "Wir haben die Annahme von Zwei eine konstitutionelle Krebsdisposition bedingenden Faktoren im Vorangehenden begründet: Eine allgemeine Blastomanlage, welche für die Krebsfähigkeit ihres Trägers ganz allgemein erforderlich ist, und eine bestimmte Organdisposition welche die Lokalisation bei gegenener Krebsfähigkeit determiniert."

The author heartily agrees with this concept in general, subject to certain reservations for changes, and intends to refer to this idea later. One change in the concept would be the introduction of the action of multiple factors or genes in each of these two groups — the systemic and the organ specific. Another change will be the introduction of the concept of biological stability and instability, some, if not all, of which may have a genetic mechanism sometimes referred to as genetic homeostasis. But there are other aspects of homeostasis in relation to cancer and the aging process which will be discussed later.

K. H. Bauer (1928, 1949a, 1949b) also expressed an early interest in the genetic concept of somatic mutation in the origin of cancer.

Bauer stated, "The mutated hereditary dispositions for cell differentiation are the ultimate material bearers of the tumor qualities and the corresponding somatic mutation is the biological process owing to which cancer cells represent a 'new race of cells', which differ genetically from their mother cells and do not adapt

themselves any longer to the existing order, but grow and spread rapidly in an egotistic and thereby destructive manner" (1949b).

The similarities of ideas between K. H. Bauer and the present author do not stop with the somatic mutation concept but continue through the inclusion of biological instability in an underlying cause in the origin of cancer. These similarities were discussed at the recent celebration of Bauer's 75th birthday at Heidelberg, Germany, 24 September 1965 (Strong, 1965b).

SECTION II

Chemical Carcinogenesis and Genetics

The author's use of genetics in investigating the problem of chemical carcinogenesis developed rather late (about 1936). This was, no doubt, due to at least two reasons, as follows: (1) The two fields of genetics and chemical induction of tumors were developing at the same time. The work of Yamagiwa and Ichikawa (1915, 1918) on the production of epidermoid cancer on the ear of a rabbit by coal tar was reported (in Japanese) in 1915 and (in English) in 1918.

It was commonly expressed shortly after the introduction of coal tar and several other chemical carcinogens that cancer could be produced at will in all experimental animals, particularly in mice. Even though strain differences for many biological characteristics including differences in the incidence of spontaneous tumors were being obtained by the use of partially inbred mice (particularly A/St and C₃H/St), it was probably not sound at this early date to venture into the field of the application of their use to chemical carcinogenesis.

As soon as it was decided that the mice had been inbred sufficiently to permit reproducible results to be obtained, the mice were made available to all investigators. At least two concepts appeared then to be evident. In the first place, the mice could serve as a constant source of spontaneous and transplantable tumors and, in the second place, it was concluded that it was time to bring the fields of genetics and of chemical carcinogenesis together.

This problem of the relation of genetics to chemical carcinogenesis was approached in a similar manner as the previous one

dealing with the control of susceptibility and resistance to spontaneous and transplantable cancer through the processes of hybridization and subsequent inbreeding. The modified new technique also included the injection subcutaneously of a standard dose of 20-methylcholanthrene (1 mg) dissolved in 0.1 cm³ of sesame oil into mice of 60 ± 5 days of age.

Hybridization and then continued inbreeding by the conventional brother-to-sister matings were employed with or without genetic or any other selection. The greater number of these mice, however, were subjected to a rigid selection toward resistance to chemically induced cancer. It was hoped that, by this means, cancer could not only be produced at will in genetically controlled mice but that also, by genetic principles, a biological state of resistance to cancer could be obtained in spite of the fact that an overwhelming dose of the most powerful carcinogen then developed had been injected into mice at a very critical period of life.

The results obtained were quite involved and unexpected but perhaps it is better to discuss some simpler experiments before attempting the more complex.

The first paper dealing with chemical carcinogenesis was published in collaboration with G. M. Smith (Strong and Smith, 1939). This was the reporting of the induction of carcinoma of the mammary gland by methylcholanthrene.

In this investigation, it was demonstrated that a strain or genetic difference was involved in the induction of carcinoma of the mammary gland by methylcholanthrene. This conclusion was based on the observation that, so far, such tumors had only been obtained in two or seven distinct inbred strains of mice treated with the same carcinogen in exactly the same manner. It is further noteworthy that these two strains in which adenocarcinoma of the mammary gland could be induced were genetically related and were both characterized by a very low incidence of spontaneous tumors of the mammary gland. It is also of interest that the normal mice of one of these strains (JK/St), when attempts had been made to subject them to long-continued estrogen treatment, had been found to be extremely intolerant to that hormone, so that death occurred at an early age without the appearance of carcinoma of the mammary gland (Gardner, 1937).

A genetic influence on chemical carcinogenesis had already been indicated by the classical works of Lynch (1924, 1937),

Andervont (1937) and Kreyberg (1934, 1938a, 1938b). One of the greatest contributions to the genetic analysis of the chemically induced tumor has been the work of Heston (1966), particularly in his demonstration of linkage between known genetic markers and new genes implicated in the inheritance of susceptibility to chemically induced lung tumors. Heston received the Pasquali Award at the recent Symposium on Lung Cancer in Animals held at the University of Perugia, Italy, for this outstanding research.

Another contribution of Heston was the demonstration of a correlation between susceptibility to spontaneous lung tumors and susceptibility to the chemically induced lung tumor, a field in which Andervont, Strong and others had similar findings. In 1946 (Strong, 1946c) data on a selection toward resistance to chemically induced tumors were published. It was reported that hybrid mice of six separate lines of descent from a common origin had been injected at 60 ± 5 days of age with 1 mg of methylcholanthrene dissolved in 0.1 cm^3 sesame oil. These mice had been continued through four to six generations of a rigid régime of selection toward resistance to the appearance of the expected local tumors at the site of the injection. No discrimination of selection was practised in the different lines. Diversified effects of selection were obtained as follows: (1) no effect in changing tumor susceptibility in one subline; (2) an intermediate or rapid decline in the incidence of local tumors in the succeeding generations in four sublines, and (3) a gradual shift toward increased local tumor susceptibility counter to the trend of genetic selection in two sublines. Data that had been reported previously (Strong, 1945e) had disclosed the appearance of a sudden change of local tumor susceptibility in another subline of methylcholanthrene-injected hybrid mice. The conclusion is drawn that two phenomena are involved in shifting susceptibility to local tumors induced by methylcholanthrene. These are: (1) segregation and recombination of pre-existing genes, partially under the control of genetic selection towards resistance in lowering susceptibility in the succeeding generations at different rates, and (2) the process of mutation (influenced by the effect of methylcholanthrene upon the germ plasm) which is acting by increasing susceptibility (counter to the trend of selection) by (a) small, gradual changes or (b) sudden changes of large magnitude.

Another experiment dealing with genetic selection and susceptibility to chemical induced tumors was reported in 1951 (Strong, 1951a). The mice between F_1 and F_{20} of the 2NHO descent were injected with 1 mg of methylcholanthrene dissolved in 0.1 cm^3 sesame oil at 60 ± 5 days of age. It was reported that a continuous selection toward resistance to all chemically induced tumors was constantly employed. Fibrosarcoma at the site of the injection of the carcinogen and lung adenomas appeared to respond to selection between F_1 - F_4 . However, beginning with F_5 there was a reversal of susceptibility to these two tumors in spite of selection toward greater resistance. Adenomatous lesions near the pylorus and papillomas of the forestomach appeared in mice only during the intermediate generations F_3 - F_{14} . Following a period of delayed latency for the appearance of fibrosarcoma as a result of selection toward resistance to such tumors, there was a progressively earlier appearance of such tumors counter to the trend of selection (providing methylcholanthrene had been injected into both parents over many generations). This phenomenon of reversed susceptibility to induced tumors has occurred in *all* lines and must be considered the rule.

But perhaps we should consider the results of the injection of methylcholanthrene into mice of the original inbred strains before any results with hybridization and selection were employed.

Two papers may suffice here. The first paper (Strong, 1952) presents the data obtained by the use of mice belonging to fifteen strains and injected with the same amount of the carcinogen at 60 ± 5 days of age. It was reported that considerable differences were obtained in the production of tumors between mice of these independent pure lines by the same technique of the injection of methylcholanthrene. The mice of the different strains possessed different combinations of genetic markers—from wild type (C_3H/St , CBA/St and CHI/St) to five recessives of the I/St strain ($ppdbbaass$). The mice developed fibrosarcomas at the site of injection of the carcinogen at different rates. Sex was an important factor, in some cases, in the induction of this neoplasm. The gene *s* (piebald) was characteristic of all strains with relative resistance to fibrosarcomas. These are the F/St , $CBAN/St$, N/St and I/St . A complete genetic interpretation of susceptibility and resistance to chemically induced fibrosarcomas is complicated by the two variables. These were found to be (1) body weight and (2) toxicity to the carcinogen. There is a correlation between

the coefficient of dispersion and the median latent period for the appearance of the tumors. This coefficient is greater in females than in males. The strains (data for males) can be classified according to susceptibility to chemically induced fibrosarcomas in the following sequence: CHI, C₃H, C₁₂I, L, C, A, C₅₇, CBA, JK, FC, JKL, N, CBAN, F and I.

Two descents were continued from mice injected with methylcholanthrene (N and CHI). These mice gave rise to four germinal mutations. The controls for N and CHI have never given rise to a spontaneous mutation during a period of 25 years. Following the appearance of these mutations, no more mutations were found in the untreated descents. Thus their appearance is associated with the injection of methylcholanthrene and cannot be due to "genic instability".

A further genetic analysis of the inheritance of susceptibility to chemically induced fibrosarcomas was reported by Gruskay and Strong (1955).

They reported that there is a correlation between tumor resistance, as measured by the latency period and the percentage of animals acquiring tumors, and the number of recessive genes in the genotypic make-up of the individual. They also presented evidence which points to the fact that the particular recessive genes studies impart more resistance on the chemically induced tumor than their dominant alleles. In addition, the relationship between the different recessive genes and resistance to chemically induced tumors seems to be an additive phenomenon, i.e. the more recessive genes in the make-up of the animal, the greater the resistance to chemically induced tumors. For example, the mice of the I/St inbreds with a genetic make-up of aabbCCppddssS^eS^e (five recessives) showed the greatest degree of resistance to methylcholanthrene induced fibrosarcomas. A discussion of these findings and those from a large literature led the authors to conclude that the process of carcinogenesis is one which there seems to be three variables: (1) the genetic make-up of the tissue affected; (2) the extra-genetic environment, such as litter frequency or number, sex, age, hormone balance, etc.; and (3) some physiological or pathological imbalance or irritation caused by an alteration in the tissue metabolism, either naturally or experimentally induced. A further elaboration of the nature of the cancer cell will be discussed in Section V.

In this 1955 (Gruskay and Strong) paper, the authors asked the

following question: What is the significance of the recessive tendency in the genetic control to tumor formation? They discussed probabilities. It may mean that the hereditary transmission of greater or lesser resistance to tumors is involved in a change in cell metabolism, or it may mean that genes can control the production of an individual with a greater or lesser resistance to malignant disease, or even to all disease through some other mechanism. The conclusion can be entertained with a certain degree of finality that the resistance to chemically induced cancer in experimental animals is, at least, partly associated with the cumulative recessive traits of the individual.

One further question can now be asked: Are recessive (rr) combinations of genes more stable in their biological function thus leading to more biological balance than combinations of dominant genes (RR or Rr)? In other words, does dominance lead to less balance and thus be conducive to a higher incidence of tumors? Homeostasis will be discussed later in Section V of this chapter.

Another facet of the genetic analysis of the chemically induced tumor with methylcholanthrene concerned the attention of Sanghvi (Sanghvi and Strong, 1958; Strong and Sanghvi, 1951) working in the author's laboratory at Yale University School of Medicine.

This investigation includes observations on 2966 mice of the pBr/St subline of the NHO/St strain of mice produced by a single mating of a JK/St mouse to one of the CBAN/St strain in the year 1937. These mice, analyzed by Sanghvi, belonged to the first twenty generations of inbreeding. A constant attempt to increase the latent periods of chemically induced tumors of succeeding generations was made by selecting breeders with increasingly longer latent periods. A statistical method was devised to study the multivariate effects of carcinogen and selection. The variables examined were anatomical sites, histological types, sexes, latent period and generations of inbreeding. This procedure left adequate numbers of mice in the three periods of inbreeding to study the changes in the variables concerned. In F_{17} - F_{20} there were two families (one with 403 mice and the other with 283 mice) which had a common descent up to the eighth generation and separated thereafter. These two families were analyzed for anatomical sites, litter seriation, and latent period

of local tumors. The method adopted for the entire analysis makes possible the visualization of the effect of carcinogen and selection in terms of probability on either the mouse, the anatomical site, or the histological type of tumor.

A single injection of carcinogen gave rise to tumors of a variety of histological types at the site of the injection and at other sites. These other sites include the surface of the body, the lung, forestomach, liver, ovary, uterus and some others. This finding suggests, among others, that the carcinogen under certain conditions has a widespread systematic effect on the production of cancer.

There were considerable differences in the mean values of latent periods for tumors at different sites: at the site of injection, the mean value was 201 days; for "surface spread" tumors it was 447 days; for the tumors of the forestomach, 478 days; for lung tumors, 510 days; and for liver tumors, 570 days. There were minor variations in the mean values of latent periods of different histological types at various sites. For local tumors the mean value for epidermoid carcinoma was 151 days; for fibrosarcomas it was 167 days. There were also differences in the sexes. For lung tumors the mean value was 460 days for females and 532 days for males. Probably there is no effect of selection in lengthening the latent period of any specific type of tumor. By selection toward resistance, the obvious finding was that a later appearing tumor would replace one that normally occurred earlier. These findings suggest that tissue susceptibility, as reflected by its latent period for the appearance of a chemically induced tumor, showed considerable differences under the conditions of the present experiment. However, these differences are probably not subject to variations brought about by the effect of selection.

There were considerable variations in the incidence of tumors at different anatomical sites and histological types during F_4-F_{20} . There were also considerable differences in the incidence of tumors between the sexes. The only major variation in the tumor incidence in males during the experiment was between F_4-F_{12} and $F_{13}-F_{16}$, reflected in a large decrease (from 56.4 percent to 39.3 percent in the incidence of local tumors). The incidence of histological types of local tumors in males does not show much variation. The tumor incidence in females, however, showed considerable variations both at different anatomical sites and in

histological types during F_4-F_{20} . The incidence of liver tumors also showed striking differences in the sexes. This would suggest that tissue susceptibility could be visualized only in terms of probability expressed here as percentage incidence. Variations in tissue susceptibility are probably the result of selection. Differences between the sexes suggested the action of some specific physiological avenues. In contrast to these variations in tissue susceptibilities during F_4-F_{20} , the variations in total tissue susceptibility (as measured by the proportion of mice that developed tumors at any site to all the mice) are not great. This would be at a different level in the mechanism of heredity than is total tumor susceptibility.

It seems to me that for the present evaluation of data, there are two conclusions from the study of chemical carcinogenesis in mice of the pBr/St strain that warrant emphasis.

1. That selection toward resistance to chemically induced tumors does not change the average mean latent period for any specific type of tumor. What actually happens is that with this selection (at least on a heterozygous background) a new tumor with a longer latent period replaces one that would occur early, but is incapable of manifesting itself by being suppressed. By genetic selection toward resistance to all tumors, the latent period for a late-appearing tumor can be reached.

2. The second conclusion is that "tissue susceptibility might be at a different level in the mechanism of heredity than is total tumor susceptibility". This conclusion was based upon the observation that during the work on induced tumors in pBr/St mice there was a continuous shifting in the types of induced tumors obtained but that the sum total of all tumors did not change through the process of inbreeding in the presence of a powerful carcinogen.

This observation recalls the conclusion arrived at by J. Bauer (1925) that in the inheritance of susceptibility to cancer there were two components, one gene determining cancer or not cancer and another that determines the specific type of cancer. Bauer's concept may in fact be too simple but it may have a kernel of truth in it—subject perhaps to the addition of the concept of multiple genes but only functioning in a standard environment.

The last series of experiments that should be included in this presentation of data bearing upon the genetic concept for the

chemically induced tumor were the ones obtained on another subline descent from the NHO/St mice which received the symbol Br/St (brown non-agouti).

The original investigation of the problem of selection toward resistance to chemically induced tumors was partially based upon the idea that if the origin of cancer was, in the last analysis, genetic then selection toward resistance should lead to a biological state in which it would be impossible for cancer to arise.

We may anticipate a general conclusion arrived at, after thousands of animals had been used, that the biological effects of a carcinogen were more complex than originally realized.

Keeping Johannsen's principle (1909) in mind that "selection within a pure line" is ineffectual in changing the incidence of a genetic character of an individual, selection toward resistance to chemically induced tumors was started in the early hybrid generations produced by originally crossing two mice of different inbred lines together.

Another point that will be emphasized later is that the relatively genetic state of the mice or experimental animals used should be continually kept in mind. Here through the process of inbreeding, the animals are gradually shifting from the heterozygous to a homozygous state, even though the actual number of genes involved is not known. There is thus the opportunity of measuring the biological effect of an experimental procedure (in this case the injection of methylcholanthrene) on a heterozygous background and then (in their descendants) on a homozygous basis. In this case this opportunity proved to be very promising.

Starting in 1939 and ending about 1948 the data obtained in this extensive series of one descent produced originally by one outcross and continued through thirty-five generations of inbreeding are reported in several articles. The only experimental techniques used were: (1) injection of each mouse at 60 ± 5 days of age with 1 mg of methylcholanthrene dissolved in 0.1 cm^3 of sesame oil and (2) subjecting all derived offspring to a very rigid selection of the most resistant-to-chemically induced tumor mice in each generation. All experimental animals were kept in breeding pairs until one of them died and their offspring continued to receive methylcholanthrene. As in the early generations of the pBr/St subline analyzed by Sanghvi and in the 2NHO/St descent as determined by Strong, this Br/St descent early increased their

resistance to several types of chemically induced tumors by this selective program. It was found that there was a kaleidoscopic appearance of many kinds of tumors each with a different latent period for their induction. The sequence of appearance of tumors was (1) local appearing tumors made up of (a) fibrosarcomas, (b) epidermoid carcinoma or (c) rhabdomyoma. With selection toward resistance these local appearing tumors became less frequent only to be replaced by (2) "surface spread" tumors of the extremities, the mouth, ears, tail, genitalia, etc. With further selection the local appearing and the "surface spread" tumors became less frequent only to be replaced (3) by tumors in internal organs, (a) in the thorax, especially the lung, and (b) in the abdomen involving the stomach, liver, ovary and uterus. Finally some mice were obtained following several generations of selection toward resistance to chemically induced tumors that did not develop any type of tumor in spite of the fact that they lived more than 1000 days following the injection of methylcholanthrene.

The experiment should have been considered complete at that time but was not since the descendants of these resistant-to-cancer mice had already been injected with methylcholanthrene when they were 60 days of age. The startling observation was made, as in the later generations of the 2NHO/St descent, of the chemically induced cancers appearing earlier than they did in the early generations of inbreeding. In other words, this whole series of chemically induced tumors was reversed from the abdominal tumors, then to be thoracic then to the "surface spread" and finally to the locally appearing tumors at a latent period shorter than in the original mice of the early hybrid generations. Another observation was also made that mammary gland tumors, which rarely appeared in the series of tumors of the early hybrid generations, now became of frequent occurrence even to having an incidence in excess of 50 percent of all tumors (in female mice only).

A point, in passing, was the obtainment of several germinal mutations in the descendants of mice which had been subjected to a rigid selection toward resistance-to-cancer, thus showing only internal tumors of the abdomen including the liver and ovary with latent periods in excess of 400 days. As far as known, even though there had been some short-term experiments dealing with carcinogen-induced mutations, the above experiment of rigid genetic

selection to the elimination of all appearance of early induced tumors has never been repeated. This being the case, the problem of methylcholanthrene-induced mutations will not be considered final. Interesting that the data may be on mutagenesis they do not interfere with the development of the original concept that a somatic mutation may be involved in the origin of cancer.

Now we are faced with a scientific dilemma. The kaleidoscopic shift in the types of chemically induced neoplasia is what one may expect by the practical application of genetic selection, i.e. variation is taking place in the direction of selection. Thus the slow accumulation of recessive genes could explain the results especially after it is known that resistance to chemically induced tumors has been brought about by the recombination of recessive genes (Gruskay and Strong, 1955). Now if the first series of chemically induced tumors can be explained by a well-known and recognized genetic phenomenon what about the second series where variation of tumor type is taking place counter to genetic selection? It has been demonstrated that after some of these tumors were induced they became hereditarily established in their descents freed from the further injection of methylcholanthrene (Strong, Collins and Durand, 1943; Collins, Gardner and Strong, 1943; Strong, 1945b; and Strong and Hollander, 1951). For example, a strain of mice characterized by a very low incidence of spontaneous tumors (0.60 percent) has been converted into one in which the incidence of spontaneous tumors is very high (66.2 percent) by subjecting the ancestry of the strain to methylcholanthrene for many generations (Strong, 1948a).

Are we to conclude that perhaps the methylcholanthrene—or one of its metabolites, since the phenomena occurs only after a long period of time—is actually affecting the germ plasm? One should also entertain the possibility of the introduction of a virus but until proof of this can be obtained in the laboratory, the genetic possibility can at least be entertained. But even with the introduction of a hypothetical virus, the problem remains the same since the virus may also affect the genic material.

The primary purpose of the research program on chemically induced tumors which has been outlined perhaps too cursorily was to ascertain whether the two fields of cancer research, genetics and chemical carcinogenesis were antagonistic to each other or complimentary. Both fields had developed simultaneously and,

at present, have reached a common goal. Without going into the discussion of the virus-induced tumors with any detail it can now be concluded that all three goals look upon a genetic change in the origin of cancer. Whether one concludes a genic change or rearrangement (spacial relation between genes in an "action and reaction" system) or even to a block in genic physiology such as a block of the information given to the transfer RNA by the DNA or any other of several possibilities, the problem remains the same. We are probably approaching the analysis of the true nature of the cancer cell, that which was brought about by a deranged genic mechanism—a change in the genome of the cell.

SECTION III

Studies on Maternal Age

The initiation of a program for the investigation of the genetic analysis of the aging process (Gerontology) was indicated by the work on chemical carcinogenesis as partly outlined in Section II (pp. 145-56). The results obtained on gerontology have been published in a series of papers between 1946 and the present time. Chapters 1, 3 and 4 of the present publication contain the latest report and analysis of the data obtained. These latest analyses should not, however, be considered final. Finality should only be reached by a complete statistical IBM or similar computer analysis, which, up to the present time, has only been applied partially to the data bearing upon age of first litters in one maternal age descent (Strong, Johnson and Rimm, 1965).

However, extensive papers (Strong, 1946d, 1948b, 1950a, 1950b, 1950c, 1951c, 1954, 1957; Strong and Fuller, 1958; Strong and Johnson, 1962; Johnson and Strong, 1963; Markello, 1958; Roman and Strong, 1962; Johnson and Strong, 1966; Strong, Johnson and Rimm, 1965) and two unpublished progress reports to the National Heart Institute (Strong and Johnson, 1964; Strong, 1965a), whose generous support for several years made the research program on the Genetic studies of the Aging Process possible, will be the foundation for the present discussion.

In 1946 (Strong, 1946a) data were published on "Mutation from Brown to Black with a Concomitant Increase of Susceptibility to Fibrosarcoma".

It was reported, in this paper, that following the subcutaneous injection of methylcholanthrene, three dominant mutations (brown to black) occurred in a single litter of a mouse belonging to the F_{14} generation of the NHO/St descent. Their ancestry had been subjected to a subcutaneous injection of methylcholanthrene for nine successive generations. Concomitant with the production of these dominant hair color mutations, there was also obtained (1) a considerable increase in susceptibility to fibrosarcomas, (2) an increased litter size, and (3) an increased vitality of the mice. All three of these biological characteristics have been transmitted through three generations of untreated descent and they all appear to be associated with the black mutant character—never with their brown litter mates. The evidence points towards the production of a widespread genetic change brought about by the effect of methylcholanthrene or one of its metabolites upon the germ plasm but a point mutation at a single locus or the loss or gain of all or part of the chromosome bearing the B locus has not, as yet, been completely excluded.

In order to analyze further the appearance of several mutations from brown to black and a change in susceptibility to chemically induced fibrosarcomas, a new F_1 generation was produced by a cross between mice of the NHO/St (brown) and C₅₇/St (black) inbred strains (Strong, 1948b). An examination of the data disclosed the fact that mice of the three groups (litters 1-2, 3-4 and 5-6) developed sarcomata at the site of the injection of methylcholanthrene at significantly different rates. The rate for the appearance of tumors was slowest in mice of litters 1 and 2, intermediate in litters 3 and 4 and highest in litters 5 and 6.

It is fair to assume that all mice of the F_1 generation produced by an outcross of mice of the two highly inbred strains are, theoretically, genetically alike or very similar if not identical. The difference of susceptibility to induced tumors observed between mice of successive litters is consequently not apparently a genetic one, or at least not a simple one. It is obvious that something which is increasing or decreasing in the mother's body is being handed down to her offspring. This transmitted principle sensitizes or changes the progeny's susceptibility to a subsequent injection of methylcholanthrene, influencing the rate at which the offspring develop sarcoma in the presence of a given amount of methylcholanthrene. This investigation is of significance, since

it demonstrates that a new principle, presumably of a biochemical nature, has influenced the production of a malignant tumor. Perhaps when it is identified, other phases of the cancer problem, such as prevention and spontaneous regression, may be elucidated. Susceptibility to cancer is, of course, only the opposite of resistance to cancer. The present evidence indicates equally clear the existence of a new *resistant* mechanism that is capable of changing the rate at which an offspring of a given female develops sarcoma in the presence of methylcholanthrene; that it is highest in young breeding females and diminishes in effectiveness in advancing age.

Two other characteristics of cancer, invasiveness and the survival time of a mouse growing a sarcoma were then investigated in relation to maternal age. As regards invasiveness, it was found that the ability of a chemically induced sarcoma to invade into the abdomen is gradually reduced in offspring of successive litters of the same breeding parents (Strong, 1950c). This suggests, therefore, that the characteristic of invasiveness of chemically induced fibrosarcomas is also partially determined by some new principle found in the host which has been derived from its ancestry and is differentially determined by litter seriation or mother's age. This influence is similar to, if not identical to, the new resistant mechanism which determines the percentage incidence of chemically induced sarcomas, and is also determined by litter seriation or mother's age. The third characteristic of chemically induced tumors, survival time of sarcoma-bearing mice, was reported in *Science* (Strong, 1950a). In this publication, it was shown that the analysis of the data indicates that the survival time of a mouse of the pBrunt/St descent growing chemically induced fibrosarcoma increases with the litter frequency of the mother. The group of mice that survive for the shortest length of time (an average of 52.4 days) with the growth of a primary induced fibrosarcoma belong to a first litter. This survival time gradually increases with litter frequency until, if the mouse belongs to an eighth litter, the average survival time is 130.0 days. After temporary growth, sixteen mice belonging to advanced litters have regressed their primary chemically induced fibrosarcomas spontaneously. Thus by controlling the ordinal sequence of the litter in which a mouse has been born, an influence on survival time with growth of a malignant tumor has been altered.

It is difficult to obtain many mice that belong to litters succeeding the eighth one. Later litters should yield interesting data about growth rates of chemically induced fibrosarcomas, retrogressive potentialities, alterations in latent period, survival time and perhaps even in the histological characteristics of invasiveness and the ability of the tumors to metastasize.

Thus through the construction by genetic principles of a biological system that shows increased resistance to the growth and survival of a malignant tumor, such as obtained in the pBr/St strain, the fate of the cancer and cancer-bearing individual can be changed significantly.

Before leaving the discussion of the chemically induced tumor, which led to the concept for the investigation of mother's age, a third contribution appears to be significant. This was also reported in 1950 (Strong, 1950a).

It was reported then that in one descent, the prunt/St, there was an increasing sexual differential in relation to chemically induced fibrosarcomas in the succeeding litters born to the same parents due to an increased susceptibility in the female. An increasing sexual differential was not encountered in mice of the related 2 prunt/St strain.

It has been encountered many times in the author's laboratory and reported by other investigators that more variations occur in the female animal rather than in the male. Thus it can be said that the male is more homeostatic than the female, a concept that will be referred to later in this report (Section V, p. 178).

Finally in 1951 another paper dealing with litter seriation in fibrosarcoma susceptibility was published in the *Journal of Gerontology* (Strong, 1951c).

The new data on litter seriation or mother's age verifies similar data obtained in earlier experiments.

As in previous experiments the characteristics of (1) latent period for the appearance of tumors following the subcutaneous injection of methylcholanthrene, (2) survival time with the growth of tumors, (3) age of death with the growth of a chemically induced fibrosarcoma, (4) a sex differential and (5) invasiveness or ability to invade into the abdomen are analyzed in terms of litter seriation. The mice belonged to the first to twelfth litters born to the same parents.

It was again found (Strong, 1951c) that mice in successive

litters show differences in susceptibility to chemically induced fibrosarcomas (latent period) and in characteristics of malignancy (survival time of tumor-bearing mice and invasiveness, or ability to invade into the abdomen). There seems to be an inverse relation between survival time and latent period, although two mechanisms appear to be evident. In the pBrunt/St strain, as in the previous experiments, female mice develop tumors earlier in succeeding litters, even though males do not do so, thus bringing about a sex differential in relation to fibrosarcomas susceptibility which increases in degree in mice of successive litters. Female mice show more tumors with the ability to invade into the abdomen than do their own litter-mate brothers. The number of mice with malignant tumors as measured by invasiveness is reduced in mice of both sexes in successive litters. Survival time of tumor-bearing mice is also influenced by litter seriation.

The present data (Strong, 1951c) indicate two hereditary (transmission from parent to offspring) mechanisms involved in cancer susceptibility and malignancy which change during the life span of a female mouse and are handed down to her progeny. This transmission is apparently not by way of the chromosomes but whose real natures are, as yet, unresolved. They are probably manifestations of the phenomenon of homeostasis or biological stability (see Section V, p. 178).

With the advances made possible in cancer research, it was not long before the concept arose that if litter frequency or mother's age could thus determine or alter the characteristics of cancer, what could be discovered if several of the normal biological characteristics of the offspring of controlled parentage were investigated?

The first problem was in surveying the literature bearing upon parental age and the possibility of this process having an effect upon the characteristics of the offspring. For this purpose a symposium was originated for the New York Academy of Sciences and the papers presented at this symposium assembled into a monograph (Strong, Editor, 1954). Since this publication is still available, no attempt will be made here to analyze the several contributions. Suffice it to say, however, that the problem of the aging process and its probable effect on the characteristics of their offspring is recognized by many investigators around the world. Considerable interest was expressed by the participants

at the New York Academy Symposium but it was widely indicated that the actual data bearing upon the problem are yet too meager for suitable analysis with any degree of finality.

Cowdry (1954), in his summation remarks, said: "In view of the widespread interest in parental age and characteristics of the offspring, as indicated by the contributions of the authors of this monograph, it appears that the topic is a timely one and that the base line has been drawn upon which further conferences and monographs may be organized."

The author then set up an experiment in order to analyze, at least partially, the biological components of the mouse. Methylcholanthrene had clearly implicated a "litter seriation" or maternal age effect in the development of the characteristics of that animal receiving it but it was decided that its continued use would only further complicate the analysis of the components of biological determination.

Further reference from now on to methylcholanthrene injection will only be made when some of the older observations on compensatory changes indicating homeostatic control have been verified by the use of other biological characteristics by further research on maternal age selection particularly in Markello's (1958) analysis as will be discussed later.

Consequently, a new hybridization cross was set up as outlined in Chapter 2 of this series (p. 19). Continued selection of maternal age has always been the rule beginning with F_1 and up to the present time. Only brother-to-sister matings were used following the original but single outcross. In one subline, the early maternal age descent, when the offspring were continued as breeders from mothers less than 100 days of age, F_{70} has been reached. In the later relatively advanced generations of inbreeding from a continued selection toward maternal age descent a new variable which was shown to influence many biological characteristics not originally anticipated was discovered. This was in the manifold or pleomorphic effects of the polydactylia gene LST as discussed in Chapter 4 (p. 53).

Thus in the final analysis of the variables involved in the development of biological characteristics in this experiment there were (1) segregation and recombination of genetic determiners through the process of inbreeding, (2) the effect of maternal age selection upon (a) a relatively high degree of heterozy-

gosity and (b) homozygosity and (3) the effects of specific genes, particularly LST and lst, upon (a) a relatively high heterozygous background and (b) on a relatively homozygous state.

The biological characteristics investigated were (1) penetrance of polydactylia, (2) age of first litters, (3) litter spacing or frequency when the sexes were kept continuously together, (4) age of mothers at the production of eighth litters, (5) longevity, (6) mammary gland tumors, (7) comparative analysis mammary gland tumor onset, mammary gland tumor incidence and longevity, (8) mammary gland tumor incidence and incidence of polydactylia, (9) spontaneous lung tumors, (10) litter size, (11) fertility, (12) fecundity (average number of litters per female) and (13) number of females producing eighth litters.

It is not the intention to discuss all the data obtained in this single selected experiment.

These have been partially published in periodic papers and reports. We shall attempt now only to outline certain aspects of the problem of maternal age selection where it appears obviously that a new force upon biological characteristics including cancer has been indicated.

Polydactylia

1. It has been shown that the polydactylia gene or genes have pleomorphic effects upon many characteristics. Its manifestation is quite variable even to being influenced by "somatic overlaps", so that its real incidence cannot be determined by gross inspection alone. In all descents, the incidence of about 2.6 percent in the F_1 has increased through the process of inbreeding but has reached 100 percent penetrance for only short periods of time. The "leveling" off period of incidence of polydactylia is somewhere between 70 and 90 percent depending upon the particular descent. This incidence, however, is never static but fluctuates from time to time even after sixty-five generations of inbreeding. Occasionally sudden changes take place. For example, it was discovered that a very high incidence of 80 percent polydactylia was reduced to 2-3 percent without any obvious reason in the descendants of one pair of mice and continued from that point onward with this reduced penetrance of polydactylia. Even after many generations of inbreeding, a very low penetrance of polydactylia can "revert" to a very high penetrance within three or

four generations. Thus the syndrome brought about by LST or its allele *lst* has not only pleomorphic effects on many biological characteristics but also is unstable in its own incidence. These findings were discussed more in detail in Chapter 4 (p. 53). A third characteristic of the biological effects of this gene will be discussed later following a brief survey of a few biological characteristics under investigation.

Age of First Litters

2. These data were published in three papers (Strong and Fuller, 1958; Johnson and Strong, 1963; Strong, Johnson and Rimm, 1965). In the first paper, written in collaboration with the late C. A. Fuller, it was reported that the analysis of the data bearing upon the age of first litters indicates that the different independent sublines based upon maternal age selection have different parabola regression curves when their successive means of age of first litters (on a generation basis) are compared to the general mean of first litters for the entire population (73.04 days). In the second paper, dealing with age of first litters, written with F. Johnson, it was reported that female mice born to young mothers between < 100–200 days of age had first litters at 79.81 ± 0.41 days between F_{16} – F_{20} ; females born to mothers between 201–400 days had first litters at 71.24 ± 0.42 between F_{16} – F_{20} ; whereas females born to mothers between 401–600 days had first litters at 68.02 ± 1.00 days. Thus there was observed variation counter to the trend of selection—a phenomenon that was first seen by the injection of methylcholanthrene into mice belonging to a relatively highly inbred or homozygous stock and observed on several other occasions. This observation on age of first litters then emphasized the conclusion that a compensatory mechanism may be responsible for the fact that late maternal age descents have first litters earlier than mice of the early maternal age classes and thus keep the strain in homeostatic "equilibrium".

This is clear evidence that even by brother-to-sister matings through the process of inbreeding there is some force counter to genetic selection that is responsible, in part, for the continuation of the species, since selection in one direction only may lead to eventual chaos.

The third paper dealing with age of first litters was written in collaboration of F. N. Johnson and A. A. Rimm. Here with the

development of the new computer centre at Roswell Park Memorial Institute, Buffalo, New York (Health Research Inc.) there was an opportunity to analyze some of the data on age of first litters by the IBM system. Only the data on the early maternal age descent were used. The analysis leads to conclusions applied to some fundamental principles of genetics. These observations and analyses lead to the following conclusions: age of first litter in mice selected for early maternal age is variable and not normally distributed (i.e. it does not have the characteristic bell-shape curve). To facilitate the study of the effect of inbreedings on days to first litter, the data were converted to logarithms.

The average age at first litter (geometric average, since the data were converted to logarithms) did not decrease or increase steadily with increased inbreeding. The variability of age at first litter gave some slight indication of decreasing as homozygosity increased. There seemed to be no relationship between sterility and homozygosity; that is, after forty generations of inbreeding there was no appreciable increase in sterility.

After fifty-four generations of brother-to-sister matings, with selection for early maternal age and polydactylism, a relatively stable or predictable response with respect to age of first litter was not attained. Thus instability was indicated in an early maternal age descent.

Spontaneous Tumors

3. Since the early maternal age descent had (1) an element of variability as measured by age of first litters and other characteristics, i.e. not being in homeostasis, and (2) that a very high incidence of spontaneous tumors was characteristic of them as compared to the older maternal age descents in the same laboratory which appeared to be in greater biological equilibrium, an attempt was made to correlate biological instability and the origin of spontaneous tumors together. This attempt was published in *Science* in 1957.

Starting with an analysis of the various types of tumors induced with methylcholanthrene by Strong, Markello (1958) arrived at the conclusion that with "selection toward an earlier and earlier litter seriation descent (early maternal age) has also produced variation in the opposite direction of all specific types of tumors

investigated: (1) squamous cell carcinoma of the skin, (2) fibrosarcoma, (3) adenocarcinoma of the lungs, (4) adenocarcinoma of the mammary gland, (5) mixed tumors and (6) rarer tumor types at other sites".

But a compensatory change or reversal of trend with maternal age selection does not only apply to neoplastic conditions. It has been encountered in the measurement of many normal biological characteristics, in addition to age of first litters as discussed above.

For example, the descents (that are selected in an early litter from an early maternal age) when the litter size is maximal have smaller litters than do the females that were taken from a late litter of a late maternal age descent where the litter size had been minimal. Here again is evidence of a compensatory mechanism influencing litter size.

It is clear by the evidence now available that the characteristics of the offspring occurring in the maternal age descents in the middle of the age distribution from the entire population do not significantly change between F_1 and F_{10} generations—it is only the offspring of the two extreme maternal age classes which deviate or vary and this deviation is sometimes counter to the trend of maternal age selection, especially when the descent has reached a certain level of inbreeding.

The obvious reason for such compensatory changes as those enumerated and referred to previously is to keep the species in equilibrium. If the practice were continued for many generations for females to have their first child (or litter) earlier and earlier, irreparable damage to the species might be the result. Similarly, the practice of bearing young long after the normal reproductive period has been exceeded might also produce disastrous results.

The present experiment indicated quite convincingly that, unless the insult by continued selection has not been too great for too many generations, a compensatory mechanism corrects for a deviation from the norm, and this variation takes place sometimes counter to the trend of selection.

In genetics there is a concept that a species is in equilibrium until a mutation occurs "to upset the apple-cart" after which a new equilibrium is established by incorporating into, or by discarding from descent, the new mutation. We no longer have to make the assumption *a priori* that the species is in equilibrium

(homeostasis). There is evidence, such as the present data and from other laboratories, to prove that a mechanism actually exists.

It is not my intention to review the literature dealing with equilibrium or homeostasis. This discussion will follow later in Section V (p. 178). To me, it is not clear whether biological or genetic equilibrium is always involved. To some investigators these two phenomena may be absolutely dependent on each other. This may be true, but certainly more data are needed for final analysis.

Since so many types of biological characteristics, such as age of first litters, litter spacing, litter size and various types of tumors, are kept in equilibrium, this homeostatic mechanism or mechanisms must be of extreme biological importance. A species could probably not be kept in equilibrium unless the individuals who make up the species are also in equilibrium at least during the reproductive period.

The various types of cancers which arise in the mammalian body have, at least, one characteristic in common. Cancer arises because the organism has lost control of a definitive part. Perhaps during the aging process the mechanism that keeps the individual in equilibrium is disrupted and as a result of this loss of control of all the parts cancer of one or more elements is able to originate and to grow at the expense of the rest of the body (Strong, 1957). It was this concept of a common bond in cancer as well as the many contributions made possible in chemical carcinogenesis that led the author to conclude: "in the genetic mechanisms involved in cancer, perhaps one or more factors control the origin of specific types of tumors, whereas another mechanism may be involved in the origin of the total number of tumors (all tumors at all sites taken together)" (Strong, 1957).

This discussion of Section III was started to analyze the genetic nature of the aging process or the effect of the aging process on genetic phenomena. It was soon realized that the established facts of cancer could not be kept separate from those involving the normal components of the individual.

In an attempt to bring the fields of cancer research and gerontology together, a paper was presented at the second International Symposium organized by Professor Lucio Severi for the Division of Cancer Research, University of Perugia, Italy in 1961. The

paper was entitled "Oncology and Gerontology—Genetic Implications" and was written in collaboration with F. N. Johnson (Strong and Johnson, 1962). The findings reported there will be given now only in résumé:

The percentage incidence of total spontaneous tumors does not vary between the four maternal age classes 101–200, 201–300, 301–400 and 401–500 which are near the middle frequency distribution of mice for the entire population. This incidence is about 34 percent. The female mice of the <100 maternal age descent which develop a very high incidence of mammary gland tumors (70 percent) may deviate from the average percentage of total tumors for the entire population, whereas both males and females of the 501–600 maternal age class may also deviate from the percentage incidence of spontaneous tumors for the entire population. In other words, female mice of an early sexually maturing class and both sexes in the late maternal age class, i.e. the extreme classes of a normal frequency distribution for the entire population, may be deviating from the "norm" for the species. It is well to keep in mind that just before early maternal age is adolescence, and just after late maternal age is senescence.

The average incidence of spontaneous tumors at about 34 percent is due, perhaps, to more than chance. The study of the mice below this level is oncology; the study of mice above the line is gerontology.

This finding of a fixed percentage of total tumors is not unique in this experiment. For example, in the extended series of mice injected with 20-methylcholanthrene at 60 ± 5.0 days of age through the process of inbreeding from an outcross between mice of the CBAN/St and the JK/St inbreds, there was a gradual shift in the histological types of chemically induced tumors. But, when all chemically induced tumors, on a generation of inbreeding basis, were added together it was found that the percentage incidence was "fixed" at about 80 percent. It was concluded that "the control of tissue susceptibility might be at a different level in the mechanism of heredity than is total tumor susceptibility" (Sanghvi and Strong, 1958).

A certain percentage incidence of spontaneous tumors was characteristic of many of the commercial strains of mice used many years ago. This fact has been somewhat neglected following the establishment of the many inbred strains of experimental animals now in existence where one obtains either with or without deliberate selection a very high or a very low incidence of specific types of spontaneous tumors, particularly of mammary gland, lung, leukemia, etc., origin. But a 100 percent incidence of either susceptibility or resistance to tumors is probably never obtained. At present after one hundred and twenty-one generations of inbreeding, female mice of the C₃H/St strain, in my laboratory, if used for breeders, show an incidence of 79.10 percent of spontaneous tumors of mammary origin; if continued as virgins 55.60 percent develop these tumors. That is 20.9 percent of all females used as breeders die of some other cause than cancer; some before the average age incidence of tumors, whereas some actually live longer. For virgins, 44.4 percent die free of cancer, some before the average age of tumors and some live longer. Spontaneous tumors occur on an average of 455.0 days of age, whereas those mice which do not develop cancer live on an average of 541.0 days.

This differential between cancer and no cancer may be due to genetic differences or perhaps to slight disturbances in a physiological homeostatic

mechanism, or some other cause between individuals. However, the problem should no longer be ignored.

Specific types of tumors seem to be characteristic of certain maternal age descents at different periods of time. For example, 70 percent of all tumors in females of the < 100 maternal age descent are of mammary gland origin. This percentage drops to zero in female mice of the 501–600 maternal age descent following nearly a straight line decline in female mice of the successive maternal age descents. The loss of mammary gland tumors in the successive maternal age groups is, however, replaced by other types of tumors particularly the leukemias, in order to keep the total number of spontaneous tumors at about 34 percent.

A suggestion may be made that perhaps the high incidence of mammary gland tumors that is found in the so-called high spontaneous tumor lines such as the C₃H/St may have been due not only to the introduction of the virus of Bittner but also to a certain degree of biological instability due to an early maternal age descent. It is a fact that when C₃H/St was being developed following an outcross between A/St and dBA/St mice, there was need to build up as many generations as possible through inbreeding in order to establish a pure line at the earliest possible time and this procedure would necessarily be the result of continuing the descendants of first or second litters, that is, an early maternal age descent.

Longevity

4. Again longevity appears to be determined, to a certain extent, by the age of the mother at which time the offspring were born. Again, the data were published in the *Symposium on The Morphological Precursors of Cancer* (Strong and Johnson, 1962) and can be located there.

There is a gradual increase in the expectancy of life beginning in the offspring of the < 100 maternal age descent and reaching a maximum in mice of the 201–300 maternal age descent. After this period of maternal age, i.e. beginning with mothers older than 301 days, life expectancy diminishes, but this decline is not yet statistically significant.

It seems that with the process of inbreeding several forces are at work that are influencing specific types of spontaneous tumors, the total numbers of tumors, and life expectancy of mice dying without tumors. Some of these factors may very well be genetic, but some may be dependent upon some selective influence of maternal age [the nature of which is quite unknown]. In this experiment, oncology and gerontology are very closely related to each other.

This brief review on the aging process should also include another paper published in 1966. This paper, entitled "Inbreeding, Spontaneous Lung Tumors, and Maternal Age Selection in Mice", was presented at The Third Quadrennial Conference on Cancer at the University of Perugia, Italy, and published in the ensuing monograph on *Lung Tumors in Animals* (Johnson and Strong, 1966).

The conclusions from the recent studies were as follows:

(1) In six descents established by selection of maternal age between F_{11} - F_{15} there is a progressive increase (with one exception) in longevity of female offspring with later and later maternal age selection; (2) the one exception is the female offspring of the 201-300 maternal age descent which outlive the female offspring of all other maternal age descents; (3) data on longevity of male offspring are more variable but increased longevity with advanced maternal age selection is also evident with one exception, the early maternal age descent; (4) there is a sex difference in longevity between F_{11} - F_{15} in all the < 100, 301-400, 401-500, and 501-600 maternal age classes—in all cases males outliving females; (5) there is no sex difference in longevity between F_{11} - F_{15} in the 101-200 and the 201-300 maternal age classes thus indicating more uniformity in these classes near the middle point of a frequency distribution for the entire population as compared to the classes on either side of these; (6) in order for either a male or a female to develop a lung adenoma it must live longer than the normal expectancy of life for its descent; (7) through the continuous process of selection for early maternal age descents and between F_1 and F_{45} , longevity of both males and females is gradually increased; (8) one exception to this rule appears between F_{11} - F_{15} where the values obtained on longevity of females appear to be maximal; (9) spontaneous lung adenomas can be eliminated through inbreeding by a continuous selection toward an early maternal age descent; and (10) spontaneous lung adenomas of the early maternal age descent are eliminated between F_{34} - F_{45} generations of inbreeding.

This is the end of the development of the research program on maternal age selection, and even though perhaps too brief and lacking in significant contributions in the literature, may serve for the time being.

In summation, then, it can be said that there are several forces at work in the development of biological characteristics both normal and neoplastic. During the process of inbreeding of genetic force of segregation and recombination of numerous (already determined) genes appear to be the dominant force. However, two other influences are also present, (1) maternal age selection and (2) specific genes such as LST or its normal allele, 1st, also function but their influence can be more clearly demonstrated on a relatively high homozygous state, that is, when the force of genetic recombination is lower. These two processes of maternal age selection and action of specific genes may influence different mechanisms but obviously some of their effect are counter to the trend of genetic selection thus keeping the species in relative biological stability. It is possible that one of the effects of the LST gene is to control the tempo of biological activity and thus increase the chances of instability in the manifestation of a

biological characteristic. The introduction of a chemical carcinogen such as methylcholanthrene also may bring about changes counter to the trend of genetic selection as well as influencing many normal and neoplastic processes, and thus may also be involved in the "tempo" of biological activity.

The significance of the present investigation is to demonstrate more precisely the mechanism or mechanisms involved in the physiological activity of normal tissue as well as in the origin of neoplasias.

Another aspect of the nature of cancer has to do with its cellular composition.

A discussion of the concept that a cancer is a mixture of populations of cell types rather than a pure culture of a single type will follow.

SECTION IV

Is Cancer a Population of Mixed Cell Types or a Pure Culture of Single Type?

In order to analyze further the nature of cancer, it is desirable to consider the concepts whether a neoplastic lesion is a population of different cells with variable potentials or a culture of a single type of cell. The literature bearing upon this concept is quite voluminous. The facts are extensive although there exists, as in many fields, a difference of interpretation.

Many tumors, the so-called collision type and the teratomata, are obviously made up of several cell types. Especially is this frequently true in chemical carcinogenesis with the local application of the carcinogens (for example, 20-methylcholanthrene) to the skin of mice, the most frequent type of tumor is a squamous cell carcinoma. However, islands of sarcoma and occasionally rhabdomyosarcoma, adenocarcinoma of mammary gland (particularly the scirrhous type) and other elements are frequently found, thus indicating a population concept.

It was found about 1910 that a transplanted sarcoma could be mixed with a carcinoma by grinding the tissues together and the ensuing injected mass would grow as a single tumor with both cell types present. Consequently, different neoplastic cells can survive together and grow as a single mass. This mixed experi-

mentally produced carcinoma-sarcoma could readily be separated into its component parts by injecting the single carcinoma-sarcoma mass into mice of two separate strains, one of which was susceptible to grow the carcinoma while the other mice of a different strain tolerated the continued growth of the sarcoma but not the carcinoma.

Very frequently, two histologically identical spontaneous tumors in mice which originated close together (especially in mice of the C₃H/St and C₃HB/St inbred strains which are characteristic of multiple malignancies) will eventually fuse together and continue to grow as a single fused mass. This phenomenon should not be considered as evidence of cell fusions as has recently been proven by Barski, Sorieul and Cornefert (1960) and Sorieul and Ephrussi (1961) and others—but merely as fusion of a cancer mass retaining the cells of both tumors. Bearing in mind the observation of Cloudman (1932b) that two genetically dissimilar tumors can arise in the same gland of a single mouse, it is obvious that some of these fused tumors may have dissimilar moieties and hence must be made up of a population of different tumor cell types.

In 1920 (Strong and Little) the author, in collaboration with C. C. Little, demonstrated that even histologically identical tumors had different physiological characteristics. Later, in 1924 (Strong and Little) it was clearly shown that these physiological differences were gene differences—gene requirements that permitted the continuous growth of the dBrA and dBrB tumors. Thus the mixture of different genetically determined cancer cells in a tumor mass must be entertained.

Many spontaneous tumors in mice have more than one center of growth. Whether this is evidence of a multicentric origin of the mass should not concern us here. In fact, some of these tumors become mulberry shaped. All centres of growth, however, are not uniform.

Some areas of growth appear to be sluggish or even "resting" while others appear to be rapidly growing. The original detection of somatic mutants that led to the investigation of histocompatibility genes was made by observing extreme variants of the growth pattern of a transplantable tumor and, therefore, any significant change of pattern of growth should be investigated from the standpoint of the origin of a mutant cell type. As a matter of fact,

the growth pattern of a transplantable tumor was shown, in 1926, to be genetically determined. Thus the conclusion reached, "The rate at which a transplant grows in an individual is a genetic problem, explained partly by the phenomenon of heterosis". Heterosis is, of course, a genetic problem (Strong, 1926a).

Some of the best evidence that a cancer is a mixed population comes from the analysis of a spontaneous tumor together with the early generations of transplantation. It has been demonstrated many times that the successful grafting of a spontaneous tumor indicates that numerous genes are involved. It has been shown that from twelve to fourteen and perhaps even more genes are involved in its initial successful growth. Within a relatively short period this required genic complex is reduced serially until finally only one or two genes are required to continue the tumor and in some cases no evidence of segregation could be detected. The long-established tumors that retain the same genic mechanism for their continued growth for several years may be uniform in cell type but the longer the transplantable tumor is continued to grow the greater is its possible deviation from the original spontaneous tumor state from which it originated. So that the analysis of these well-established tumors is no criterion for the analysis of the nature of the spontaneous tumor. It is inconceivable that these rapid serial changes in gene requirement for tumor survival do not overlap in appearance—and every new appearance of a mutant type would produce another cell type thus leading to a mixed population of cells only to be replaced later by another cell population.

It is necessary to record, however, that in the author's opinion, the analysis of the early transplant generations of a spontaneous tumor has not been too exhaustive, so that the complete state of cancer cell populations in this material is not completely known.

Sanford (1958) among others has shown a possible way by which different populations of cells may be derived from a single cell at least by resorting to tissue culture. For example, by using clonal cultures, i.e. several cultures from an original single fibroblast, she was able to demonstrate the production of different neoplastic conditions. She concludes that

through these studies we have shown that neoplastic transformations *in vitro* in C₃H mouse fibroblasts are reproducible and can occur within a clone of cells. From one cell, variant cell lines differing in sarcoma-producing capacity

were derived. Cells of the two populations were found to differ in morphology, chromosome numbers and types, metabolic pattern, and in their responsiveness to growth restraints *in vivo*. These findings suggest that the two lines from one cell have undergone diverse neoplastic transformations *in vitro*, that cells of the low line as compared with the high may be less altered from the normal in their responsiveness to growth restraints *in vivo*, and that this characteristic may be associated with their different metabolic patterns. The study also revealed hereditary changes in morphology, probably antigenicity, chromosomal constitution, and enzyme activity within clones of mammalian tissue cells.

While this line of approach to the analyses of the population nature of the cancer tissue was continuing, the cytogeneticists had not been idle. Apparently the first one to draw a correlation between abnormal chromosome pattern and carcinogenesis was von Hansemann (1890). He was followed by Boveri (1914) and Winge (1930). The field has now rapidly expanded by the addition of many scientists.

Reference will, however, be made primarily to the 1958 Symposium on Genetic Approaches to Somatic Cell Variation held under the auspices of The Department of Biology, Oak Ridge National Laboratory, and published as a supplement to the *Journal of Cellular and Comparative Physiology*. References to Hauschka (1961), Ford (1958) and Braun (1958) alone will be used.

Hauschka (1961) concludes that "the exact chromosome analyses of diverse mammalian tumors support the conclusion that most cancers are multiclonal mosaics of altered karyotype. Tumor progression toward physiologic anatomy is facilitated by cytogenetic instability."

Ford *et al.* (1958) conclude that:

There is very little (if any) aneuploid variation in chromosome number in the reticular tissues (bone marrow, spleen, lymphnode, and thymus) of normal mice. No alterations of chromosome forms have been observed in these normal tissues. In a relatively small proportion of primary reticular neoplasms of the mouse, there is no direct evidence that the chromosomes differ from those observed in the corresponding normal tissue. The great majority of these neoplasms, whether spontaneous or radiation induced show a real variation in chromosome number that is usually confined to an over-all range of five or less. They also usually also differ from the normal in that (1) the mode about which variation occurs is often in the range of forty-one to forty-five and (2) one to six distinctive new (i.e. morphologically changed) marker chromosomes may be present as a regular and characteristic feature of the chromosome set. In many of the neoplasms of this majority group, the combination of a particular modal number with a group of new marker chromosomes gives each a distinct cytogenetic individuality. On continued

transplantation, the distinctive chromosomal properties of the primary growth are maintained, except that there may be occasional shifts in modal number and even more occasional incorporation in the modal set of new chromosomal types.

Braun (1958) in conclusion of his presentation states:

permit one to append a plan, which is directed principally to those currently engaged in certain applied areas of somatic variation. As you know, there has been a rapid increase in efforts directed toward identification of potential antitumor agents with the aid of test systems involving all lines of malignant and normal origin maintained in tissue cultures. Most of these test systems involve cell cultures consisting predominantly of one cell type. To anyone who has seen, in the course of microbial population studies, how different inhibitory effects can be in mixed cultures compared to cultures consisting of one cell type, the present tissue culture test systems seem at times dangerously oversimplified. These investigators should, therefore, be urged to develop adequate procedures for assaying the fate of somatic cell types in mixed populations containing known proportions of different cell types. Unless such known mixed population systems, corresponding as closely as possible to those present under natural conditions, are used for the study of environmental agents capable of interfering with the multiplication of undesirable cell types, the information obtained may be comparable to that obtained by an observer who attempts to judge the shape of an object by its shadow at 5 minutes before noon.

Klein, in 1959, considering the tumor cell as being made up of a population of cancer cells, continued the discussion of the control of the progression of tumors by taking into consideration the present status of the understanding of their probable nature. He states: "The unit characters involved in progression [of tumors] are quite diversified and include such properties as responsiveness to hormonal controls, morphological and functional differentiation, growth rate, invasiveness, metastasizability, sensitivity to various drugs, homotransplantability, etc."

Later G. and E. Klein (1959), in discussing the possible mechanism or mechanisms involved in changes in the population of cells that make up a tumor derived from an F_1 individual, stated: "Thus while the appearance of specific variants compatible with one of the parental strains seems to be a phenomenon limited to heterozygous F_1 tumors and is distinctly different from the development of 'false positives', growing in spite of a homograft reaction, and from immunological enhancement, the details of the cellular mechanism underlying the change and its genotypic or phenotypic nature remains to be investigated." The Kleins point out, however, that their discussion applies to the specific tumor they were working with and may not serve as a model for all.

Thus it is clear that many accept the population concept for the tumor mass but that there are many divergent opinions in an attempt to explain them.

A further elaboration of Hauschka's important work on karyotype analysis is contained in a paper published in 1961. His concluding remarks were:

disregarding the data for transplantable tumors, we may accept the abnormal karyotypes in most primary malignancies and in early stages of carcinogenesis as inferential support for Boveri's hypothesis: *oncogeny by chromosomal mutation*. Conversely the diploid karyotype is stable in normal somatic tissues and in benign growths. The absence of microscopically detectable changes in the latter [diploid tumors without any detectable chromosome rearrangements] nevertheless leaves the back door open for the somatic mutation hypothesis. This furtiveness of the genes, coupled with the crudity of chromosomal hieroglyphs, irks the quantitatively minded investigator who wants to attack neoplasia in more articulate, molecular terms. Proponents of the biochemical approach admit, however, that the "fundamental biochemical lesion" of cancer is an "embarrassingly elusive" goal, and that the neoplastic transformation "has to be something which eventually results in effects on the genome" [quote from Heidelberger]. A hereditary growth advantage could originate within the nuclear DNA, enter the cell from without as an infectious nucleic acid, or follow in the wake of a cytoplasmic (enzymatic or antigenic) deletion. . . . The unresponsiveness of malignant tissue to growth control by homeostatic mechanisms requires no specific metabolic lesion. Isolation from the host might begin by deletion of any one of the genetic or epigenetic links in the chain of homeostasis. This chain can be broken one link at a time by events at the molecular level or shattered by gross changes in chromosomal organization.

The introduction of homeostasis mechanisms in oncogeny will be taken up later in Section V (p. 178).

However, the naive concept, that by "disregarding the data for transplantable tumors" we may arrive at a certain conclusion regarding the nature of the cancer process, cannot be taken too literally.

The characteristics of the transplantable tumor dependent so much on "histocompatible genes" has received widespread attention. These genes have been shown to segregate and to recombine. They show linkage relations to several "so-called" normal genes and are as real as the genes for black eyes in mice and men and for growth patterns in kidney beans. They certainly do not arise *de novo* in the process of transplantation and must be present in the spontaneous tumor from which the transplant was derived. Whether they function in the actual origin of neoplasia is still, however, a disputed question.

In the final analysis at attempting to visualize the actual process in the origin of neoplasia there must be determined the relationship between genetic and cytogenetic influences perhaps more clearly oriented than at present sometimes expressed. To put the question that is involved: What is the relation between the gene and the chromosome? This is an old but apparently persistent problem. We know that specific genes are involved in the determination of some characteristics of the cancer cell. We also know that very striking and unquestionably very significant variations of the karyotype are also involved in the determination of some characteristics of neoplasia. Does the chromosome, as such, have any specific function other than being the bearer of genes which are linearly arranged down to its core? It is not feasible to conclude that what is involved in an altered karyotype are changes in deletions or additions or interference with specific genes and thus modified "action and reaction systems"—and the great mass of the extra-chromatid material may be inactive in neoplasia? Without becoming too pedantic, therefore, in a problem that will perhaps eventually be resolved to the satisfaction of all "quantitatively-minded investigators", it is well to go on to the consideration of what perhaps is the goal of both approaches—the genetic and the cytogenetic—of whether homeostatic mechanisms may or may not influence "populations of cells".

The purposes of the discussion on the topic—is cancer a population or pure culture of single type cell?—were twofold. In the first place, it is realized that to influence cancer in any way, in either a negative or positive direction, one may have to depend upon the evaluation of the true nature of the lesion. This is not necessarily so but failing in the "rule of thumb" or other approaches, science may have to depend upon the slower approach to prevention and control. If cancer is a culture of a single type cell its eventual control may be quite different than if it was found to be made up of a population of cells even though the nature of this population is quite unresolved to the satisfaction of all. In the second place, it has been shown that the growth rate of individual tumors even from mice of the highly inbred strain now available are quite variable and even unpredictable. It has also been shown that mice of the same homozygous strain and bearing the same histological type of tumor do not respond alike to the injection

of many drugs or chemicals. All that we can positively say about the use of mice of the inbred strain is that these mice show less variability than mice that were not inbred. Even the use of inbred mice for therapeutic investigators has been criticized for the fact that they do *not* show the variability of response that human beings show. So that the problem of evaluation of the results, particularly if the advances are small but cumulative, are involved. If one could depend upon an all or none response, i.e. continuous progressive growth of a tumor or complete inhibition, it would be fine. But no one has been able to do this and hence the present attempt of building upon small effects that are shown to be significant from the investigation of a system that leads itself to a statistical approach of evaluation.

In summation, then, it is probably true that all spontaneous genetic changes (somatic mutations), all transductions, transformations, tumor cell fusions, the introduction of viruses (infectious DNA and even RNA) or the application of chemical carcinogens to bind with the cellular DNA or its associated protein in nucleoprotein, or block transfer RNA in any manner or do any one or more of a large number of effects to the complicated system of cellular physiology (part of which may be genetic and part even non-genetic) will lead to the production of populations of tumor cells. This is true since all the cells of the entire mass of either normal or neoplastic tissue cannot possibly be affected or change simultaneously. Thus a population of cells with different potentialities are present at the origin of physiological change. Whether this alteration leads to the continuation of the population or will gradually be reduced to only a few or one cell type remains to be further investigated, but its solution may not change the concept for the fundamental nature of the spontaneous cancer itself.

Certainly, it is obvious that such a population of different cells may lead, in most cases if not in all, to unstable (i.e. to continuous variable) biological states. But instability implies a break in the equalizing or homeostatic mechanism or mechanisms which are already known to have widespread effects on biological systems.

The study of the concept of populations of cells with different genetic contributions and different potentialities in the neoplastic process naturally leads to the study of Homeostasis.

SECTION V

Homeostasis

Since Cannon's (1929, 1932, 1944) pioneer work on physiological mechanisms of control of biological stability, there has been extensive research along similar lines. The literature is enormous on the subject but it is not the intention of a review at this time. We will refer only to that part of homeostasis that may be considered as having a bearing upon the problem at hand, that of attempting an analysis of the cancer process on a genetic basis. A portion of the problem of biological stability and instability was covered by a recent symposium of the Society of Experimental Biology and published by the Cambridge University Press, entitled, *Homeostasis and Feedback Mechanisms*, edited by G. M. Hughes (1964). There have been other symposia and reviews on the same problem.

Cannon defined homeostasis as "the totality of steady states maintained in an organism through the coordination of its complex physiological processes". Lerner (1954) amplified this definition by stating that "homeostasis refers to the property of the organism to adjust itself to variable conditions, or to the self-regulatory mechanisms of the organism which permits it to stabilize itself in fluctuating inner and outer environments".

Cannon, in developing his ideas on homeostasis or biological control of stability, emphasized the constancy of the composition of extracellular fluid. It is difficult to visualize, however, how this factor could explain the variable products of neoplastic tissue where changes in the karyotype pattern and genic constitution or alterations of the genome of the cell are occasionally being produced even though the derived cellular mass may continue *in status quo* for long periods of time.

Also it is difficult to apply the concepts outlined in the very excellent monograph chairmanned by E. F. Adolph (1960) and published by the Czechoslovak Academy of Sciences, entitled *The Development of Homeostasis: with Special Reference to Factors of the Environment* to the cancer problem as it is usually defined.

With reservations that will be outlined presently, the monograph on "Rhythmic Functions in the Living System" (Wolf, 1962) contains several significant contributions.

The concept of "rhythms of growth" of the transplantable tumor apparently originated by Bashford *et al.* (1905). They stated

while for any one sporadic tumor the average percentage of success of a large number of experiments is fairly constant through many generations, considerable variations in success are frequently accompanied by differences in the rate of growth of the tumor and do not remain constant for their descendants in further transplantation. Thus one series of inoculations may give a small percentage of slow-growing tumors which at a subsequent period may begin to grow rapidly, or on transplantation while still growing slowly give a high percentage of quickly growing tumors.

Calkins (1908) criticized these interpretations of rhythmic growth of the transplantable tumor on the grounds that they were observed not on a single animal but in many. The problem was soon resolved, especially by Woglom (1913, 1919), by dividing the problem into the observations on early transplantations and on late. Woglom (1913) stated that "although all observers agree that once a mouse tumor has been transplanted it generally yields an increasing number of daughter tumors upon the cultivation". The "rhythmic after effect" in later generations, however, continued to be observed in some laboratories.

Woglom (1919) then attempted to analyze the problem of successful growth in terms of "Virulence or Adaptation", and arrived at the conclusion that "the presence (or absence) of power to adapt themselves to new hosts appears to be the deciding factor in the success (or failure) of most spontaneous mouse carcinomata after transplantation".

The interpretation of these observations on both the early increase of successful growth and the subsequent "rhythmic" activity of transplantable tumors were considerably affected by the production of inbred strains of experimental animals (Strong, 1942, 1955). The rapid increase in the number of mice that would successfully grow the tumors in successive generations of transplantation were shown by Strong to be brought about by serially altered gene requirements in the phenomenon of changes in tissue specificity and this concept of genetic analysis of increased success of growing a transplantable tumor later developed into the very fruitful field of histocompatibility and changes in histocompatibility.

The use of the inbred mice where biological variability had been reduced to a minimum also made possible the observation

that "periodic rhythms" of the percentage of mice showing successful "takes" of a transplant do not exist. This interpretation was verified and emphasized by Bittner (1932, 1934).

In 1934 Bittner concluded that the fundamental concepts on the nature of the tumor cell were: "(1) 'rhythms of growth' do not occur in transplanted tumors; (2) the tumor cell does not become 'adapted'; (3) the tumor cell acquires no change in 'virulence'; (4) hereditary factors determine the characteristics of both the host and the tumor cell; and (5) animals of controlled genetic constitution are necessary for tumor transplantation investigations."

However, with the investigation of the transplantation of chemically induced neoplasia where different levels of malignancy may be involved, the problem of variability of successful takes in successive generations of mice needs to be reinvestigated. In 1959 Strong reported an extensive experiment using 19,340 mice which had been injected with a transplantable anaplastic carcinoma. This malignant tumor had occurred originally in the forestomach of a mouse following the parental injection of 20-methylcholanthrene. He reported that the tumor showed a limited breach of tissue specificity (growth in a particular mouse) as soon as it was tested out in mice of several foreign strains. When the tumor was placed in mice of strains other than the strain of origin (pBr/St) a temporary enhancement of growth capacity was obtained. The tumor, following a period of rather uniform growth rate as measured by the weights of the tumors at 14 days, acquired the capacity of showing significant fluctuations from time to time. These fluctuations of growth occurred in mice of all inbred strains that would tolerate the tumor as well as in the derived hybrids of a cross between the pBr/St and N/St strains, both ancestral stocks which had been rigidly inbred previously. Fluctuations, however, were not encountered in the F₂ generation of a cross between mice of the pBr/St and F/St strains. This last observation of no fluctuations in an F₂ where a large amount of biological variability is to be expected may be very significant, and perhaps indicates a biological peculiarity of the F/St inbreds.

This work on a transplantable anaplastic carcinoma should not be interpreted as support of the old "periodic rhythm" concept of the English school of cancerologists, Bashford, Murray and Cramer. The fluctuations of growth capacity of this anaplastic

carcinoma which persisted for at least 248 transfer generations could not be interpreted, in any manner, as being periodic. They occurred frequently, it is true, in some cases, but apparently at random (i.e. not periodic). In view of the more recent ideas on cell populations in cancerous tissue, as advocated, especially by Hauschka, G. and E. Klein, and many others and discussed in Section IV of this summation review, the most likely interpretation for fluctuations in growth capacity of the tumor under consideration would be a shift, from time to time, in cell populations. As far as the evidence goes this shift could be brought out either in the karyotype or by genic change comparable to the sudden changes that are known to occur in histocompatibility genes. In either case, the change may be spontaneous and sporadic and perhaps could occur without any immediate effect from the environment.

These changes are sporadic and it is known that, for long periods of time, the reaction capacity for the growing tumors are quite uniform and indicate a homeostatic mechanism which may perhaps only occasionally be broken.

The reservations expressed on the monograph "Rhythmic Functions in the Living System" (Wolf; Chairman, 1962) can now be given. It is clear that the changes in the characteristics of cancer (growth capacity, percentage incidence of successful takes, survival time of a tumor-bearing mouse) are not periodic or rhythmic phenomena. These occur sporadically and are probably associated with occasional shifts of cell populations as mentioned previously. The exact environmental or external agents, if any, that bring these changes about are quite unknown. But perhaps research on the viruses and chemical carcinogenesis is leading the way for final analysis and evaluation.

Quite another approach to homeostasis in relation to the determination of characteristics of neoplasia has been taken by several investigations. These attempts have been made literally from all aspects of biology and biochemistry that may have a bearing on cancer.

Since a very large portion of the research program on cancer has been done on spontaneous and their derived transplantable mammary gland adenocarcinoma in mice, it is well to introduce the concept of homeostasis with the paper by W. U. Gardner (1948a). His summary was as follows:

Tumors of four endocrine glands arise under conditions of hormonal imbalances in experimental animals. The gonadotropic hormones of the pituitary, in part by experimentation, and in part by assumption, have been associated with tumors of the testes and ovaries. Pituitary chromophobe adenomas develop in estrogen-treated mice of some strains but not in others and the tendency to acquire such tumors is transmitted by both male and female mice to their hybrid offspring. An environment deficient in gonadal hormones and high in gonadotropic hormone (F. S. H.) results in adrenal cortical tumors in mice of some strains and in hyperplastic growth of the adrenal cortex in mice of other strains.

Reference should be made to the multiple malignancies that occurred in a single mouse where it was obvious there must have been hormonal imbalance (Gardner, Strong and Smith, 1936). This mouse, No. S88685 of the EI/St partially inbred strain developed by Strong, developed

a pituitary adenoma, bilateral ovarian granulosa-cell tumors, and multiple mammary adenocarcinoma.... No definite physiological activity was observed that might be attributed to the pituitary adenoma. A uterine endometrial hyperplasia and a general cystic hyperplastic condition of the mammary glands were observed and ascribed to a hyper-ovarian state associated with the ovarian tumors. Though it is hazardous at the present time to state that mammary tumors are induced by a hyper-ovarian condition, the role of the ovarian hormone in the stimulation of the initial growth of the mammary glands must be a necessary factor.

In addition to developing multiple malignancies in a system when hormonal imbalances were evident, it is of more than passing interest that this mouse undoubtedly possessed a peculiar genetic constitution as a result of being produced by multiple outcrosses to mice of two foreign strains and a subsequent partial inbreeding. Similar observations would obviously lead to a synthesis of genetic constitution, hormonal imbalances and multiple malignancies. Unfortunately, the mouse S88685 was the last of her line and no attempt has been made, as far as the author knows, to repeat an experiment which may take a few years, but in which most if not all of the variables involved are already known.

(While the analysis of the pathological lesions that developed spontaneously in mouse S88685 was being done by Gardner and G. M. Smith with the advice of Dr. James Ewing of Memorial Hospital, New York City, the endocrinologist, Dr. Ed Allen, told the author that on the basis of this one mouse alone he could raise \$100,000 to investigate the hormone concept for the origin of cancer. It gave the author a warm feeling of accomplishment that by the combination of a genetically determined peculiar

adrenal physiology of mice of the E/St stock (Woolley and Little, 1945; Bittner and Frantz, 1954), to a genetically determined stimulation of neoplasia entirely from mice of the I/St mice and the subsequent opportunity of mendelian segregation and recombination to take place for four generations that he had obtained this mouse of multiple malignancies.)

A second contribution dealing with the possibility that hormonal imbalance may be involved in the origin of mammary gland tumors in mice was as follows: (1) It was early discovered by several investigators, Lacassagne, Gardner and others, that mammary gland tumors could be induced in male mice by estrogenic hormone provided, of course, that the mouse belonged to a cancer-susceptible strain, such as C₃H/St. (2) It was then discovered that the onset of mammary gland tumors in mice could be delayed by the injection of the male hormone, testosterone (Gardner *et al.*, 1953). The injection of the same male hormone into mice after a spontaneous mammary gland tumor had originated did not have any inhibitory effect on the growth rate of that tumor or the survival time of the mouse harboring that tumor.

Here is clear evidence, therefore, that there are two mechanisms, perhaps both influencing hormonal balance and imbalance in the cancer process. One is the mechanism of the origin of that neoplastic state, and the second is the continuation of the tumor after its origin. But this idea of more than one mechanism in the tumor process is widespread and is derived therefore from many sources.

Again, Gardner (1948b) reported that hypophysectomy, if performed after the mammary gland has fully formed but before tumors appear, prevents mammary tumors, but once tumors have formed does not prevent the growth of the tumor or the progressive growth of some of the hyperplastic nodules.

Another aspect of a balanced or imbalanced mechanism in the injection or acceptance of a tumor or normal tissue graft has recently been presented by Jonas Salk (1965). There the attempt was made to develop the concept of a balance between a "tissue element" and a "serum factor". In the preliminary presentation at the lecture before the Eastern Seminar of Allergy, he stated

in summary of this preliminary account, that the hypothesis that the delayed-allergy system is counterbalanced by an anti-delayed allergy system, which is maintained through an humoral factor, accounts for facts which are paradoxical according to other hypothesis; it unifies a variety of seemingly different and unrelated phenomena, and it provides direction for observations and

for experimentation of theoretical and practical importance. It now remains to apply the acid test of experimentation and to dissolve or to substantiate the broadly outlined view of a two-factor mechanism operative in rejection or retention of grafts, tumors and normal (self) tissue.

Attention should now be drawn to the work of Hartwell, Vogt and Dulbecco (1965) on polyoma virus inducing changes in cellular enzymes which was referred to previously. Their conclusion was: "The possibility must therefore be entertained that a viral genome is not capable of specifying all the enzymes which are evaluated in the infected cells. Rather it may act by inducing the synthesis of enzymes which are specified by cellular genes. This does not seem too unlikely since the virus has also been shown to induce the synthesis of cellular DNA."

Unless there is a homeostatic mechanism immediately present to correct for any change in an enzyme system, be it either quantitative or qualitative, there must be produced, by the invasion of a virus, biological (i.e. enzyme) imbalance. Whether this imbalance persists or is corrected by subsequent events need not concern us here except to say that correctional procedure would certainly involve the mechanism or mechanisms of homeostasis.

In these brief references to biological balance and imbalance, hormones, enzymes and antigens (both tissue and humoral) have been mentioned. The list could be radically expanded, but perhaps enough has been said to arrive at the conclusion that an approach to genetic homeostasis is called for whether or not this genetic approach will involve hormones, enzymes or antigens. This has been outlined recently by Lerner (1954). All three of these entities are intimately associated (if not controlled) by gene action.

The discussion of Lerner's *Genetic Homeostasis*, important as it is in the development of the concept for biological stability and instability, cannot be followed too critically for the problem at hand. Many of his observations and critical analyses of observations of many geneticists seem quite inappropriate in the interpretation of the origin of cancer from an unstable biological (and perhaps) genetic state.

Lerner outlined his views (1) by discussing developmental homeostasis of the individual and (2) the application of these principles to populations of individuals. In order to develop his viewpoint that "heterozygosity means maximal individual fitness", he

emphasized the need of sexual reproduction. Thus he states: "Sexual reproduction and Mendelian heredity made possible by it have been generally viewed as progressive forces in evolution."

Thus if both of these forces are necessary for the interpretation of a biological population, then our concept of the origin of cancer must be changed—unless somatic fusions of cancer cells are more frequent and more important than are at present realized. And there is no evidence that a fusion of normal somatic cells was involved in the origin of neoplasia although this possibility must be kept in mind. Perhaps, however, there is a loophole, since Lerner admits that the problem of homeostasis in plants which reproduce by self fertilization may be of another order; and perhaps, we may add, neoplasias.

Several references to Lerner's analysis of homeostasis from the viewpoint of the geneticist will be given. At the outset it is clear that cancerologists may be restricted in their analysis if they apply too literally to cancer Dobzhansky's (1951) definition of a Mendelian population as "a reproductive community of sexual and cross-fertilized individuals which share a common gene pool". But cancerologists perhaps can apply to the cancer process Wright's (1951) description of evolution as "an irregularly shifting state of balance".

Again in the observation reported previously by Strong that "biological variation may take place counter to the trend of selection", (1) in the presence of a carcinogen and (2) by having the mice exposed to differential maternal age selection, the conclusion of Lerner and Dempster in 1951 seems appropriate "that natural selection for fitness does indeed operate in the opposite direction from artificial selection".

Also in the research outlined in Chapters 1, 3, and 4 of this book, it was indicated that the descent from mothers of the 201-300 maternal age descent were less variable, lived longer and developed fewer spontaneous tumors. Lerner's references to balanced phenotypes are very significant.

Lerner states:

The best adapted individuals in a population are those which exhibit a harmonious combination of all characters leading to maximum fitness. Natural selection working on the whole complex of traits will then tend to favour either organisms clustering around mean values for all characters (see e.g. Fisher, 1930), or those in which extreme deviations for one trait are compensative for some form of deviation in another.

As Wright (1951) has stated:

the best adapted form in a species is usually one that is close to the average in all quantitatively varying characters. The intermediate values towards which selection is directed are not static, but represent optima which may change with changes in genetic frequencies (Fisher, 1932). When mean values of particular characters move in one direction, there may be compensatory movements in others. For preserving a balance specific genes may be necessary (Haldane 1932) [the 1st gene discussed in Chapter 4 may be one of these specific genes for biological balance *par excellence*] to achieve the balance between different characters, extreme phenotypic deviants must be produced, only to be sacrificed to natural selection (Schmalhausen, 1949).

Several examples of the elimination of individuals of the two extremes of a population are cited by Lerner thus bringing about less variability of the individuals around the mean. These need not be repeated now as they are well known.

The observation of Strong, Johnson and Rimm (1965) on the continued variation of age of first litters even after fifty-four generations of inbreeding emphasizes one of the main themes in Lerner's analysis that the heterozygous individual is more uniform or balanced than a homozygous one, i.e. possesses more buffering properties. A similar situation was expressed by Strong (1957) when he found that the effect of maternal age selection was more pronounced on a homozygous than on a heterozygous background thus indicating more homeostasis (less subject to change) in the heterozygous state. But here again may be a complicating factor, by the presence of polydactylia (Strong's luxoid syndrome). According to the evidence available this mutant should be considered a phenodeviant. Lerner has expressed the opinion as to the nature of the phenodeviant by stating that: 'Irrespective of the physiological basis for this phenomenon, the important fact that is suggested by intra-individual variation is the lability of the organism endowed with genetic potentialities for phenotypic deviation. This indeed may be considered as an added point of strong evidence for an interpretation based on developmental homeostasis'.

Sufficient references of Lerner's classic biological thesis have been given to arrive at some concrete conclusions. It is obvious that there has been observed in the present experiment by Strong *et al.*, extending over many years and outlined in Chapters 1, 3 and 4 of the present volume, many observations that have a bearing upon the problem of genetic homeostasis. The similarity

of results obtained from various laboratories has been visualized by Lerner and presented in masterly fashion. The limiting factor for the present discussion is whether these findings of Lerner and others can be applied to the cancer problem. The present author thinks so and has tried to organize his conclusions for this purpose. That spontaneous cancer in mice develops less frequently and the animals live longer when they were produced by mothers near the middle of a frequency distribution of many characters for the entire population (i.e. between 201-300 days of life) must be very significant. Just what this means in terms of homeostasis is not clear since there was always present the pheno-deviant gene complex, Strong's luxoid syndrome. The problem is being resolved, however, by freeing many of the maternal age descendants from polydactylia.

At present the conclusions on genetic homeostasis do not take into account the many other contributions on the subject that have been observed with hormones, enzymes and antigens or those bodily components that are known to have a genetic basis. Perhaps the molecular biologists working with more favorable material may be able to advance the problem further.

In the meantime, it is time to analyze the problem further whether the contributions obtained by the investigation of homeostatic states may advance the analysis of the problem of cancer (and its associated problem of age) or whether the investigation of new mechanisms may be fruitful.

SECTION VI

The Development of the Concept for the Use of a Liver Extract for the Inhibition of Spontaneous Tumors

From the second International Cancer Congress held at Brussels, Belgium, in 1936, Dr. G. M. Smith of Pine Orchards, Connecticut, brought back to America two new chemical compounds and, at least, one idea for cancer research. The use of one chemical and the practical application of the idea changed considerably the program of the present author for the investigation of cancer. The two chemicals were 3,4,5,6-dibenzcarbosole (a gift of Professor E. Boyland of Chester Beatty Institute, London) and colchicine (from the laboratory of Professor A. P.

Dustin in Brussels). The idea was derived from the observation of Vlès (Vlès and Ugo, 1936) demonstrated at the congress that a wave of fluorescence occurred down the bile duct following the subcutaneous injection of a carcinogen.

The first chemical, 3,4,5,6-dibenzcarbosole, was given to me. This compound, dissolved in benzene, was painted on the skin of mice and a variety of tumors were produced such as (a) epithelioma of cutaneous tissues, (b) carcino-sarcoma of the subcutaneous tissues and (c) sarcoma. In addition there were also obtained a low-grade inflammation of the liver and a hyperplasia of biliary ducts. Following an injection of the carcinogen, eight cases of benign circumscribed hepatomas were obtained in mice of the CBA/St strain. No hepatomas were noted in mice of the A/St strain receiving the same treatment. Thus a probable genetic difference was detected.

About the same time the idea of a carcinogen followed by fluoroscopic inspection of the bile duct was performed by the author with a complete verification of the fact that the carcinogen or a metabolite of the carcinogen had passed through the liver.

These two experiments were the basis of the author's interest in the physiology of the liver and its bearing upon the cancer problem, at least, in the mouse which had always been his point of interest.

The continuation of the work on chemical carcinogens and its genetic analysis (a strain difference being discovered between CBA/St and A/St strain mice with 3,4,5,6-dibenzcarbosole) took some 10 years (1937-47) and the focusing of attention on the liver some 29 years (1937-66) and continues even now with no abatement.

In some ways, the liver had already received some attention for a program of cancer research but only in a cursory manner. For example, the idea was early entertained that organ extract therapy may aid as an adjunct to fortify a possible resistant mechanism against cancer that apparently is present in old mice. It had been provisionally concluded from many observations that old mice had a mechanism of resistance to spontaneous tumors even if only in a weakened condition. Some observations bearing on this concept were as follows:

It had been commonly known that old female mice of the A/St strain developed spontaneous adenomas (of a lower degree of

malignancy) rather than adenocarcinomas that occurred in female mice of younger ages. Also it was known that an old female mouse of the C₃H/St strain would occasionally regress a small spontaneous tumor of mammary gland origin. This regression was sometimes permanent and sometimes only of a temporary nature, since recurrences at the site of the appearance of the original tumor sometimes took place. Now the program of tissue or organ extract (usually from old animals) as supplemental therapy had been used on mice bearing spontaneous tumors ever since their original appearance in adequate numbers about the year 1920. At any one time there were always a few mice available particularly of the A/St and C₃H/St strains, and these were used for the purpose of a possible inhibitory action of some organ extracts. Among the organ extracts produced, some had been prepared by a variety of means on several organs including the liver, but the results obtained never showed any encouraging degree of inhibition of spontaneous tumors. The liver had been used frequently for these extracts. Unfortunately, these early records on organ or tissue extract therapy of experimental spontaneous cancer were lost in the recent transfer of the author's laboratory from Springville, New York, to La Jolla, California, and are thus not available for comparative studies of more recent data.

More recently, however, the later observations on (1) the genetic nature of the cancer cell, (2) the concept of populations of cells in spontaneous tumors and (3) the idea of homeostasis and its bearing upon biological stability and instability and the origin of spontaneous tumors all emphasized anew the possibility that the liver and perhaps other organs could be more intimately involved in the cancer problem than was at first realized.

In this brief discussion, an attempt will be made to indicate how this renewed interest in the liver was developed—taking into consideration these new findings.

This latest emphasis upon the liver and its intricate physiological behavior on the cancer problem was influenced by several facts not yet known when the original work on liver extracts therapy had been fostered. We shall attempt to outline the development of this change of emphasis upon the liver.

Following the subcutaneous injection of methylcholanthrene, its passage through the liver and its final elimination as such,

by detoxification from the organism, it was found that the liver had been permanently altered or damaged.

Some of these changes were described by Hoch-Ligeti (1954) as follows: "Fatty changes and cirrhosis with the deposition of ceroid and cholangio-fibrosis were found in the livers of rats fed the basic diet with and without MC, [methylcholanthrene] although these changes were manifest in all rats, their severity was enhanced by the feeding of MC."

The injection (subcutaneous, etc.) and the feeding of methylcholanthrene by mouth produces liver damage as observed by many investigators. The mice eventually either develop liver hepatomas or liver sarcomas, or in our own extensive series, induced tumors at many different anatomical sites. Even if the mouse lived more than 1000 days following the subcutaneous injection of methylcholanthrene, the histology of the liver of the treated animal was never normal the mouse developed a variety of malignant tumors.

According to Vest and Rossier (1963):

one of the functions of the liver is to perform transformation of endogenous or exogenous compounds so that their excretion is enhanced or their toxicity is reduced. The reactions involved are oxidations, reductions, hydrolyses, and synthetic or so-called conjugation processes. The latter include combinations with glucuronic acid, amino acids like glycerine, cysteine or acetylcystein (mercapturic acid synthesis) sulphate methyl and acetyl-groups.

Many of these processes must be affected by an altered or damaged liver by an injected carcinogen.

Eisen (1946) has expressed the opinion that "the liver is endowed with no general resistance to carcinogenic agents such as might be linked with its extensive detoxifying powers is clearly evident from the case of production of parenchymal cell neoplasia in the rat with azo compounds administered orally or parenterally".

Another series of experiments that appear to emphasize the role of the liver in cancerous states is the grafting of ovarian tissue into the spleen thus altering the flow of the estrogens from the ovarian graft to the liver.

The research is reported in a series of papers by W. U. Gardner and Min Hsin Li between 1947 and 1955 (Li and Gardner, 1947, 1948, 1949; Li, Gardner and Kaplan, 1947; Gardner, 1955).

According to Li and Gardner:

the present experiments are based on two principles: (a) the capability of the liver to inactivate ovarian hormones, i.e. estrogen and progesterone, when the

hormones circulate through the hepatic portal system and (b) the increase of intrinsic gonadotropic hormones subsequent to castration as determined by bioassay of urinary, blood and hypophyseal gonadotropins and in experimental parabiosis of an intact with a castrated or roentgen-rayed animal. A condition of endocrine imbalance might be produced by the transplantation of ovaries with spleens of castrated mice. Such conditions should permit the study of continuous action of endogenous pituitary gonadotropins in ovarian tumorigenesis.

Their conclusion was practically an acceptance of their original concept that "the ovarian hormones produced by the intrasplenic ovarian transplants are inactivated by the liver before entering the systemic circulation while the hypophysis, under such conditions as physiological castration, produces larger amounts of gonadotropic hormones to further stimulate the ovarian transplants. As a result of this procedure of altering the normal detoxification of estrogen by the liver, a malignant ovarian neoplasm was produced."

Interesting as these observations have been in focusing attention on the liver in changing the appearance of both hormone-dependent and chemically induced neoplasias the greater emphasis on the liver was arrived at from the consideration of the genetic nature of the cancer process.

It is now very widely, if not universally, accepted that a change in the genome of the cell must be involved, not only in the origin of the cancer cell but also in its continuation. This, of course, implies that nucleoproteins are involved. There is a difference of opinion whether DNA or RNA or relationships between the two nucleic acid moieties are involved. But nucleoproteins cannot be considered without reference to their origin and their final elimination from the body and this cannot be discussed without reference to the liver. True it is that these entities are self perpetuating entities in the cells of the body but they cannot do this without building materials and the eliminating of wastes, where again the liver is involved.

But to go into the discussion of the role of the liver in protein metabolism both catabolic and anabolic or even restricting discussion to nucleoproteins would involve more time than can be given here. Many excellent discussions such as *The Liver Morphology, Biochemistry and Physiology*, edited by Ch. Rouiller and published by Academic Press, New York-London, 1963, are available and the author would mostly quote from these reviews in any case.

The liver possesses hundreds of enzymes and is involved in numerous reactions involved in both catabolism and anabolism. Many cases of balance and imbalance, particularly of the hormone-relationships, are known and discussed in a section of pages 430-3, (Rouiller, 1963). On page 92, Ch. Rouiller (1963) states: "Answers have been sought to questions concerning the role of different tissues, including liver, in maintaining homeostasis of biologically active steroids and their derivatives in blood and tissue fluids, and the influence of environmental and dietary factors or disease states."

In the liver, therefore, we have possibly an organ that possesses many characteristics needed for the control of abnormal growth, including cancer.

We have innumerable reactions conditioned, in part, by a very large number of enzymes, an organ involved in the synthesis of the building blocks of tissues including the nucleoproteins, and its partial elimination from the body the very significant function of detoxification of both endogeneous and exogeneous poisons and tissue components including the hormones.

Many of these processes continue in a normal or balanced (homeostatic) sequence but at times in quite an imbalanced path particularly when the insult or injury has been large. Since so many processes are going on in one organ for its own economy and also for the physiological behavior of many other parts of the body these homeostatic mechanisms must be numerous. One could even speculate whether a super-homeostatic mechanism may be present to keep the whole elaborate system functional and in health, but such must be considered merely as a suggestion.

The problem whether one or more of these homeostatic mechanisms could be extractable from liver tissue is an intriguing one. The idea was foremost in the mind of the author even involving another idea that this extractable entity may affect the origin and fate of a neoplastic condition at least in mice where so many of the factors involved in its characteristics are so clearly indicated. The observations already reported in "Biological Equilibrium and the Origin of Cancer" in *Science* in 1957 (Strong) emphasized our attention to this possibility. In this paper, two conclusions were reached which seemed significant. (1) It was observed that more spontaneous tumors occurred in

mice which belonged to a genetic descent characterized by biological variability, i.e. imbalance. (2) "That the various types of cancer which arise in the mammalian body have at least, one characteristic in common. Cancer arises because the organism has lost control of a definitive part. Perhaps during the aging process the mechanism that keeps the individual in equilibrium is disrupted, and as a result of this loss of control of all the parts cancer of one or more elements is able to originate and to grow at the expense of the rest of the body."

The analysis of the data on maternal age selection, as discussed in Chapters 1, 3 and 4 of this present publication, indicated possibly the place to look for a controlling influence on spontaneous tumors in mice—in the liver of a mouse which belonged to the 201-300 maternal age descent where a maximal degree of biological equilibrium (homeostasis) had been encountered in the analysis of many biological characteristics.

This, in brief, was the origin and the development of the concept for cancer research discussed in Chapters 5 and 6 of this book where the attempt has been made to influence the origin and fate of spontaneous cancer in mice by the injection of liver extracts.

The preparation of tissue or organ extracts has varied considerably, but has now narrowed down to the method of lyophilization, differential solubilities and finally the taking up in distilled water to which a little thymol has been added as a preservative.

The preparation of a pure chemical from a tissue or organ is usually specific for that entity and for its separation from other components, its purification and final production, if possible, in crystalline form. When one is not looking for a specific entity of known chemical structure and suspects only that one or more may be present in a complex mixture of countless entities the above procedure must be changed. In the present experiment techniques of preparation of several fractions containing possibly many active, both inhibitory and even stimulatory, some inactive and/or inert components was designed to protect the integrity of an unknown entity in the condition that it probably existed in the intact organ. This technique was followed by further separations of component parts into active and inactive fractions, thus emphasizing the active entity, i.e. an inhibitor of neoplastic growth through several separations.

The use of a test animal, that of a mouse growing a spontaneous tumor which may live 5 months, consumes much time and progress is consequently slow—hence the need to identify the biochemical or chemical nature of an inhibitor even though necessary eventually may not, in itself, lead to immediate progress of controlling cancer in an experimental animal. If the problem of inhibition of a spontaneous tumor is only partially indicated by the injection of the liver material then a program of complete chemical and biochemical analysis of that entity or entities is amply justified.

In a preliminary discussion on reporting (1) the inhibition of the growth of spontaneous tumors in mice and the increased survival time of a mouse growing a spontaneous tumor, (2) the complete regression of not only one spontaneous tumor but also three in the same animal and (3) a significant decrease in the appearance of multiple malignancies in mice of the C₃H/St and C₃HB/St strains by the injection of liver extracts as outlined in Chapters 5 and 6, one comment was that you may be approaching a goal of success in the control of cancer by following a faulty premise. This is possible but not probable. When this opinion was expressed to me, an old opinion occurred to me that "there are a thousand ways to death", or to put the same thought in another language, "The Porches of death are manifold". It would not be too far afield to substitute the word death by cancer, since if left to itself, cancer always ends in death. The reverse is probably not true. That is, there are no thousand approaches to the solution of the cancer problem. Perhaps there may be five or six or even nine or ten but not a thousand. But really it does not make any difference in the philosophical interpretation of the attainment of a scientific goal. If the solution to the problem of control or only part control of that dread disease, cancer, is obtained by any means, then and not till then should there be an attempt made to evaluate any concept that led to success no matter by what devious road of approach.

It then becomes desirable to summarize the facts obtained by the injection of liver extracts into mice bearing spontaneous tumors of mammary gland origin.

SECTION VII

Recent Evaluation of Liver Extracts on Cancer in Mice

The evaluation of a possible effect of an experimental procedure on such a disease as cancer is sometimes based upon the prevention or delay of onset of the neoplastic lesion and/or its disappearance and the patient living a certain length of time free of any disease symptoms. In the case of human cancer, the American Cancer Society and other interested groups have set up a period of 5 years for the patient to be considered free of any recurrence. It is known that some cancers in the human subject may occur after 5 years of regression but this occurrence is rare. In terms of mouse physiology, a period of 60-75 days is probably comparable to 5 years in the life of man.

In dealing with new techniques in pioneer research, it may be too much to expect, in such an animal as a mouse, where the growth capacity of a malignancy is greater than it is in a human patient, the application of this "all or none" evaluation of human statistical evaluation.

Perhaps the discovery of small effects on either prevention of a lesion or its disappearance, or even if inhibited to a certain extent is all that should be, at first, expected. Then by the accumulation of small effects on the neoplastic process, one should eventually expect to obtain highly significant end results. Due to the very nature of the problem in mice, this latter approach has had to be followed. An old expressed opinion was that, if the growth of a spontaneous tumor would remain stationary then the experimenter should be satisfied. We have gone much beyond this old expressed hope.

Some of the reasons for the present approach should be obvious to the reader when the problems of the nature of the cancer process in mice have been taken into consideration. The difficulties have centered around (1) the analysis of the genetic nature of cancer, (2) the consideration of the concept that in its origin and continuation, cancer is a continuum of a population of cells with different physiological capacities and (3) the recognition of the possibility that the mechanism or mechanisms of homeostasis (of yet unknown origin) are apparently involved. As the investigation proceeded, it was found that certain liver extracts prepared

by a special technique as outlined in Chapter 5 had an inhibitory effect on spontaneous tumors of mammary gland origin in mice. It is still quite unknown what the chemical nature of these extracts are, either by direct chemical analysis or by inference on their effects on biological systems other than the single test used in this investigation, the effect on spontaneous tumors. It is also still an unknown problem whether the liver extracts have any significant effects on homeostasis or on mixed populations of cells or even on genetic phenomenon.

Positive effects on, at least, (1) partial prevention of primary and multiple spontaneous mammary gland tumors in mice, (2) their inhibited growth pattern in varying degrees and (3) their complete disappearance in 15 percent ($15.1 \pm 1.9\%$) of all cases have already been obtained by the injection of liver extracts. The final analysis of the liver extracts in relation to the control of spontaneous tumors of mammary gland origin in mice will eventually be known through the cooperation of chemists, physiologists and perhaps other scientists. The continuation of the present technique should also produce additional significant observations on cancer.

For the time being, therefore, the recording of facts, rather than theoretical considerations, should be sufficient. The data will not be presented in chronological sequence, however, but in relation to the criteria set up to evaluate the effects of an experimental procedure on cancer.

A. *Prevention*

For a pilot experiment, on 19 August 1964, twenty-four virgin female mice of the C₃H/St strain were started on a program of receiving 0.1 cm of liver extract No. 12A in distilled water subcutaneously every week. This extract was prepared according to the outline given in Chapter 5.

The controls of twenty-three virgin female mice of the same strain were isolated at the same time. To date, all mice are either dead or have developed a spontaneous tumor of mammary gland origin. Of the twenty-four experimental mice receiving the injection of liver extract (No. 12A), four mice developed mammary gland tumors at an average age of 14.5 months. Of the twenty-three controls, ten developed spontaneous tumors at an average

age of 16.5 months. The difference between the incidence of tumors in the controls and the experimental groups was 3.31 times the probable error, as follows:

	Total	Free of tumors	With cancer	% Tumors
Controls	23	13	10 ± 1.6	43.5 ± 6.9
Experimentals	24	20	4 ± 1.2	16.7 ± 5.0
DIFFERENCE or $3.31 \times P.E.$				26.8 ± 8.1

The pilot experiment was highly significant in that a positive effect had been obtained in preventing spontaneous cancer of mammary gland origin in mice. The experiment should, therefore, be repeated on a larger scale, but for now the time element is too limited, for either preparing the suitable extracts from mice of suitable age groups or in performing research on spontaneous tumors in mice, which is a program that would require at least 2 years.

B. *Complete Regression of Tumors*

Of the 172 tumor-bearing mice that have received intra-peritoneal injections of one or the other of the various liver extracts (either singly A or W or in combinations A and W) used in this research, twenty-six or 15.1 ± 1.9 percent of them have had a complete disappearance of their spontaneous tumors. Of the forty-five controls, none has ever regressed their tumor. (This observation on mammary gland tumors in controls could be expanded to the hundreds of C₃H/St mice which have been observed in the author's laboratory for many years.) But the comparison between forty-five controls in the Del Mar series and the 172 experimental tumor-bearing mice in the present series is obviously a very significant observation. Of the 172 experimental animals receiving liver extracts, 146 showed various degrees of inhibited growth of spontaneous tumors, as discussed in Chapters 5 and 6 and will be briefly referred to later. The statistical analysis of the data bearing on complete regression of spontaneous tumors under experimental procedure is therefore:

Class	Number of tumors	Inhibited growth	Complete regressions	Percent complete regressions
Experimentals	172	146	26 ± 3.2	15.1 ± 1.9
Controls	45	0	0	0

Thus 15.1 ± 1.9 percent of all tumor-bearing mice receiving liver extracts had disappearance of spontaneous tumors compared to none in the controls.

The twenty-six mice with disappearance of their original spontaneous tumor of mammary gland origin lived, on an average, of 123.5 days and four of these mice are still alive free of any tumor at 98, 204, 252, and 319 days following the discovery of their original tumor. The controls for these mice with the growth of their tumors lived, on an average, of 66.7 days. There was an improvement in the survival times of these mice regressing spontaneous tumors with the injection of liver extracts following the observation that the small effect on inhibition of tumor growth could be increased by the use of different combinations of the A and the W liver fractions prepared according to the outline in Chapter 5. For example, the first thirteen mice to regress spontaneous tumors had received either solution 8 (before separation into A and W), 9W₃-10A and 10W-10A. These thirteen mice lived, on an average, of 78.5 days following the appearance of their primary tumor, whereas the second series of thirteen mice that received later prepared solutions 12W-13A, 12A-13W, 16W-17A or 16A-17W, lived, on an average, of 168.6 days with four of these mice still living, receiving occasionally 0.1 cm³ of liver extract, at the advanced ages listed above.

Twenty-five of these regressions of spontaneous tumors occurred in mice that received either the combined No. 8 solution, or both A and W on alternate injection periods, spaced two days apart. With one exception, therefore, obtained in one mouse with solution 12W, it is probably true that both moieties (A and W, either combined or with alternate injections) are necessary for the complete regression of a spontaneous tumor of mammary gland origin in mice.

It is of further significance that fifteen of these twenty-six mice regressing spontaneous tumors following the injection of

liver extracts developed only a single primary tumor. The other eleven developed multiple primary tumors. Some of these multiple tumors also regressed under treatment while others grew slowly for a short time only to become stationary in size. The reduction of the total number of multiple appearing tumors following treatment with liver extracts will be discussed later.

Mouse No. S554,777, receiving solutions 16W and 17A on alternate injections, was the prize so far. She developed three primary tumors all of which regressed under treatment and she lived for a period of 234 days following the discovery of her original primary tumor.

C. *Multiple Tumors*

In a discussion of this topic it is well to emphasize the following: it has been known for some time that some mice develop more than one primary spontaneous tumor of mammary gland origin. Whether these multiple tumors are all primary or due to metastasis has been discussed at length by several investigators with the usually accepted conclusion that these multiple tumors, even in the same gland, are primary, i.e. in the mouse, mammary gland adenocarcinoma may have a multicentric origin.

The primary purposes for the selection of the C₃H/St, and its derived subline C₃HB/St strain, for the supply of spontaneous tumors were as follows:

(1) Female mice of these strains (both virgins and breeders) develop a very high incidence of spontaneous tumors of mammary gland origin with progressive growth rates and,

(2) Many of these mice develop many multiple primary malignancies. (As a matter of fact, their common ancestor No. F₁ S79 developed, over a period of time, following several surgical removals of tumors, some fourteen primary spontaneous tumors of mammary gland origin.)

In this present experiment, no mouse ever developed more than five primary tumors.

Of the 172 female mice receiving the injections of combinations of liver extracts, 122 developed only a single tumor and 50 ± 2.3 developed multiple tumors. Of the forty-five controls, only twelve developed single tumors, whereas 33 ± 2.0 gave rise to multiple primary tumors.

The data are:

Class	Total	Single	Multiple	Percent single	Percent multiple
Controls	45	12	33 ± 2.0	26.7	73.3 ± 4.5
Young W: Old A	72	44	28 ± 2.8	61.1	37.5 ± 5.6
Old W: Young A	53	27	26 ± 2.5	50.9	49.1 ± 4.7

DIFFERENCES

Control: Young W: Old A 35.8 ± 7.2 or $5.0 \times$ P.E.
 Control: Old W: Young A 24.2 ± 6.5 or $3.7 \times$ P.E.
 Old W: Young A: Young W: Old A 11.6 ± 9.6 or $1.2 \times$ P.E.

D. Inhibition of Tumor Growth

The inhibition of growth rate of spontaneous tumors in mice has been discussed in Chapters 5 and 6. There the attempt was made to determine the many variables involved in the production of inhibition of growth by the injection of liver extracts prepared in different ways and stored under different conditions. Many of these variables are now known and several others are still under investigation. By taking these facts into consideration, it has been possible not only to increase effective inhibition of tumor growth, but also to obtain tumor regressions in 15.1 ± 1.9 percent of all liver-treated mice, to bring about the reduction of the number of multiple primary tumors from 73.3 ± 4.5 percent in the controls to 29.0 ± 1.4 percent in the experimentally treated mice, and the prevention of spontaneous tumors in 26.8 ± 8.1 percent of all mice, as outlined in sections A, B and C of Section VI.

The degree of inhibition of tumor growth has indeed been a variable quantity in the different series of treated mice, possibly influenced not only by the variable nature of a mixed population of tumor cells in a tumor mass from time to time but also in the variations in the preparation and storage of the liver extracts used.

Perhaps it is well to summarize briefly the variables encountered by the use of the injected material, some of which have already been discussed earlier and incorporated into the experimental approach to liver extract used, but some are still being

encountered in the laboratory and, therefore, have not as yet been combined with previous experience in order to possibly obtain a greater degree of control of cancer in mice.

There appears to be, at least, two moieties of the liver extracts that have had an effect upon the characteristics of cancer. One of these is water soluble (W) and decreases its effectiveness with the advancing age of the mouse from which the liver was obtained. The other moiety is alcohol soluble, which in these experiments had been reduced to dryness under reduced atmospheric pressure and taken up in a standard amount of distilled water, 2 cm³ per liver from one mouse. In this condition, the A moiety is obviously an emulsion whose physical properties change somewhat when kept under refrigeration for any length of time.

Either moiety, the W or A, has an inhibitory effect on tumor growth (i.e. on averages of more than one mouse), but their use on alternate days of injection (spaced 2 days apart) apparently gives a greater effect of inhibition than either one used alone. This conclusion, so far, is derived from observations when a W solution from a young mouse is alternated with an A "emulsion" from an old mouse (see series 1-6 of 17W-16A discussed in Chapter 6).

However, the combination of a young liver W alternated with an old liver A injection does not give the same result on inhibition as combining an old liver W with a young liver A. In some ways a paradox has developed which has not been completely resolved at this time.

Data obtained on series 1-6 (24 mice) for series 16A (old liver) and 17W (young liver) have already been presented. Data for series 7-8 (8 mice) for 16A-17W are complete and series 1-8 (32 mice) for 16W (old liver) and 17A (young liver) have now reached near completion and can now be discussed.

In series 1-6 of 16A-17W when the obvious "aging" of the solutions under refrigeration was the only apparent variable, there was obtained an increasing degree of inhibition of tumor growth (on an average of four mice) with time. The solutions for series 1-2 had been aged 28 days, for series 3-4, 62.5 days; and series 5-6, 120.5 days (see Chapter 6).

After the solutions had been aged for 240 days, series 7 and 8 with eight mice bearing spontaneous tumors were injected with

the liver extracts (16A-17W) on alternate periods, spaced 2 days apart. It was found that the inhibition of tumor growth was less than it had been for mice in series 5 and 6. Thus the solutions had lost part of their inhibitory capacity. But even after 240 days of aging of solutions, the inhibition of tumor growth was greater than it had been in series 1 and 2 or even in series 3 and 4. It is not clear whether one or both of the liver extracts change with storage time. The only obvious physical change is that the A solution becomes pearly white and more viscid with storage.

In the use of solutions 16W and 17A another situation of inhibition of tumor growth developed. There never was obtained an increased inhibition of tumor growth in the successive series of four mice each. The loss of inhibition of tumor growth apparently began much earlier than it did in the series of 16A-17W. This loss of inhibitory action on tumor growth was, however, never complete nor has there been any evidence that by this successive change an inhibitor of tumor growth was ever converted into a stimulator of tumor growth — the growth rate of a tumor in an experimental animal was always slower than in the controls.

For example, the average total increments of growth expressed as square millimeters per unit of time for (1) series 1-4 (16 mice), (2) series 5-8 (16 mice) for mice alternately injected with solutions 16W-17A and (3) the controls (45 mice) are presented in tabular form at 20-day intervals and discloses inhibition of tumor growth by liver extract injections and never a stimulatory effect, as follows:

16A-17W	20 days	40 days	60 days
Series 1-4 (16 mice)	66	223	229
Series 1-8 (16 mice)	83	266	464
Controls (45 mice)	206	497	605

The most recent experiments now under way (in anticipation of Chapter 8) are also showing some new significant results on tumor inhibition but whose final analysis must wait.

These new observations in brief, are as follows:

1. There is a sex difference in the inhibitory action of liver extracts on tumor growth. The male liver extracts produce more inhibition than does the liver from a female mouse, when compared on an age basis.

2. This sex difference is greater at 250 days of age than it is at 75 days.

3. This increase of a sex difference with age of mice is probably brought about, in part, by the fact that the inhibitory agent in female liver extract does not change much with age of the donor (at least between 75 and 250 days of age) but the male liver extract does. In other words, the effect on tumor growth from a male liver at 250 days is more inhibitory of tumor growth than that obtained from a male mouse at 75 days of age.

4. Liver extracts from other species, especially Mastomi, or South African Rat, and beef also have some inhibitory action on tumor growth in mice but here again the final analysis of the data must wait.

5. Boiling of solutions for 30 minutes alters the inhibitory reaction on tumor growth but here again the experiments are not complete.

In brief summation then it can be emphasized that one approach to the solution of the cancer problem, at least, in mice has been made and progress obtained even though many years have been used in preparation of suitable experimental animals, the analysis of the biological nature of cancer itself and the preparation of suitable tissue extracts.

Whether the final goal can be reached by this approach or even by a slight or radical detour from the mainstream or whether an entirely different approach will be needed, time alone can tell.

Postscript

THE author desires to express at this time a challenge to the young scientist now entering or who will shortly enter the field involving the problem of cancer, including its prevention and cure in any species. This challenge is, that if your generation will do as much for cancer research as my generation (which, fortunately, is not yet ended) then the solution to the problem will be available and perhaps, at the same time, a better appreciation of the aging process will be available.

*Authorem praesentis justitiae habes, sponsorem futurae
non habes.*

References

- ADOLPH, E. F. (1960) *The Development of Homeostasis: With Special Reference to Factors of the Environment*. Academic Press, London and New York. 218 pp.
- AMOS, D. B. (1956) Seriological differences between comparable diploid and tetraploid lines of three mouse ascites tumors. *Annals N.Y. Acad. Sci.* **63**, 706-10.
- AMOS, D. B. (1958) Preliminary studies on the antigenic relationship between compatible tumors of different chromosome number. *Annals N.Y. Acad. Sci.* **71**, 823-6.
- ANDERVONT, H. B. (1937) Pulmonary tumors in mice. II. The influence of heredity upon lung tumors induced by the subcutaneous injection of a lard-dibenzanthracene solution. *U.S. Pub. Health Repts.* **52**, 304-15.
- AUERBACH, C. (1949) Chemical mutagenesis. *Biological Reviews* **24**, 355-91.
- BARSKI, G., SORIEUL, S. and CORNEFERT, F. (1960) Production of cells of a "hybrid" nature in cultures *in vitro* of 2 cellular strains in combination. *C.R. Acad. Sci., Paris* **251**, 1825-7.
- BASHFORD, E. F., MURRAY, J. A. and CRAMER, W. (1905) *Transplantation of Malignant New Growths*. Second Sci. Repts. Imp. Cancer Res. Fund, London. Part 2, p. 48.
- BAUER, J. (1925) Das Wesen der vererbaren Krebsdisposition. Beiträge zur klinischen Konstitutionspathologie XVI. *Zeitschrift für Konstitutionslehre* **II**, pp. 147-65.
- BAUER, K. H. (1928) Mutationstheorie der geschwulstentstehung Übergang von Körperzellen in Geschwulstzellen durch Genänderung. Verlag von Julius Springer, pp. 1-72.
- BAUER, K. H. (1949a) *Das Krebsproblem Einführung in die allgemeine Geschwulstlehre: Für Studierende, Ärzte und Naturwissenschaftler*. First Edition, Springer-Verlag, Berlin, ix + 758 pp.
- BAUER, K. H. (1949b) *On the Cancer Problem* (English), reference to Das Krebsproblem. Springer-Verlag, Heidelberg. 20 pp.
- BITTNER, J. J. (1931) A genetic study of the transplantation of tumors arising in hybrid mice. *Amer. J. Cancer* **15**, 2202-47.
- BITTNER, J. J. (1932) Genetic studies on the transplantation of tumors. III. Interpretation of apparent rhythms. *Amer. J. Cancer* **16** 1144-52.
- BITTNER, J. J. (1933) Jackson Memorial Laboratory, Staff of the: The existence of non-chromosomal influence in the incidence of mammary tumors in mice. *Science* **78**, 465-6.
- BITTNER, J. J. (1934) Genetic studies on the transplantation of tumors. VIII. The genetic explanation of "rhythms of growth". *Amer. J. Cancer* **20** 834-47.
- BITTNER, J. J. (1936) Some possible effects of nursing on the mammary gland tumor incidence in mice. *Science* **84**, 162.
- BITTNER, J. J. and FRANTZ, M. J. (1954) Spontaneous mammary cancer in mice of the CE stock. *Cancer Research* **14**, 81-85.
- BLAKESLEE, A. F. (1954) The aging of seeds and mutation rates. In: Parental Age and Characteristics of the Offspring. *Annals N.Y. Acad. Sci.* **57**, 488-490.

- BOVERI, T. (1914) *Zur Frage der Entstehung maligner Tumoren*. Gustav Fisher, Jena. 119 pp.
- BRAUN, W. (1958) Cell population dynamics and somatic change. *J. Cell. and Comp. Physiol.* **52**, suppl., pp. 337-69.
- BROOKES, P. (1966) Quantitative studies of the reaction of some carcinogens with nucleic acids. Symposium: *Interaction Between Chemical Carcinogens and the Living Cell*, Denver Meetings of A. A. Can. Res. 27 May 1966.
- BROOKES, P. and LAWLEY, P. D. (1964a) Reaction of some mutagenic and carcinogenic compounds with nucleic acids. *J. Cell. and Comp. Physiol.* **64**, suppl. 1, pp. 111-28.
- BROOKES, P. and LAWLEY, P. D. (1964b) Evidence for the binding of polynuclear aromatic hydrocarbons to the nucleic acids of mouse skin: relation between carcinogenic power of hydrocarbons and their binding to deoxyribonucleic acid. *Nature* **202**, 781-4.
- BURNS, R. (1785) "The best laid plans of mice and men gang aft agley." In: *To a Mouse. Bartlet's Familiar Quotations*, Little, p. 284.
- CALKINS, G. N. (1908) The so-called rhythms of growth energy in mouse cancer. *J. Exp. Med.* **10**, 283-307.
- CANNON, W. B. (1929) Organization for physiological homeostasis. *Physiol. Rev.* **9**, 399.
- CANNON, W. B. (1932) *The Wisdom of the Body*. Norton, New York. xv + 312 pp.
- CANNON, W. B. (1944) Homeostasis; the maintenance of steady states in the organism. *Colloid Chemistry, Biol. and Med.*, Reinhold Publishing Corporation, pp. 529-44.
- CLOUDMAN, A. M. (1932a) A comparative study of the transplantability of eight mammary gland tumors arising in inbred mice. *Amer. J. Cancer* **16**, 568-630.
- CLOUDMAN, A. M. (1932b) Agenetic analysis of dissimilar carcinomata from the same gland of an individual mouse. *Genetics* **17**, 468-80.
- COLLINS, V. J., GARDNER, W. U. and STRONG, L. C. (1943) Experimental gastric tumors in mice. *Cancer Res.* **3**, 29-35.
- COWDRY, E. V. (1954) Summary and general conclusions of monograph parental age and characteristics of the offspring. *Annals N. Y. Acad. Sci.* **57**, pp. 451-614.
- DI MAYORCA, G. A., EDDY, B. E., STEWART, S. E., HUNTER, W. S., FRIEND, C. and BENDICH, A. (1959) Isolation of infectious deoxyribonucleic acid from SE polyoma-infected tissue cultures. *Proc. Nat. Acad. Sci.* **45**, 1805-8.
- DOBZHANSKY, T. (1951) Mendelian populations and their evolution. In: *Genetics in the 20th Century*. Macmillan, New York, pp. 573-89.
- EAST, E. M. (1922) As genetics comes of age. *J. Hered.* **13**, 207-14.
- EAST, E. M. and JONES, D. F. (1919) *Inbreeding and Outbreeding*. Lippincott, Philadelphia. 285 pp.
- EISEN, M. J. (1946) Induction of sarcoma of the liver in the rat with methylcholanthrene and benzpyrene. *Cancer Res.* **6**, 421-5.
- FISHER, R. A. (1930) *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford. xiv + 272 pp.
- FISHER, R. A. (1932) The bearing of genetics on theories of evolution. *Sci. Progress* **27**, 273-87.
- FORD, C. E., HAMERTON, J. L. and MOLE, R. H. (1958) Chromosomal changes in primary and transplanted reticular neoplasms of the mouse. *J. Cell. and Comp. Physiol.* **52**, suppl. 1, pp. 197-233.

- FORSTHOEFEL, P. F. (1962) Genetics and manifold effects of Strong's luxoid gene in the mouse, including its interactions with Green's luxoid and Carter's luxate genes. *J. Morph.* **110**, 391-420.
- FORSTHOEFEL, P. F. (1963) The embryological development of the effects of Strong's luxoid gene in the mouse. *J. Morph.* **113**, 427-52.
- FRANCIS, L. D. and STRONG, L. C. (1938) Hemoglobin studies on the blood of female mice of the CBA strain; effects of age, diet, strain and reproduction. *Amer. J. Physiol.* **124**, 511-16.
- GARDNER, W. U. (1937) Influence of estrogenic hormones on abnormal growths. *Occasional Publications of the American Association for the Advancement of Science* **4**, 67-75.
- GARDNER, W. U. (1948a) Hormonal imbalances in tumorigenesis. *Cancer Res.* **8**, pp. 397-411.
- GARDNER, W. U. (1948b) Hormones and experimental carcinogenesis. *Extrait de Acta de l'Union Internationale Contre le Cancer* **6**, pp. 124-33: Redaction 61, Voer does Capucius, Louvain, Belgique.
- GARDNER, W. U. (1955) Development and growth of tumors in ovaries transplanted into the spleen. *Cancer Res.* **15**, 109-117.
- GARDNER, W. U., PFEIFFER, C. A., TRENTIN, J. J. and WOLSTENHOLME, J. T. (1953) Hormonal factors in experimental carcinogenesis. In: HOMBURGER, F. and FISHMAN, W. H. (Eds.) *The Physiopathology of Cancer*. Paul H. Hoeber, Inc., New York, pp. 225-97.
- GARDNER, W. U., STRONG, L. C. and SMITH, G. M. (1936) An observation of primary tumors of the pituitary, ovaries, and mammary glands in a mouse. *Amer. J. Cancer* **26**, pp. 541-6.
- GORER, P. A. (1942) The role of antibodies in immunity to transplanted leukaemia in mice. *J. Pathol. Bacteriol.* **54**, pp. 51-65.
- GREEN, C. V. (1931) Size inheritance and growth in a mouse species cross (*Mus musculus* × *Mus bactrianus*). III. Inheritance of adult quantitative characters. *J. Exp. Zool.* **59**, pp. 213-45.
- GRUSKAY, F. L. and STRONG, L. C. (1955) Correlation between mendelian recessive traits and the resistance to fibrosarcoma in mice. *Yale J. Biol. and Med.* **27**, pp. 441-50.
- HALDANE, J. B. S. (1932) *The Causes of Evolution*. Longmans, Green & Co., London. vii + 235 pp.
- HARTWELL, L. H., VOGT, M. and DULBECCO, R. (1965) Induction of cellular DNA synthesis by polyoma virus. II. Increase in the rate of enzyme synthesis after infection with polyoma virus in mouse kidney cells. *Virology* **27**, 262-72.
- HAUSCHKA, T. S. (1958a) Present status of the relationship between chromosome constitution and transplantation specificity of tumors. *Annals N.Y. Acad. Sci.* **71**, 821-2.
- HAUSCHKA, T. S. (1958b) Correlation of chromosomal and physiologic changes in tumors. *J. Cell. and Comp. Physiol.* **52**, suppl., pp. 197-233.
- HAUSCHKA, T. S. (1961) The chromosomes in ontogeny and oncogeny. *Cancer Res.* **21**, pp. 957-74.
- HEIDELBERGER, C. (1966) Summary and Interpretations of Symposium Interactions Between Chemical Carcinogenesis and the Living Cell. A. A. Can. Res. Meetings, Denver.
- HESTON, W. L. (1966) The genetic aspects of lung tumors in mice. *Lung Tumors in Animals Third Quadrennial International Conference on Cancer*. Division of Cancer Research, University of Perugia, Italy, pp. xlivi-lvi.
- HOCH-LIGETI, C. (1954) Effect of prolonged feeding of methylcholanthrene to rats kept on a low protein diet. *Cancer Res.* **14**, 749-52.

- HUGHES, G. M., Editor (1964) *Homeostasis and Feedback Mechanisms*. Cambridge University Press. viii + 460 pp.
- JOHANNESEN, W. (1909) *Elemente der exakten Erblichkeitslehre*. G. Fisher, Jena. 516 pp.
- JOHNSON, F. N. and STRONG, L. C. (1963) The effect of maternal age on time of first litters in inbred mice. *J. Gerontol.* **18**, 246-9.
- JOHNSON, F. N. and STRONG, L. C. (1966) Inbreeding, spontaneous lung tumors and maternal age selection in mice. In: *Lung Tumors in Animals*. Division of Cancer Research, Perugia, Italy, pp. 257-72.
- KENNAWAY, E. L. (1924a) On the cancer-production factors in tar. *Brit. Med. J.* **1**, 564-7.
- KENNAWAY, E. L. (1942b) On cancer-producing tars and tar-fractions. *J. Industr. Hyg.* **5**, 462-88.
- KIT, S. (1960) Nucleic acid synthesis in the neoplastic cell and impact of nuclear changes on the biochemistry of tumor tissue: a review. *Cancer Res.* **20**, 1121-48.
- KLEIN, G. (1959) Variation and selection in tumor cell populations. *Proc. Third Canadian Cancer Conf.*, Academic Press, New York, pp. 215-40.
- KLEIN, E. and KLEIN, G. (1959) The use of histocompatibility genes as markers for the study of isoantigenic variation in populations of tumor cells. *Biological Problems of Grafting. Les Congrès et Colloques de l'Université de Liège*-**12**, pp. 1-18.
- KREYBERG, L. (1934) On the genetic factors in the development of benign tar tumors in mice. *Acta. Path. Microbiol. Scand.* **11**, 174-82.
- KREYBERG, L. (1938a) The genetic and constitutional aspects of spontaneous and induced tumors. *A Symposium on Cancer*, University Wisconsin Press, pp. 3-19.
- KREYBERG, L. (1938b) The influence of extrinsic factors on the development of induced tumors in animals. *Acta. Path. Microbiol. Scand. suppl.* **37**, pp. 317-38.
- LEDERBERG, J. (1946) A nutritional concept of cancer. *Science* **104**, p. 428.
- LEDERBERG, J. (1952) Cell genetics and hereditary symbiosis. *Physiol. Reviews* **32**, 403-30.
- LEDERBERG, J. (1958) Genetic approaches to somatic cell variation: Summary comment. *J. Cell. and Comp. Physiol.* **52**, suppl., pp. 383-401.
- LEDERBERG, J. (1959) A view of genetics. *Stanford Medical Bulletin* **17**, pp. 120-32.
- LERNER, I. M. (1954) *Genetic Homeostasis*. Oliver & Boyd, Edinburgh, London. viii + 134 pp.
- LERNER, I. M. and DEMPSTER, E. R. (1951) Attenuation of genetic progress under continued selection in poultry. *Heredity* **5**, 75-94.
- LI, MIN HSIN and GARDNER, W. U. (1947) Experimental studies on the pathogenesis of ovarian tumors in mice. *Cancer Res.* **7**, pp. 549-66.
- LI, MIN HSIN and GARDNER, W. U. (1948) Malignant granulosa-cell tumor in an intrasplenic ovarian graft in a castrated male mouse. *Amer. J. Obs. and Gyn.* **55**, 316-20.
- LI, MIN HSIN and GARDNER, W. U. (1949) Further studies on the pathogenesis of ovarian tumors in mice. *Cancer Res.* **9**, 35-41.
- LI, MIN HSIN, GARDNER, W. U. and KAPLAN, H. S. (1947) Effects of x-ray irradiation on the development of ovarian tumors in intrasplenic grafts in castrated mice. *J. Nat. Cancer Inst.* **8**, 91-98.
- LITTLE, C. C. (1920) Factors influencing the growth of a transplantable tumor in mice. *J. Exper. Zoöl.* **31**, 307-26.

- LITTLE, C. C. (1951) Genetics and the cancer problem. In: *Genetics in the 20th Century*, L. C. DUNN, Editor, Macmillan, New York, New York, pp. 431-72.
- LITTLE, C. C. and STRONG, L. C. (1924) Genetic studies on the transplantation of two adenocarcinomata. *J. Exper. Zoöl.* **41**, 93-114.
- LURIA, S. E. (1958) Mutations of viruses in relation to normal and abnormal cell function. *Annals N.Y. Acad. Sci.* **71**, 1085-91.
- LYNCH, C. J. (1924) Studies on the relation between tumor susceptibility and heredity. *J. Exp. Med.* **39**, 481-95.
- LYNCH, C. J. (1937) Studies on the relation between tumor susceptibility and heredity. VI. Lung tumors in mice with respect to the phenomena of maternal influence. *Amer. J. Cancer* **31**, 77-84.
- MARKELLO, R. (1958) Maternal age selection and chemically induced tumors in mice. *Annals N.Y. Acad. Sci.* **71**, 897-930.
- MORGAN, T. H. (1935) The relation of genetics to physiology and medicine. Nobel Lecture, 4 June 1934. *Scientific Monthly* **41**, 5-18.
- MURRAY, J. A. (1908) Spontaneous cancer in the mouse. Histology, metastasis, transplantability and relations of malignant new growths to spontaneously affected animals. *Sci. Report Imp. Can. Res. Fund* **3**, 69-115.
- OCCAM (ca. 1300-49) "of 2 solutions to a problem choose the simpler."
- ROMAN, L. and STRONG, L. C. (1962) Age, gestation, mortality and litter size in mice. *J. Gerontol.* **17**, 33-39.
- ROUILLER, CH., Editor (1963) *The Liver Morphology, Biochemistry, Physiology*. Academic Press, New York and London, Vol. I, p. 683, Vol. II, p. 673.
- SALK, JONAS (1965) Immunological paradoxes Theoretical considerations in the rejection and retention of grafts, tumors and normal tissue. The Von Pirquet Lecture, presented at: The Eastern Seminar on Allergy, Grossinger, New York, pp. 1-29.
- SANFORD, K. K. (1958) Clonal studies on normal cells and on their neoplastic transformation *in vivo*. *Cancer Res.* **18**, 747-52.
- SANGHVI, L. D. and STRONG, L. C. (1958) Effects of selection on chemically induced tumors in mice. *Annals N.Y. Acad. Sci.* **71**, 839-78.
- SCHMALHAUSEN, I. (1949) *Factors in Evolution*. McGraw-Hill Book Company, Inc., New York, New York. xiv + 327 pp.
- SHAKESPEARE, W. *Henry V*, Act III, Scene 6, line 36.
- SHAKESPEARE, W. "That she (Fortune) is turning, and inconstant, and mutability, and variation." *Complete Concordance or Verbal Index to Words, Phrases and Passage and Dramatic works of Shakespeare with a supplement concordance to the poems*.
- SMITH, J. B., FREEMAN, G., VOGT, M. and DULBECCO, R. (1960) The nucleic acid of polyoma virus. *Virology* **12**, 185-96.
- SMITH, F. W. and STRONG, L. C. (1949) Studies on gastric neoplasia in mice. The histogenesis and influence of some endocrine factors. *J. Nat. Cancer Inst.* **10**, 423-7.
- SNELL, G. D. (1953) The genetics of transplantation. *J. Nat. Cancer Inst.* **14**, 691-704.
- SORIEUL, S. and EPHRUSSI, B. (1961) Karyological demonstration of hybridization of mammalian cells *in vitro*. *Nature* **190**, 653-4.
- SPORN, M. B. (1966) Carcinogenesis and gene action. Symposium "Interaction Between Chemical Carcinogens and the Living Cell." A.A. Can. Res. Meetings, Denver, Colorado, May 1966.
- STRONG, L. C. (1922) A genetic analysis of the factors underlying susceptibility to transplantable tumors. *J. Exper. Zoöl.* **45**, 231-53.
- STRONG, L. C. (1926a) A genetic study of the growth of a transplantable tumor (adenocarcinoma, dBrB). *J. Exper. Zoöl.* **45**, 231-53.

- STRONG, L. C. (1926b) Changes in the reaction potential of a transplantable tumor. *J. Exper. Med.* **43**, 713-24.
- STRONG, L. C. (1926c) On the occurrence of mutations within transplantable neoplasms. *Genetics* **11**, 294-301.
- STRONG, L. C. (1926d) Genetic studies on the nature of cancer. *Amer. Naturalist* **60**, 201-26.
- STRONG, L. C. (1929) Transplantation studies on tumors arising spontaneously in heterozygous individuals. *J. Can. Res.* **13**, 103-15.
- STRONG, L. C. (1935) The genetic appearance of spontaneous carcinoma of the mammary gland in the C₃H mice. *Amer. J. Cancer* **25**, 599-606.
- STRONG, L. C. (1936) Hemoglobin levels in various degrees of susceptibility to spontaneous tumors. *Amer. J. Cancer* **27**, 500-9.
- STRONG, L. C. (1937) The age distribution of 1250 spontaneous mammary carcinoma in female mice of the A strain. *Amer. J. Cancer* **30**, 527-9.
- STRONG, L. C. (1938) Latent period in growth of spontaneous mammary carcinoma in female mice of the A strain. *Arch. Path.* **26**, 814-19.
- STRONG, L. C. (1940a) Chemical studies on the nature of susceptibility to spontaneous carcinoma of the mammary gland in mice. *Yale J. Biol. and Med.* **12**, 255-68.
- STRONG, L. C. (1940b) A genetic analysis of the induction of tumors by methylcholanthrene. I. With a note on the origin of the NH strain of mice. *Amer. J. Cancer* **39**, 347-9.
- STRONG, L. C. (1941a) *Ibid.* II. The influence of spindle cell sarcoma and of carcinoma of the skin upon each other. *Cancer Res.* **1**, 572-4.
- STRONG, L. C. (1942) The origin of some inbred mice. *Cancer Res.* **2**, 531-9.
- STRONG, L. C. (1943) *Ibid.* V. Absence of sex influence when a large dose of a carcinogen is administered. *Arch. Path.* **36**, 58-63.
- STRONG, L. C. (1944a) *Ibid.* VII. Primary carcinoma of the liver following subcutaneous injection of methylcholanthrene. *Arch. Path.* **37**, 131-5.
- STRONG, L. C. (1944b) The influence of methylcholanthrene on the genetic constitutional state of susceptibility to cancer of the stomach in mice. *Rec. Genetics Soc. Amer.* **13**, 39.
- STRONG, L. C. (1945a) *Ibid.* VIII. Two mutations arising in mice following injection of methylcholanthrene. *Arch. Path.* **39**, 232-6.
- STRONG, L. C. (1945b) *Ibid.* IX. Induced and spontaneous adenocarcinomas of the stomach in mice. *J. Nat. Can. Inst.* **5**, 339-62.
- STRONG, L. C. (1945c) *Ibid.* X. Carcinoma of mammary gland following injection of methylcholanthrene into mice of the NHO strain. *Proc. Soc. Exp. Biol. and Med.* **59**, 217-20.
- STRONG, L. C. (1945d) *Ibid.* XI. Germinal mutations and other sudden biological changes following the subcutaneous injection of methylcholanthrene. *Proc. Nat. Acad. Sci.* **31**, 290-3.
- STRONG, L. C. (1946a) *Ibid.* XII. The effects of selection towards resistance. *Yale J. Biol. and Med.* **18**, 145-55.
- STRONG, L. C. (1946b) *Ibid.* XIII. Mutation from brown to black with a concomitant increase of susceptibility to fibrosarcoma. *Yale J. Biol. and Med.* **18**, 359-66.
- STRONG, L. C. (1946c) Mice, men and malignancy. *Minnesota Medicine* **29**, 413-18.
- STRONG, L. C. (1947a) Observations on the genetic nature of gastric cancer in mice. *Surg. Gynecol. and Obs.* **84**, 727-9.
- STRONG, L. C. (1947b) Further observations on the genetic nature of gastric cancer in mice. *J. Nat. Can. Inst.* **7**, 305-8; Discussion, pp. 395-400.

- STRONG, L. C. (1947c) The induction of germinal mutations by chemical means. *Amer. Naturalist* **81**, 50-59.
- STRONG, L. C. (1948a) Conversion of a cancer-resistant to a cancer-susceptible strain of mice by chemical means. *Cancer* **1**, 120-4.
- STRONG, L. C. (1948b) A new influence on chemically induced sarcoma. *Science* **108**, 688-9.
- STRONG, L. C. (1949a) Genetic changes in gastric lesion and fibrosarcoma susceptibilities. *Proc. Soc. Exp. Biol. and Med.* **69**, 521-4.
- STRONG, L. C. (1949b) The induction of mutations by a carcinogen. Proc. 8th International Congress of Genetics, *Hereditas Suppl.* **1**, pp. 486-99.
- STRONG, L. C. (1949c) A new theory of mutation and the origin of cancer. *Yale J. Biol. and Med.* **21**, 293-9.
- STRONG, L. C. (1950a) The control of survival time of mice bearing methylcholanthrene induced fibrosarcoma. *Science* **111**, 381-82.
- STRONG, L. C. (1950b) A sex differential for chemically induced fibrosarcoma associated with litter seriation. *British J. Cancer* **4**, 315-20.
- STRONG, L. C. (1950c) Litter seriation and the invasion of fibrosarcomas in mice. *Yale J. Biol. and Med.* **22**, 303-7.
- STRONG, L. C. (1951a) Susceptibility to fibrosarcomas in 2NHO mice. *Yale J. Biol. and Med.* **24**, 109-15.
- STRONG, L. C. (1951b) Invasiveness of fibrosarcomata in mice. *Proc. Soc. Exp. Biol. and Med.* **78**, 269-71.
- STRONG, L. C. (1951c) Litter seriation phenomena in fibro-sarcoma susceptibility. A contribution to the subject of cancer susceptibility in relation to age. *J. Gerontology* **6**, 340-57.
- STRONG, L. C. (1952) Differences in response among mice of fifteen inbred strains to the subcutaneous injection of methylcholanthrene. *Yale J. Biol. and Med.* **25**, pp. 34-43.
- STRONG, L. C., Editor (1954) Parental age and characteristics of the offspring. *Annals N.Y. Acad. Sci. (Symposium)* **57**, 451-64.
- STRONG, L. C. (1955) The origin of some inbred mice. II. Old techniques and new. *J. Nat. Cancer Inst.* **15**, 1417-26.
- STRONG, L. C. (1957) Biological equilibrium and the origin of cancer. *Science* **125**, 595-6.
- STRONG, L. C. (1958) Genetic concept for the origin of cancer. Historical review. *Annals N.Y. Acad. Sci.* **71**, 839-78.
- STRONG, L. C. (1959) Recent contributions on the genetic concept for the origin of cancer-Studies on the nature of adaptation of the transplantable tumor. *Proc. 3rd Canadian Cancer Research Conference, Honey Harbor, Canada*. Academic Press, pp. 99-134.
- STRONG, L. C. (1962) Mouse No. 46801. *Lavori dell'Istituto di Anatomie e Istologia Patologica dell'Università degli Studi di Perugia* **22**, pp. 73-90.
- STRONG, L. C. (1965a) Continuation of report of progress on Genetic studies of the aging process. Unpublished data.
- STRONG, L. C. (1965b) Biological balance and imbalance and the origin of cancer. 75th birthday celebration of Prof. K. H. Bauer, Heidelberg, Germany.
- STRONG, L. C. (1966a) Balance and imbalance and the origin of cancer. Springer-Verlag, Heidelberg, pp. 25-34.
- STRONG, L. C. (1966b) *Aging and Cancer*. Chapter 4, pp. 53.
- STRONG, L. C. and FRANCIS, L. D. (1937) The blood of female mice (breeders) of cancer-susceptible (A) and cancer-resistant (CBA) strains. *Arch. Path.* **23**, 202-6.

- STRONG, L. C. and FRANCIS, L. D. (1940) Differences in hemoglobin values in the blood of breeder female mice; a comparison between cancer-susceptible and cancer-resistant strains. *Amer. J. Cancer* **38**, 399-403.
- STRONG, L. C. and FULLER, C. A. (1958) Maternal age at the time of first litters in mice. *J. Gerontology* **13**, 236-40.
- STRONG, L. C. and HOLLANDER, W. F. (1951) Spontaneous gastric neoplasia in mice of the Br-S strain: Incidence and genetic linkage tests. *Cancer Res.* **11**, 94-99.
- STRONG, L. C. and JOHNSON, F. N. (1962) Oncology and gerontology—genetic implications. *The Morphological Precursors of Cancer, Division of Cancer Research, Perugia, Italy*, pp. 119-51.
- STRONG, L. C. and JOHNSON, F. N. (1964) Report of progress on continuation of Grant H-2666. Unpublished data.
- STRONG, L. C. and LITTLE, C. C. (1920) Tests for physiological differences in transplantable tumors. *Proc. Soc. Exper. Biol. and Med.* **18**, 45-48.
- STRONG, L. C. and LITTLE, C. C. (1924) Genetic studies on the transplantation of two adenocarcinomata. *J. Exp. Zoöl.* **41**, 93-114.
- STRONG, L. C. and SANGHVI, L. D. (1951) Wirkungen der Auslese auf chemisch erzeugte Tumoren bei Mäusen. *Ztschr. Krebsforsch* **58**, 1-27.
- STRONG, L. C. and SMITH, G. M. (1939) The local induction of carcinoma of the mammary gland by methylcholanthrene. *Yale J. Biol. and Med.* **11**, 589-92.
- STRONG, L. C. and WERNER, T. H. (1936a) Precipitation tests in mice. I. Cancer. II. Comparative study of cancer-susceptible and immune mice. *Amer. J. Can.* **26**, 767-9.
- STRONG, L. C. and WERNER, T. H. (1936b) Precipitation tests in mice. III. A disturbance between two hundred and three hundred days of life. IV. Determinations of mice belonging to an immune-to-cancer stock CBA. *Am. J. Cancer* **27**, 115-19.
- STRONG, L. C. and WILLIAMS, W. L. (1941b) *Ibid.* III. Local and remote inductions of carcinoma of the mammary gland. *Cancer Res.* **1**, 886-90.
- STRONG, L. C., COLLINS, V. J. and DURAND, E. A. (1943) *Ibid.* IV. The probable remote induction of various types of gastric lesions. *Cancer Res.* **3**, 21-28.
- STRONG, L. C., JOHNSON, F. N. and RIMM, A. A. (1965) The effects of fifty-four generations of inbreeding on age of first litters. Selective influences of early maternal age and polydactylism. *J. Gerontology* **20**, 405-9.
- STRONG, L. C., SMITH, G. M. and GARDNER, W. U. (1938) Induction of tumors by 3:4:5:6-dibenzcarbazole in male mice of the CBA strain, which develops spontaneous hepatoma. *Yale J. Biol. and Med.* **10**, 335-46.
- STURTEVANT, A. H. (1965) *A History of Genetics Modern Prospectives in Biology*. Harper & Row, New York. 165 pp.
- TYZZER, E. E. (1916) Tumor immunity. *J. Can. Res.* **1**, 125-55.
- VEST, M. F. and ROSSIER, R. (1963) Detoxification in the newborn: the ability of the newborn infant to form conjugates with glucuronic acid, glycine, acetate and glutathione. *Annals N. Y. Acad. Sci.* **111**, 183-98.
- VLES, F. and UGO, A. (1936) Sur la fluorescence des souris provoquée par les carbures cancérogènes. *International Cancer Congress, Brussels*, 2, pp. 104-5.
- VON HANSEMANN, D. V. (1890) Über asymmetrische Zellteilung in Epithelkrebsen und deren biologische Bedeutung. *Virch. Arch. Path. Anat. Physiol.* **119**, 299-326.
- WHITMAN, WALT (1892) "And a mouse is miracle enough to stagger sextillian of infidels." *Bartlett's Familiar Quotations*, Little.

- WILLIAMS, W. L. and STRONG, L. C. (1944) A genetic analysis of the induction of tumors by methylcholanthrene. VI. Epidermoid carcinoma and associated tumors in mice of the F₄-F₇ generation of the NH descent. *Cancer Res.* **4**, 11-17.
- WINGE, Ö. (1930) Zytologische Untersuchungen über die Natur maligner Tumoren. II. Teerkarzinome bei Mäusen. *Ztschr. Zellforsch u. mikroskop. Anat.* **10**, 683-735.
- WOGLOM, W. H. (1913) *Studies in Cancer and Allied Subjects*. Columbia University Press, New York.
- WOGLOM, W. H. (1919) Virulence or adaptation. *J. Can. Res.* **4**, 1-18.
- WOLF, W., CHAIRMAN (1962) Rhythmic functions in the living system. *Annals N.Y. Acad. Sci.* **98**, 753-1326.
- WOOLLEY, G. W. and LITTLE, C. C. (1945) The incidence of adrenal cortical carcinoma in gonadectomized male mice of the extreme dilution strain. *Can. Res.* **5**, 211-19.
- WRIGHT, S. (1951) The genetical structure of populations. *Ann. Eugenics* **15**, 323-54.
- YAMAGIWA, K. and ICHIKAWA, K. (1915) Über die künstliche Erzeugung von Papillom. *Verhandl. Jap. Path. Ges.* **5**, 142.
- YAMAGIWA, K. and ICHIKAWA, K. (1918) Experimental study of the pathogenesis of carcinoma. *J. Can. Res.* **3**, 1-21.

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