THE CORRELATION BETWEEN CHROMOSOME BEHAVIOUR AND SUSCEPTIBILITY TO MAMMARY GLAND CANCER IN MICE

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CHROMOSOME BEHAVIOUR AND SUSCEPTIBILITY TO MAMMARY GLAND CANCER

IN MICE

by

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TABLE OF CONTENTS

INTRODUCTION

Any survey of the occurrence of neoplastic growth in the animal kingdom brings to light two fundamental facts. The first is that the ability to produce such growths is present in a very wide variety of organisms. Malignant tumours are found in every class of vertebrates and in many invertebrates. The second is that although tumours are known to occur in many different groups, their frequency and kind in each of these is markedly different. The ability to form neoplasms is a probably universal characteristic of organized tissues, but some tissues and some genetic constitutions have greater tendencies towards tumour production than others. This difference in tendency is in itself evidence of an hereditary influence on tumour susceptibility. That such an influence does exist has been amply demonstrated in the case of a number of organisms.

In mankind, such data as exist on the heritable nature of a tendency towards neoplastic growth have been derived from mass population statistics, from individual family pedigrees, and from the behaviour of monozygotic twins. Several investigators have been led to the opinion that certain races, particularly the northern ones, show a cancer frequency appreciably higher than others (cf. Niceforo and Pittard, 1936, who base their conclusions on the behaviour of northern and Mediterranean races in colonial countries). Other authors consider that in some cases at least the statistical differences observed between races are due to differences in environment or custom (Afifi, 1934). Conclusions on such points are necessarily of limited value because of the widespread admixture of races and the different conditions under which people live in various parts of the world. However it is a well-established fact that within a population the

tendency towards tumour formation is limited to a noticeable degree to certain lines of descent. C. C. Little (1923) made an investigation which showed conclusively that cancer is more frequent in families already showing it than in the population in general. The same conclusion was arrived at by Hoffman in an investigation of 100,000 negro inhabitants of the United States Registration Area.

More specific evidence on the inheritance of a cancer tendency has been reported by a number of authors from isolated family trees. It has been summarized by Schinz and Buschke (1935). Allowing always for the undoubted importance of particular environments found in certain occupations, it shows for example that when several instances of a rare type of cancer are found they very often occur in related individuals. In monozygotic twins the time and place of occurrence of tumours in the two members are frequently alike (Bauer, 1932; Schinz, 1936; Militzer, 1935). Interpretations of these pedigrees which attempt to explain the detailed mode of inheritance of the tendency have led for the most part to conflicting results, but this in no way affects the conclusion that in man an inherited susceptibility, at least to certain forms of cancer, does exist, although its mode of operation may be different in different cases. This fact, though not in itself of much direct practical benefit, is nevertheless extremely important since, together with the close histological resemblance of human to animal tumours and their similarity of behaviour, it justifies an attack on the cancer problem through experimental work on laboratory animals. The mode of inheritance of cancer tendency and its <u>modus operandi</u> in some experimental animals may therefore be briefly reviewed.

The only cases of tumours known to be controlled by simple mendelian genes are those found in Drosophila. Two distinct types have been described by Stark (1918 and later). One is due to a sex-linked recessive mutation, lethal Morgan-Stark-1 and therefore affects all males and all homozygous females carrying it. The tumour appears during larval life and the larva dies before pupation, but the gene may cause death of the egg long before the tumour stage is reached. Its manifestation is subject to the modifying influence of its genotypic milieu, as shown by the effect of outcrossing to flies of different constitution. Although it is possible that the effect is due to two very closely linked genes, one a lethal and one producing the tumour, the assumption that a single gene is present is a more plausible one since it is unlikely that two mutations would take place simultaneously.

The other tumour (Stark, 1919) is due to an autosomal gene which appeared in the same line of flies as the first. This Morgan-Stark-II tumour is clinically benign, since the larvae in which it appears later develop into normal flies. Histologically, and from its metastasis, it must be classed as malignant. It is ectodermal in origin and is unspecific as to position. The gene is recessive and has a "penetrance" of only 10%; that is, only 10% of flies homozygous for the gene show its effect. Its action is modified by other autosomal genes.

The general interest of these Drosophila tumours lies in the fact that they constitute the only clear cases showing that a tumour can be controlled by a single gene which represents the sine qua non of tumour formation. It is noteworthy that these genes, even when present, express themselves phenotypically as tumours only when acting under certain genetically controlled conditions.

In mice, the frequency of appearance of spontaneous cancer is higher than in any other species on which data have been collected. (Schinz and Buschke, 1935). This fact and the suitability of the mouse for genetic experiment and laboratory work have made it the best known and most widely used animal for cancer research.

An essential step in the work was the establishment of closely inbred lines having different predispositions to cancer. This was begun by mouse fanciers in the last century and has been greatly extended since. As early as 1904 Miss Lathrop, in Granby, Massachusetts, had laid the foundations of several inbred lines. After this date she continued the selection and inbreeding in conjunction with Loeb. By 1921 they had examined some 12,000 females of cancer age, among them about 3500 bearing mammary gland cancers. Meanwhile many other lines were being built up by Maud Slye (Cancer Laboratory of the S. A. Sprague Memorial Institute, University of Chicago), Clara Lynch (Rockefeller Institute for Medical Research, New York), L. C. Strong (now of the Yale Medical School, New Haven, Connecticut), C. C. Little (now of the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine), and others. Since 1936 more strains and many genetical experiments have been reported by Madame Dobrovolskaia-Zavadskaia, of the Laboratoire Rosenthal, Institut de Radium, Paris.

The records accumulated by these investigators have furnished considerable data on the relative frequency of various kinds of cancer in mice. The general frequency for all kinds of tumours found by Slye was 5000 in 75,000 animals examined: i.e. about 7%. 90% of those found by Slye up to 1916 were mammary adencarcinomata

(compare the case in rats, where the majority of tumours are of connective tissue origin, 20% of them originating in the thymus). Next to these in frequency come adenoma of the liver, tumours of the lungs, of the thymus, and of the ovaries and testes. Occasionally tumours of the uterus or kidneys are found; and in very exceptional circumstances, intestinal cancers. Epithelial tumours are by far the most frequent. Among 12,000 autopsied mice Slye (1917) found only 87 sarcomata. The work of other authors leads to the same general conclusion, although there is some indication that the frequency of internal tumours may be somewhat higher than had previously been suggested.

Dr. C. C. Little has developed a large stock both of his own inbred lines and of some of those originated by L. C. Strong, and made them available for purchase. These lines are now being used in a number of laboratories both in America and in Europe. Brother-to-sister matings continued over a large number of generations, together with selection of the progeny of those individuals bearing mammary cancers, or entirely free from them, has ensured the relative homozygosity of these stocks. (Jennings, 1916; Haldane, 1936 and 1937, q. v.) The result is that among the stocks now generally available there are some in which every breeding female living to the end of the cancer age develops mammary gland cancer, and others in which no cancer is found. It is safe to assume that the susceptible strains are genetically pure for those genes whose presence is necessary for cancer production, but the selection of a strain completely lacking susceptibility factors is of course more difficult. It is clear that the method of inheritance of cancer susceptibility is not a simple one, and the possibility is always present

that the so-called "resistant" strains possess cancer-permitting genes which have no phenotypic effect (since other necessary genes are lacking) and which are therefore not eliminated by selection. For this reason hybridization experiments for the purpose of determining the method of cancer inheritance have so far led to no conclusive results on the questions they were designed to answer. Nevertheless a large amount of data has been collected, and many investigators have drawn detailed conclusions as to the number and behaviour of genes involved. The fact that the majority of these conclusions are contradictory indicates that the method of inheritance cannot be explained by the assumption of a small number of mendelizing genes. Although the exact manner of transmission has not been elucidated, the experiments of Lynch, Slye, Little, Dobrovolskaia-Zavadskaia and many others have brought to light certain fundamental points. It is clear, for instance, that all kinds of cancer occurring in mice are not controlled by the same factors nor inherited in the same way. Furthermore, a strain with low cancer frequency may carry genes for cancer which causes hybrids to show a higher frequency than that of the susceptible parent strain (Cloudman and Little, 1936; Murray and Little 1936). Probably most "resistant" mice have at least some such factors. Furthermore the importance of the age incidence of cancer must not be overlooked in breeding experiments since factors which influence longevity or viability necessarily affect those forms of cancer which appear at advanced ages. In fact, the age factor has probably been a

large source of error in much of this work since absolute frequencies of spontaneous tumours can be compared only within well-defined age groups. This point was discussed and made particularly clear by Korteweg (1936a).

Probably the most important discovery which has arisen from hybridization experiments is that of C. C. Little (1933b and later) that breast cancer in mice is inherited to a very large extent maternally. In reciprocal crosses of a high tumour strain with a resistant one, the progeny of susceptible mothers showed a much higher frequency of mammary gland cancer than did the progeny of the resistant mothers. This finding represents a great step forward in our knowledge of cancer inheritance. As Little concluded, it indicates the presence of non-chromosomal factors in the inheritance of this type of cancer, though until further experiments had been carried out the possibility of a maternal effect due to the delayed action of genes could not be eliminated. The results of reciprocal crosses between the low-tumour line C 57 Black and the susceptible line dba, reported by Murray and Little (1935 and 1936) led the authors to state that genic effects are relatively unimportant. The incidence of breast cancer was 39.82% in virgin female hybrids from the dba mother, and 6.06% in those from the C57 Black mother. This difference persisted into the F2 generation. Little's results have been confirmed beyond question by his own further work (Murray and Little, (1935a and b)) and that of his colleagues, and by others (Korteweg, 1936b). Hagedoorn (1937a),

on the other hand, believed that the extra-chromosomal theory was unnecessary, and that the differences observed by Little in the reciprocal hybrids could be explained by the poor nutrition of embryos and young by genotypically aberrant mothers (cf. Hagedoorn, 1937b).

More recently Bittner (1936f and 1937) has discovered that the extra-chromosomal influence is transmitted, at least in part, through the mother's milk. He found that young mice of a resistant strain fostered from birth on a female of a susceptible strain show significantly more cancer than their unfostered sisters. The reverse is also true. He concludes that these differences are sufficient to account for the whole of Little's observations on reciprocal hybrid groups. His results for breeding females are shown in the following table (from Bittner, 1937).

STRAIN	NURSED BY	NUMBER OF MICE	% WITH BREAST TUMOURS
A	A ♀	788	83.8
A	C57 Black ♀	45	4.4
C57 Black	C57 Black ♀	386	1.0
C57 Black	A ♀	8	37.5

Bittner's work also shows that if hybrids from a resistant mother and a susceptible father are fostered on a female of

the father's strain, their mammary cancer incidence increases. Mutatis mutandis, the reverse may also be true, but as yet the experiment has not gone far enough to furnish conclusive evidence on this point. Bittner and Little (1937) also have evidence to show that a diminishing of susceptibility can be brought about by transplanting fertilized eggs into the uterus of resistant females.

These experiments leave no room for doubt that the mother's milk is a very important factor in determining whether or not breast cancer will later make its appearance. It is of course well known that maternal milk carries with it, apart from nourishment, many factors of physiological importance. Hain (1936) has shown that oestrone injected into lactating rats affects the young suckling females. The concentration of oestrone reached is apparently more important than the duration of its activity. Presumably the agent affecting cancer development which is transmitted through the milk is in the nature of a hormone, but no analytical work on this point has as yet been reported.

The most frequently occurring spontaneous tumours in mice after mammary adenocarcinoma are those of the lung. In males they are the most common of all tumours, and occur especially in males of strains the females of which show a high percentage of mammary cancer (v. Bittner, 1936a). This fact, however, does not necessarily indicate that both are controlled by the same genetic factors since two of the most susceptible mammary tumour lines (A and C3H) are derived from a strain which shows high lung tumour frequency (the Bagg Albino).

In 1907 Tyzzer had already started selecting for lung cancer susceptibility. In 1914 Slye reported that the frequency of primary lung tumours among 6000 autopsied mice was 2.7%. Since that time Slye and others have developed a number of strains differing greatly in their lung tumour incidence (5.9 % in strain 1194 agouti as compared with 66.4 % in the D albino strain: Lynch, 1937).

Susceptibility to tumours of this kind after tar painting is apparently not transmitted maternally, as concluded from Lynch's recent crosses (1937), and from Bittner's work (1936d). In fact there is no evidence that any tumours other than epithelial mammary gland ones are subject to such control (v. Cloudman, 1936; Murray and Little, 1936). There is, however, a clear maternal effect upon the incidence of leukaemia and upon longevity in mice (MacDowell, 1935), although these two factors are causally unrelated.

Though genetic factors leading to tumour formation may be present in a given mouse line, they can act only under certain physiological conditions. In mammary gland cancer a controlling factor is the activity of the female sex hormones. Few breast tumours appear, even in the most susceptible strains, in males or, as a rule, in virgin females. Lacassagne (v. infra) has demonstrated that this is related to the lower oestrone level in such animals. For this reason the incidence of spontaneous mammary gland cancer in a strain is usually quoted as the percentage of breeding females developing it.

The second major endogenous factor affecting the appearance of a given type of tumour is age. The average age at onset of breast cancer in various lines of mice is characteristic for the strain, and ranges from about 8 months in C3H to 11-12 months in dba and still later in other

strains showing lower frequencies. Internal tumours appear later, particularly the rarer forms. It is perhaps significant that the strain which shows most internal tumours (CBA) was selected over a number of years for longevity. Mice of this strain develop tumours at an age beyond the life span of most inbred mice. It is probable that its higher internal cancer record depends entirely on this fact. It has a low frequency of mammary gland tumours.

The question arises as to whether all differences between strains with regard to cancer behaviour might not be attributed to differences in some such characteristic as age at puberty, rate of breeding, or fertility. It may be that there is some necessary threshold of activity which resistant mice never reach but which susceptible ones habitually pass. H. J. Bagg (1936) has designed a "functional test" to examine this possibility. By rapid breeding and removal of young soon after birth, Bagg claims that he has lowered the threshold so that tendencies towards spontaneous cancer have been exposed in otherwise "resistant" strains. This is the case in C57 Black, described by Little as showing "during the last ten years....no recorded incidence of carcinoma of the breast" (Roscoe B. Jackson leaflet sent out in 1935). Bagg and Jacksen (1937) find about 15% of mammary tumours in tested females of this strain, and these tend to be localized in certain families.

Fekete and Green (1936) have shown that production of cancer is stimulated not by the nursing of young but by the activity of the mammary gland itself and probably by the collection and stagnation in the gland of some constituent of the milk. After sealing the mammae of one side of

the body in a number of females and allowing them to nurse their young, they found many more tumours on the sealed side than on the unsealed. Their conclusion is in agreement with the behaviour of mice injected with large doses of oestrin, which indicates that the stimulation of the mammary glands by ovarian hormone is important in the development of mammary cancer. Suntzeff, Burns, Moskop and Loeb (1936) concluded from a study of the effect of oestrin injections in mice of several strains that in susceptible females a large dose both lowers the age of incidence and increases the frequency of spontaneous mammary cancer. Males of these strains develop mammary tumours after oestrin treatment at least as readily as do non-breeding females. They point out that in some strains there is much cancer in breeding females and none in non-breeding (Murray, 1937, suggests that this may have some connection with the luteal phase of pregnancy) while in other strains there is a considerable incidence in non-breeding females, though never as great as in the breeding ones. In the former type of strain oestrin treatment of non-breeders raises their breast cancer frequency only to the level of that of the breeders. Breeding mice of high tumour strains do not show an appreciable increase with oestrin injection. The conclusion drawn is that breast cancer is due to oestrin acting on mammary tissue having an inherited irritability towards it. The reaction of other female tissues (vagina and cervix) to oestrin does not indicate the presence of inherited differences as clearly as does that of mammary tissue (Suntzeff, et al., 1938.)

Gardner, Smith, Allen and Strong (1936) have shown that given suitable genetic constitution, very large doses of oestrin (10,000 to 18,000 international units of keto-oestrin benzoate in oil, injected in weekly doses of 500 units) and a sufficiently long time of reaction (up to 362 days after completion of dosage), mammary gland carcinoma will develop in probably 100% of male mice. Lacassagne (1934) believes that differences in the behaviour of different strains is only one of speed of reaction (cf. Lacassagne, 1933) and that a high frequency of tumours can be obtained even in resistant mice if sufficient time is allowed. Nevertheless different strains react differently to a given amount of the hormone (Loeb et al.,1937). But the simple assumption that a faster natural output of oestrin in susceptible strains will account for their higher cancer frequency does not suffice. Burns, Moskop, Suntzeff and Loeb (1936) find that ten inbred lines indicate no relation between the sex cycle differences which they show and their differences of spontaneous mammary cancer incidence. Also the development of the mammary glands in young females of different strains shows nothing which could suggest a recognizably precancerous condition (Gardner and Strong, 1935).

Cramer and Horning (1938) have reported evidence which suggests that an important factor in the production of mammary cancer is an upset in the endocrine balance between the ovary and pituitary. They found that treatment just before puberty of females of the R-III strain of Dobrovolskaia-Zavadskaia (which normally shows about 60% mammary cancer at 7-9 months) with thyrotropic hormone is followed by an absence of tumour development even in animals well past the cancer age. The treatment also causes sterility, but 50% of untreated

sterile females normally show tumours at the same average age as fertile ones. The authors (Cramer and Horning, 1937) find that strain R-III shows as a spontaneous occurrence the "brown degeneration" of the adrenals which follows oestrin injection in other mice (cf. Dobrovolskaia-Zavadskaia, 1937), and are exceptionally sensitive to oestrin.

Pertinent to this discussion is the fact that carcinogenic and oestrogenic compounds are chemically closely related. They are both characterized by the presence of the anthracene system with rings at the 1:2 and 5:6 positions. However, carcinogenic and oestrogenic activity do not necessarily coincide. For example, while 1:2-benzpyrene and 1:2-dibenzanthracene are oestrogenic, 1:2:5:6-dibenzanthracene is not (Loeb, 1935). Lacassagne (1936a) points out that many of the commonest forms of cancer are associated with glands in which hormones are allowed to collect, and suggests that on stagnation some hormones, notably oestrin, may form a carcinogenic agent. This view is particularly interesting in the light of the later work of Fekete and Green (v. supra).

The work of Strong and Bittner on the incidence of mammary cancer in mice kept on various types of food suggests that diet may have some effect on spontaneous cancer development or at least on the age at which it appears. Bittner (1935b) reported an increase in tumour incidence in strain A females following a change in diet from Rolled Oats to Purina Fox Chow. More recently Strong's results (1938a and b)have indicated

that only longevity is affected, although no definite conclusions can as yet be drawn. This work was done on strain CBA, where tumours appear in any case only at advanced ages, and are of many different types, mostly internal. Possibly other strains react differently. Cameron and Meltzer (1937) claim that certain diets cause a difference in the time of onset of tar-induced tumours, though not in their ultimate frequency.

In 1932 Strong suggested that oil of gaultheria added to the diet of susceptible mice brings about a delay and lessening in frequency of mammary tumours. His subsequent results on the same strain (dilute brown) made this suggestion appear invalid (Strong 1934a), and he concluded that the effect was really on longevity. Later he started work on strain A, using oil of allspice (1935a) and oil of thyme (1935e). In the following year (1936i) he reported that natural oil of wintergreen (gaultheria) has an effect on the growth rate of the tumour and on the survival time if given to young mice over a period of weeks before malignancy appears. Synthesized oil had no effect. By distillation of the natural oil Strong has recently determined (1938c) what part is active in this respect. The active fraction, when added to the diet, caused slowing of the growth rate of established tumours and complete regression in 4 out of 34 cases. This is remarkable since the mammary gland tumours of strain A are of exceptional malignancy and under usual conditions always prove fatal within a short space of time.

Other diet changes, both of a general kind and of specific elements, are known to have an effect on established tumours of whatever origin, but evidence that a real control by this means

can be exerted on subsequent spontaneous tumour appearance has yet to be provided. Where diet does seem to have an effect it is most probably a secondary one.

Whether inherited tendencies have any fundamental influence on the reaction of tissues to carcinogenic agents is a matter of some controversy. The evidence indicates that they are important in some instances at least. Andervont (1937a) found that by injection of young mice with 1:2:5:6-dibenzanthracene he could induce a much higher percentage of lung cancer in strain A (which shows a high spontaneous frequency at a later age) than in any other inbred strain. When crossed with a resistant strain (C57 Black) this susceptibility seemed to be inherited in a dominant manner, and was unrelated to colour, sex or maternity. In general Andervont (1935a) found strains with high spontaneous frequencies to be more susceptible to dibenzanthracene than other strains.

Rheinhard and Candee (1932) concluded from tar experiments on strains with different spontaneous cancer rates that if any difference between the strains exists, it is only one of speed of reaction. Injection of dibenzanthracene into older mice shows (Andervont, 1935a) that spontaneously non-susceptible strains may in later life react to this compound with the formation of neoplasms at the site of injection. The genetic constitution of even the most resistant mouse does not prevent its cells undergoing the change to malignancy if suitable treatment and sufficient time be afforded (Andervont, 1934a).

On the other hand, Kreyberg (1935) finds the opposite

of Andervont's correlation. Of two lines of an inbred albino strain, the one which had been selected towards low frequency of spontaneous mammary carcinoma showed earlier and more frequent tar cancers than the high spontaneous frequency line. Dobrovolskaia-Zavadskaia (1936), working on her own inbred lines with minimal doses of 1:2:5:6-dibenzanthracene, found no correlation between spontaneous cancer record and reaction to injection. The same situation was reported by Lynch (1925) for two lines of mice treated with tar. Later work of Lynch (1927) proved that the organ disposition to cancers induced by tar painting is heritable, and that the reaction of one organ to treatment is independent of that of other organs. (Lynch, 1933)

Curtis, Dunning and Bullock (1933, 1934 a and b) have shown that in rat sarcomata induced by the presence of cysts of Cysticercus the important factor is the duration of the irritation. Hereditary factors apparently have an effect only in so far as they influence longevity and susceptibility to cysticercus infection. In dibenzanthracene- and benzpyrene-induced tumours in mice the probability of an individual's suffering a malignant change is not influenced by sex, age or heredity, though the latter two factors affect the speed of reaction (Dunning, Curtis and Bullock, 1936).

It is worthy of note that carcinogenic agents have been found to give rise only to those types of tumour already known to occur spontaneously in the species. This indicates that

induced and spontaneous malignancies are products of the same disturbance, and that the carcinogenic agent acts simply by stimulating or augmenting some process which occurs naturally, but which, in an unfavourable endogenous environment, would of itself come to nothing. The endogenous environment is ultimately under genetic control.

Studies on the behaviour of transplanted tumours have been carried on since the first really successful transplantation was made by Hanau in 1889. The large amount of work which has been done has shown that the success of a transplant, with the exception of certain heterotransplantable forms, depends primarily upon the similarity of the genotypes of donor and recipient. Loeb and Wright (1927) concluded that the strength of reaction of the host against the transplant is determined by the number of genes foreign to the host which are present in the fragment of tissue implanted. This statement is well substantiated, although genetic constitution has by no means an unalterable effect. The influence of the host both on the initial "take" and on the subsequest growth behaviour of a transplanted tumour is strongly affected by its age (particularly its pre- or post-pubertous condition), its endocrine balance, and whether or not it has borne or is bearing a tumour or has previously been inoculated with the same tumour or other fast-growing tissue. Certain treatments may also have an effect. Only with elimination of differences in these respects can consistent results be obtained in transplantation experiments; and similarly, only in genetically

homogeneous material can the importance of other factors be estimated.

With the establishment of inbred cancer and non-cancer lines of mice the problem of possible histological and physiological differences associated with a pre-cancerous condition was opened to attack. The comparison of normal susceptible with normal resistant animals is important because any differences which may be found add to our knowledge of the origin of cancer and the manifestations of the genes controlling it, and because the establishment of a recognizable criterion of pre-cancerous conditions would be of great clinical value. On the whole the work done along these lines has not as yet been of much theoretical value, although many descriptive facts about the strains have been collected. Gardner and Strong (1935) examined the developing mammary glands of young females of ten different strains. They found no anatomical or pathological peculiarities specifically correlated with the mammary cancer behaviour of the strain, although abnormalities such as retardation of failure of growth of one or more glands were more frequent in susceptible than in resistant females. Davis (1937) in examining biochemical differences between normal mice of different strains found that susceptible animals consume less oxygen, have lower haemoglobin amounts and lower red cell count, larger livers, more calcium in the soft tissues and a more alkaline pH than resistant mice. He does not state what strains he examined. Barry and Kennaway (1937) failed to find any differences in the thyroid reaction of mice

of different strains to skin application of oestrone, benzene or alcohol. Little (1934 a) crossed a dilute brown susceptible strain (dba) with a resistant yellow one and examined the yellow and non-yellow segregates in F-1 and F-2 for mammary cancer. Although he found significantly less cancer in yellows than non-yellows, he concluded that this was not due to genetic linkage between genes controlling cancer and the gene at the A locus (controlling coat colour), but to a more complicated physiological correlation, since the two groups have different rates of development, and the tumours of the yellow mice, though rarer, were earlier and just as malignant as those of the non-yellows. Cloudman and Little (1936) found no correlation between mammary cancer and the gene "T" for brachyury.

Strong (1936e) reports that there is a lower volume of precipitable haemoglobin in the blood of susceptible mice than in that of resistant ones (cf. the work of Casey (1927) on rabbit breeds susceptible to the Brown-Pearce tumour). He obtained this correlation in eight distince inbred lines. A more detailed examination of one susceptible line (A) and one resistant one (CBA) (Strong, 1936 c and d; Strong and Francis, 1937) revealed that in strain A there is a precocious drop in haemoglobin level between 200 and 300 days of age, after which it continues to fall, whereas in CBA there is a steady rise in level from 100 to 500 days' age. There is evidently a fundamental difference between the strains which is correlated with mammary gland cancer.

Sinay (1936) finds certain changes in the rate of metabolism of liver, muscle and kidney tissues in mice bearing tumours.

With transplanted tumours these changes become more marked as the tumour grows: with spontaneous tumours they appear later than the morphological development of the tumour. They may be considered secondary effects, and of no value in diagnosing a precancerous condition.

Cramer and Horning (1938) find regular degeneration of the adrenals in the R-III stock of Dobrovolskaia-Zavadskaia, which they find particularly susceptible to the action of oestrin. Dobrovolskaia-Zavadskaia (1937) also finds abnormality of the adrenals in this strain, though in only about half the individuals and with considerably greater frequency in females than in males. The condition is common in many pathological states other than cancer, and its relation, if any, to malignancy is not clear.

A significant difference in the bioelectric properties of mice of resistant and susceptible strains has been reported by Burr, Smith and Strong (1938). For axial measurements on the ventral side of the body, strain CBA (resistant) mice have much higher potential differences than strain A (susceptible). Across the groins, the potential difference for A is higher than for CBA. The electric pattern varies with age. Mice bearing tumours show a characteristic potential gradient pattern between the tumour and the normal areas, especially when the tumour first appears. The significance of these results is not known.

In spontaneous leukaemia, to which a tendency is also inherited (MacDowell and Richter, 1935) some work has been done on the metabolism of normal lymph nodes and on preleukaemic changes.

Victor and Potter (1935 a) made a comparison of the glycolysis rates of lymph nodes of young normal and older leukaemic mice of the highly susceptible strain C58. They found that the rate of oxygen consumption and glycolysis is higher in lymph nodes of leukaemic than of normal mice. In work on the normal lymph nodes of five different strains comprising C58 and four resistant ones, Victor and Potter (1935 b) found that the higher aerobic and anaerobic glycolytic rates found in older mice were peculiar to this strain. In other strains these rates decrease with advancing age. This means that before any morphological or cytological sign of malignancy appears, there has already been an alteration in the metabolism of the tissues which will produce it. Potter and MacDowell (1936) later reported observations on perivascular reticular cells of strain C58. The first disturbance observable in preleukaemic mice was a hyperplasia of these cells, with a loss of syncytial arrangement and an increase in number of free undifferentiated cell types, until finally typical lymphoid cells were seen to divide. This condition is not found in non-leukaemic strains.

The work of Huskins and Hearne (1936), of which the present is a continuation and extension, shows a significant difference in chiasma frequency between certain strains susceptible and resistant to spontaneous breast cancer.

The foregoing brief review, which only touches on the fast-accumulating literature on this subject, is sufficient to show that genetic factors are important in the occurrence of malignancy,

although the expression of the inherited tendencies is highly modifiable by environmental factors, both exogenous and endogenous, and little is known of the genetic mechanism involved. It is furthermore clear that there are recognizable differences between normal individuals having an inherited cancer susceptibility and those not having it. These two conclusions are fundamental to the present attack on the cancer problem.

The distinction, and the dangerousness, of malignant tissue lies in its property of unlimited growth and consequent invasion of surrounding structures. There is much to indicate that this characteristic is one of individual malignant cells rather than of the tissue as a whole. Tumours can be propagated for many generations by successive transplantation of small fragments, and will still retain their original characteristics of growth behaviour and histological picture. Furth, Kahn and Breedis (1937) proved that a strain of leukaemia can be transplanted by the inoculation of a single malignant cell. These facts point to the conclusion that malignancy is a property of single cells which have undergone a specific irreversible change inherited by all daughter cells derived from them.

The somatic cell mutation hypothesis of the origin of cancer postulates that cancerous growths arise from a single cell which has become malignant through some form of genetic mutation. If this term (i.e. genetic mutation) is taken in its broadest sense, and the exact nature of the change is not too closely specified, the hypothesis fits very well with much of what is known of malignancy from both observational and experimental evidence. Like gene mutation, the malignant change is a specific one. It occurs more frequently against some genetic backgrounds than others, and with constant frequency under constant genetic and environmental conditions. It can be induced by certain external agencies, including some known to produce gene mutation, and it reacts quantitatively, not qualitatively, to these treatments. It may be progressive, since cells already malignant sometimes undergo a further alteration which shows up as

a difference in growth energy or transplantability (Putnoky, 1938; Strong, 1926b; Strong and Hill, 1937).

Mutation of known genes is a familiar occurrence in somatic tissue and has been analyzed and described in a number of cases in plants and animals (in the latter, particularly in Drosophila). With regard to genes associated with cancerous growth, Strong (1926 c) gave experimental proof that changes of a heritable nature (in the sense of cell generations) may take place in somatic cells. He transplanted two tumours which originated in the same pure-line mouse into a number of other mice, some of which were refractory to them and others not. By selection he established familes, one susceptible to one tumour and refractory to the other, one susceptible to both, and one refractory to both. Since both tumours were always transplanted into each host, the fact that such lines could be developed proved that the tumours were of different constitutions and therefore that at least one was different from the animal which produced them. Actually, both were different, since neither reacted in the same way as the normal cells of the original animal.

An experiment of the same kind was described by Cloudman (1932) who found that three mammary tumours which arose in a single mouse differed in the genetic factors which they required in the host for successful growth, and were therefore physiologically and genetically dissimilar.

Since the time of its inception the somatic cell mutation theory has served as a working hypothesis for a number of lines of investigation on the problem of the origin of cancer. Although by no

means universally supported, it has received the endorsement of many distinguished authors and has the virtue of accounting for the observed facts more fully than any other single hypothesis. It has been suggested that the change in the cell is one of chromosome constitution. Boveri (1914) considered that the cause of malignancy lay in the loss of chromosomes bearing genes necessary for normal growth. This conception has since been supported by Winge (1930). It has, however, been amply shown that many malignant cells have normal chromosome numbers (v. Goldschmidt and Fischer, 1929; Levine, 1931), and that benign tumours, inflammatory tissues, and probably many physiologically normal cells (apart from tissues regularly showing polyploid or non-polyploid deviations : v. Belar, 1928) may exhibit gross variations from the normal chromosome constitution (Lewis, M.R. and Strong, 1934; Potter and Richter, 1932; Andres and Jiv, 1936; Schiwago and Paschlowskaia, 1936; Caffier, 1931; Karplus, 1929: contrast Kemp, 1930; Mendelsohn, 1935). These findings preclude Boveri's hypothesis. Malignancy, at least in those cases so far investigated, is clearly not determined by any visible change in the chromosomes. Where such changes are present they are more probably one of the results of the malignant condition.

The strongest objections to the somatic cell mutation theory have arisen out of the discovery that in fowl tumours exist which can be propagated by cell-free filtrates and hence are presumably caused by a virus. One mammalian tumour has been found which behaves in a similar way (Shope, 1932). To explain these cases on the somatic cell mutation theory subsidiary assumptions must be made. A possible one is

that the virus (or other active agent of the tumour filtrate) is capable of producing a specific mutation in normal cells. In this case there must be several different viruses and several different specific mutations, since the filterable tumour types known reproduce themselves specifically. A second possible assumption is that in the case of each filterable tumour line a mutation originally gave rise to the virus. In this connection it may be pointed out that the somatic cell mutation theory does not preclude an ultramicroscopic virus or an enzyme as the immediate cause of malignancy. Indeed, if a mutation is involved there must be some intermediate step or steps which connect it with its phenotypic effect in altering the growth behaviour of the cell. The discovery that in some cases such an active agent can be shown to be responsible therefore does not mean that the somatic mutation hypothesis must be discarded.

It has been argued that if malignancy is due to a genic mutation in a somatic cell, then this mutant must be dominant to its normal allelomorph since it would otherwise have no phenotypic effect. The malignant "mutation" may of course be a dominant one (dominant mutations predominate in fowl and are common in man) but even if it is recessive the objection does not necessarily hold since it is known that an originally heterozygous gene can become homozygous in patches of somatic tissue, and thus express itself phenotypically even though recessive. One mechanism by which this can occur is through somatic crossing-over in two strands of a four strand group of chromatids (Stern, 1936; Jones, 1936). Somatic crossing-over merits particular consideration in this respect since the only other known mechanism by

which a recessive mutant gene could show itself is through loss of a chromosome or part of a chromosome carrying the normal allelomorph, and this, unless the lost part were small, would necessarily lead to a visible alteration in the chromosome constitution of malignant cells.

The idea that cancer cells arise through somatic mutation is easily reconciled with the heritable nature of cancer susceptibility. That all susceptible genotypes do not express themselves in progressive malignant growth is apparent from the behaviour of both experimental animals and man. It has been suggested that malignant changes in cells take place very much more frequently than is supposed, but that certain organisms and tissues possess some kind of protective mechanism against neoplastic growth. In this case the appearance of cancer would be due to an inherent defect in the protective mechanism. This point of view explains particularly well some facts which appear in genetic and induced resistance to transplanted tumours.

An alternative possibility is that inherited cancer susceptibility is due to a mutability factor leading to somatic mutation under certain conditions, while resistance is a lack of mutability. Such factors are known to exist (v. Demerec, 1929).

There is some evidence (particularly from the effects of X-radiation on slow and fast-growing tissues) that mutation occurs predominantly during the process of cell division. If so, the frequency of mutation in a tissue should be proportional to the number of mitoses taking place in it. This affords a possible explanation of the fact that malignancy very often originates in regenerating or reparative tissues, and that some carcinogenic agents also stimulate growth

(v. Lits, 1936; Dodge and Dodge, 1937; Creech, 1938). This possibility is worthy of consideration since the prevalence of chromosomal irregularity in regenerative tissue and the cytological upsets caused by many carcinogenic agents have been interpreted by some authors as an indication that abnormality of chromosome constitution is fundamentally related to malignancy.

The question arises as to how malignant cells differ from normal cells. The fundamental distinction is one of growth behaviour. A number of authors have claimed that they have found some morphological or physiological characteristic specific to cancer cells, but in few cases has such a claim been substantiated by the work of others. MacCarty (1927 et seq) believes that the nucleolus has a larger volume proportionate to the volume of the cell in malignant than in normal cells, and that intranucleolar bodies are more numerous (Page, Regan and MacCarty, 1938). These conclusions are based on a study of fresh unfixed tissues of various malignant and non-malignant types. However Guttman and Halperin (1935) find that although hyperplastic tissue in general tends to show larger nucleoli than normal, there is no difference in this respect between malignant and non-malignant cells.

Brues and Masters (1936) find no difference between fibroblasts of rat and chick embryo heart and of the Walker rat tumour 256 in their permeability to water. Malignant cells are however apparently more permeable than others to fat-soluble substances (Ludford, 1934a). They show greater basophilic properties than normal epithelial cells (v. Andres, 1932) and, in tissue culture, they are known to liquefy the clot more readily and with greater speed than normal tissues (Fischer,

1932), and to be less resistant to changes of temperature and other environmental conditions (Fischer, 1929; Lambert, 1912). While there are a number of more or less well-defined characteristics combined (see Ludford, 1933; Mendelsohn, 1934; Lewis, W.H., 1935) which make the malignant cell recognizable to the practised eye, there is no single qualitative characteristic peculiar to it. Those quantitative ones which do exist can most reasonably be explained as results rather than causes of malignancy.

A number of authors have noted that dividing cells of malignant growths very often show chromosomes which are widely split at metaphase before the anaphase separation of halves has begun. Precocious splitting of chromosomes has not been described in the normal tissue of mammals, except in a few isolated cases, such as the amnion of rodents examined by Painter (1924) and considered by him to be cytologically abnormal, or at any rate atypical. Crew and Koller (1932), describing the normal mitotic chromosomes of mice, state that the split is not visible until separation of the halves takes place at the beginning of anaphase. This remark is borne out by the figures of Masui (1923), Minouchi (1928, a and b), Painter (1924, 1928), Cross (1931) Cutright (1932) and Butarin (1935). In malignant tissue, on the other hand, the phenomenon of early splitting, although by no means a universal attribute, is sufficiently striking (v. Ludford, 1930; Lewis, M.R., 1932, a and b; Lewis, M.R. and Lewis, W.H., 1932; Potter and Richter, 1932 and 1933) to suggest that there may be a correlation between it and the growth behaviour of cancer cells.

While the spindle, whatever its structure or origin, has the obvious function of separating the daughter halves of the chromosomes at anaphase and ensuring their arrival at the poles, the formation of the halves by splitting is apparently an autonomous function of the chromosome or is at any rate unrelated to spindle action. This is the view subscribed to by Belar (1933), Ludford (1930), Andres (1932), Darlington (1936), and others. For normal division, chromosome splitting and spindle formation must be co-ordinated in time. If splitting takes place, but the halves do not go to the poles due to lateness or failure of the spindle to function, then the split chromosomes lie in the cytoplasm and give, in extreme cases, the appearance of tetraploidy. Indeed, the term "precocity of splitting" necessarily means a precocity in relation to the other processes of division, and can probably be accurately replaced by the term "lateness of anaphase". There is little doubt that many polyploid somatic cells, especially in tumour tissues, arise through the formation of a single restitution nucleus after failure of the spindle to function. That this process sometimes takes place as a regular and normal occurence is indicated by the work of Berger (1937). Experimental arrest of mitosis at metaphase can be brought about by ether and ammonia (Rosenfeld, 1932), sodium cacodylate (Ludford, 1936), colchichine (Ludford, 1936; Blakeslee and Avery, 1937; Nebel, 1937; Brues and Cohen, 1936; Brues and Jackson, 1937; Nebel and Ruttle, 1938), weak acid, heat, bichloride of mercury, and alchohol (Lewis, M.R., 1933), pressure (Ellenhorn, 1933), and other substances including nicotine, caffeine and hypertonic sugar solution followed by water (Shiginaga, 1937). All these agents act on the spindle and cause apolarity of the cell, and their action, if not too severe, is often followed by the restitution of a

hyperploid nucleus.

Whatever the connection may be between the occurence of precociously split chromosomes and malignancy, it is clear that their presence in a cell indicates that some derangement of the normal division mechanism is at hand which might well be expected to lead to more serious cytological abnormalities.

In cytological literature there are a number of instances known where the processes of cell division and chromosome behaviour at certain stages of the life history are under the control of well-defined genes. Such a case is that in maize described by Beadle (1929 and 1931) where a single recessive gene causes a number of supernumerary divisions of the tetrad without splitting of the chromosomes, and the consequent production of sub-haploid cells. Homozygotic plants are pollen-sterile. Beadle (1932) has also described a simple recessive gene in maize causing "sticky" chromosomes, with consequent increase in non-disjunction and translocation in both somatic and meiotic divisions.

Again in maize, a case has been found (McClintock, 1937) where the loss of certain genes in patches of somatic tissue due to the behaviour at division of a ring chromosome results in a number of changes of growth capacity, among them an excessive proliferation of cells at the surface of the affected area.

Further, it is well known that chromosome length, behaviour at synapsis, and frequency of chiasma formation are under genic control. It is therefore not unreasonable to suppose that time of splitting may be subject to the same sort of regulation as other

kinds of chromosome behaviour. The assumption that this is so was made by Huskins and Hearne (1936). They made the hypothesis that if early chromosome splitting is associated with the appearance of malignant growth, and is controlled by hereditary factors, then normal dividing cells of an organism susceptible to malignancy should show earlier chromosome splitting than those of a resistant organism. By testing this hypothesis they examined the possible causal relationship of precocity of splitting to the onset of malignancy.

If splitting of the chromosomes in meiotic prophase takes place before pairing is completed, it interrupts the pairing and prevents formation of chiasmata in the unpaired regions of the chromosomes (Huskins and Smith 1934; Darlington 1935). By making the further assumption that chromosomes which split early in meiosis do the same in mitosis, Huskins and Hearne were led to the postulate that animals constitutionally susceptible to malignant growth should have a lower chiasma frequency than resistant animals, and tested this hypothesis by an examination of the reduction division in young males of inbred strains of mice having different spontaneous mammary gland cancer frequencies. Their results on eight distinct lines showed that these conformed to their hypothesis: two other strains were exceptional (Huskins and Hearne, 1936), but were related to each other and have, together with collateral lines, a somewhat doubtful record of cancer resistance (v. Huskins and Hearne, and below).

The purpose of the present work was, first, to test the correlation found by Huskins and Hearne both for new representatives of the same strains and for additional strains not previously examined.

The work was designed to establish the consistency of strains after several further generations had been bred, to reexamine the strains found not to conform to the hypothesis of Huskins and Hearne, and to test two strains of Strong's which had recently been made available.

Secondly, an investigation was undertaken of the normal tissue of representative strains to examine Huskins and Hearne's fundamental hypothesis that precocious chromosome splitting is associated with genetic susceptibility to malignancy. At the same time the possibility was examined of an association with mitotic chromosome length.

In the light of Little's discovery (v. supra) that breast cancer is inherited to a large extent maternally, first generation hybrid mice from crosses of high and low chiasma frequency parents were tested to determine whether or not chiasma frequency is inherited in a similar manner. Finally an investigation was undertaken on the influence of the milk of a foster mother of another strain on chiasma frequency.

MATERIAL

Most of the cytological observations reported here have been made on spermatogonial or spermatocyte divisions in the testes of young mice. This tissue has the advantage of showing both mitotic and meiotic divisions, and is more suitable for the study of mitotic chromosomes than most adult tissues since dividing spermatogonial cells can be found large numbers and are of exceptional size. Since spontaneous tumours are much less frequent in males than in females, and appear at later ages, the tests were made on males in order to eliminate any possibility that the results might be prejudiced by the presence of a developing tumour.

To get good preparations of spermatogenic divisions in mouse testis, healthy animals must be used. Mice brought from other laboratories were not killed until at least five days after their arrival, when the disturbances of the train journey had presumably ceased to have an effect. Throughout all the recent work, mice of 6 to 8 weeks of age have been used. Huskins and Hearne's work is on mice of similar ages (usually 1-2 months: v. their Table 1). The possibility has thus been eliminated that the chiasma frequency results have been prejudiced by the effects of age (v. Bryden, 1933 b).The origin of the mice used for chiasma frequency counts is mentioned in the tables showing the results of the tests.

Observations on chromosome splitting were made on lymph node and spermatogonial divisions from six weeks old mice received from the Roscoe B. Jackson Memorial Laboratory in November, 1935.

A brief description follows of the strains used; other data concerning them have been cited in the Introduction.

1. Strain A. Colour Genotype: aa bb cc DD. Tests A-1, etc.

Representatives of approximately the sixtieth generation of this strain were obtained from Dr. C. C. Little of the Roscoe B. Jackson Laboratory, Bar Harbor, Maine. The mice used in the first experiments (chiasma frequency tests A-1, A-2, and A-3, and the chromosome splitting tests) were young males born in Dr. Little's colonies; subsequent tests were made on mice raised in Montreal.

The strain originated (Strong 1936 b) from a mating of two albino mice, in 1921, one from Dr. Little's colony, then at Cold Spring Harbor, Long Island, N. Y., and the other from Dr. H. J. Bagg's colony, which had been brought to Cold Spring Harbour in 1918 from the Memorial Hospital, New York City. Dr. Bagg's colony had been inbred for eight years, though not by strict brother-to-sister matings. Since 1921 the stock has been closely inbred, brother-to-sister matings having been accomplished in all but six cases, where daughters were mated back to their fathers. After the twenty-seventh generation Strong selected two lines, one towards the early incidence of such tumours (Line A), and one towards the greatest longevity without tumour development (Line A-2). The ineffectiveness of this selection in producing any difference in the age of cancer incidence in the two lines was taken as evidence that the strain had already reached a high degree of homogeneity for this character (Strong, 1934 b). In a recent report, however, Strong (1937 a) concludes that after his colony had been moved to New Haven in June 1933, a slight age of onset difference appeared in the cancer development of lines A and A-2, kept on the same mixed oatmeal diet. The fact that line A, selected for early

cancer incidence, is now showing a later average age (415.0 days) of cancer development than line A-2 (355.8 days), selected for longevity without cancer, is not commented on by Strong. This difference is presumably due to an independent mutation.

Every female in the direct line of strain A from the 36th to the 55th generations inclusive, has developed primary carcinoma of the mammary gland. These cancers are the most malignant of all the mouse tumours investigated by Strong (Lewis, M. R. and Strong, 1934). Mice survive on the average for 45 days after the tumour appears, and show frequent occurence of multiple nodules and metastasis into the lungs. The incidence of cancer in non-virgin females was reported by Strong and Werner (1936) as more than 90%. The average age of cancer development under "normal" conditions of diet etc. was quoted by the Roscoe B. Jackson Laboratory in 1934 as nine to twelve months; by Bittner (1935 c) as 10.7 months, and by Strong (1937 a), on a commercially prepared diet, as 338.8 days.

As well as a high incidence of mammary gland cancer, strain A exhibits also a marked tendency towards the development of primary carcinoma of the lung, in both sexes. Bittner (1935 a) found that in males living ten months or more, 55% developed primary lung tumours at a mean age of 15.2 months. 36% of females with mammary gland cancer also had lung nodules. In 1936 (a) Bittner reported 71.6% of lung tumours in males and 77% in breeding females, all pulmonary tumours being bronchial adenocarcinomas.

Following out his discovery (1936d) that mice of susceptible strains show a lower volume of blood precipitate with trichloracetic

acid than do resistant mice (v. supra) Strong found in strain A (Strong, 1936 d and Strong and Francis, 1937) a variation in this volume dependent on age. Females of 200 days' age or more show a steady decline in haemoglobin level, whereas in females of a resistant strain (CBA) there is a continual slow increase up to 500 days. After this age the level rises in A and drops in CBA. The mortality records of strain A (Strong, 1936 c) reveal that A females go through a "depression period" every six months and show high frequency of breast cancer development in the inter-depression periods. The first period of depression coincides with the drop in haemoglobin level. The relationship of these facts to tumour development is as yet unknown.

Hybridization experiments between strains A and C57 Black (resistant: v. infra) were the first to show maternal inheritance of breast cancer susceptibility (Little, 1933 b). Much of the more recent work on the question of maternal inheritance and the influence of milk has been done with these two strains.

Injection of large doses of keto-oestrin benzoate into males of strain A causes growth and proliferation, formation of adenomas, and eventually cancer in the mammary glands (Gardner, Smith, Allen and Strong, 1936) although these normally undergo little if any development after three or four weeks of age (Gardner, Diddle, Allen and Strong, 1934) and seldom or never show spontaneous cancers in any strains.

Strain A may be briefly described as highly susceptible to mammary gland carcinoma and susceptible to pulmonary cancer. It is highly inbred and may be considered homozygous for genes controlling these types of cancer. Because of its relatively good breeding qualities, it is one of the best strains of cancer-susceptible mice obtainable for experimental work.

2. Strain dba. (listed by some authors as Strain D).

Colour genotype aa bb CC dd. Tests D-1, etc.

This strain is a continuation of the Little dilute brown strain, which has an unbroken inbred line of descent, mainly of brother-to-sister mating, extending over the last 30 years. Strain dba was obtained by L. C. Strong about 1930 and further inbred by him. The incidence of mammary gland cancer in breeding females is very high, approaching 100%. Only a few die from other causes. Strong (1932) reports a 75% incidence in females reaching eight months of age. The average age of onset of cancer was reported by Murray (1934) as 10.5 months, and by Strong (1936 g) as 11-12 months, for breeding females. In virgin females the average age is 16.6 months (Murray and Little, 1935 b).

3. Strain C3H or Z. Colour genotype AA BB CC DD. Test C3H.

This strain was developed by L. C. Strong from a mating, in 1920, of a male of the Little dilute brown strain, and a female of the Bagg albino strain, both of these being already to some extent inbred. Since then the line has been continued as far as possible by brother-to-sister mating and selected towards high incidence of mammary gland tumours. These are usually medullary carcinomata or adenocarcinomata of moderate malignancy. The mean age of tumour appearance is 8 months, lower than that for any other strain (Strong, 1936 g). Mice live an average of 51 days after the appearance of the tumour, and infrequently show metastasis into the lungs (Lewis, M. R. and Strong, 1934).

Strong (1935 b) states as his opinion that with regard to susceptibility to breast cancer, C3H may be considered genetically pure, and that all female mice of this strain that have been used for breeding and are kept under suitable hygienic conditions, develop carcinoma of the mammary gland (Strong 1935 c).

Gardner, Smith, Strong and Allen (1936 a) obtained mammary gland tumours in virgin C3H females by weekly injections of 100 international units of hydroxyoestrin benzoate. In males, after treatment with theelin followed by continued large doses of keto-oestrin benzoate, they obtained rapidly growing spindle-cell sarcomas. These developed in the subcutaneous tissues at the site of injection, and not in connection with mammary tissue.

Andervont and McEleney (1937) report the tumour incidence in breeding females of strain C3H as 66.1 to 88.2%. By selection over a number of generations they succeeded in lowering the age of incidence in a subline of this strain.

4. Strain C57 Black. Colour genotype aa BB CC DD. Tests CB1-I etc.

This strain has been inbred by C. C. Little since 1921. It is classed by the Roscoe B. Jackson Memorial Laboratory as having a very low incidence of gland tumours, and a medium to high incidence of internal tumours. It has now reached approximately its 45th generation of inbreeding.

In 1934 Murray and Strong apparently considered C57 Black

as practically immune to all neoplasia (Lewis, M. R., and Strong, 1934). When Bagg submitted females of this line (obtained from Little) to a "functional test", they showed up to 15% of spontaneous mammary gland tumours. This behaviour is unlike that of other resistant strains (F and JK) under similar treatment (Bagg and Jacksen, 1937). The tumours showed a tendency to be concentrated in certain families, and the question arises as to whether, among the large numbers of mice raised by the Roscoe B. Jackson Laboratory, some degree of mutation or segregation has not caused sublines with distinct characteristics to appear. In 1936 it was reported from Bar Harbor that only one malignant tumour had appeared in the strain during the last ten years, but Dr. Alexis Carrel stated that 18-30% of internal tumours had arisen in mice derived from Little's C57 Black obtained in 1928 (Huskins and Hearne 1936).

Korteweg has reported verbally to Dr. Huskins that he finds leukaemia in 10% of mice of a substrain of C57 Black, but no tumours. No leukaemia is found at Bar Harbor.

Branch (1936) obtained a higher frequency of dibenzanthracene-induced tumours in C57 Black than in the susceptible strain A. This higher frequency may represent only a difference in speed of reaction, since Branch apparently did not keep his mice until the end of the cancer period.

Strain C57 Black has been used extensively in connection with strain A in experiments on the maternal inheritance of tumour susceptibility and the influence on subsequent tumour formation of the

mother's or foster-mother's milk. In these experiments C57 Black is treated as, and appears to behave as, a resistant strain. For this reason it was used for the first tests on the effects of maternity and fostering in the present work. But the possibility of distinct variations, perhaps due to mutation, in the cancer resistance of different sublines which have been isolated for some time from the stock has not yet been ruled out. There is reason to believe that the substrain now being bred by Dr. Bagg behaves differently from Little's stock (v. supra). Chiasma frequency tests have been made on mice obtained from each of these colonies. Huskins and Hearne also made a test (included in Tables V and VI, qv.) on Carrel's subline of this strain, mentioned above.

5. Strain C57 Brown. Colour genotype aa bb CC DD. Test C-Br.

This strain is derived from a mutant brown-coated mouse which appeared in 1924 in the C57 Black line. It is therefore inbred to the same extent as the parent strain (c. 45 generations) but has been isolated from it for the last thirteen years. It is described by the Roscoe B. Jackson Laboratory pamphlet (1934) as having "tumours at an advanced age", and in a later circular (1937) as showing a medium incidence of mammary gland and a low incidence of internal tumours.

6. Strain C57 Leaden or M-Leaden. Colour genotype aa bb CC DD ll. Tests M-1 etc.

Derived from a mutant which appeared in strain C57 Brown in

1929-30, and claimed by the Roscoe B. Jackson Laboratory in 1934 to have an "extraordinarily low cancer incidence". Murray has remarked (Huskins and Hearne, 1936) that there are a number of tumours in old females of this strain. A more recent circular sent out by the Roscoe B. Jackson Laboratory (1937) describes M-Leaden as having a medium to low breast cancer incidence and a medium internal tumour incidence.

7. Strain CBA. Colour genotype AA BB CC DD. Tests CBA-1, etc.

This strain has been inbred by Strong by brother to sister matings since 1920. He selected only descendants of that mouse which lived longest in each generation. By 1930 he had obtained stock in which the average length of life was more than 24 months with no sign of tumours. In collateral lines tumours occurred sporadically but the descendants of these mice were always discarded (Strong, 1936 a). In Strong's colony no instance of any carcinoma of any kind had been found up to 1936 (Strong and Werner, 1936).

Recent evidence has shown, however, that at an advanced age mice of strain CBA develop various kinds of tumours with considerable frequency. Strong (1936 a) reports that of 71 females which were placed in reserve on a uniform diet, two developed mammary gland carcinoma at 732 and 557 days' age respectively, and two developed hepatoma or adenoma of the liver at 602 and 581 days respectively. Uterine and ovarian carcinoma have also been observed. Between 1933 and 1936 Strong and Smith (1936) found 14 solitary benign hepatomas in CBA. Since they were confined to two families they were probably

determined by genetic factors. Hepatomas are more frequent in CBA than in any other of Strong's strains. Such tumours appear at an advanced age (25-35 months).

An epithelial metaplasia has been induced by Lacassagne (1936 b) in the uterus of a six months old female derived from strain CBA, by injection during alternate weeks of benzoate of oestrone and alcalin extract. Lacassagne's mice were descended from a line obtained from Little and bred in the Institut de Radium, Paris, by Madame Dobrovolskaia-Zavadskaia, who had never observed a spontaneous cancer in it.

Andervont (1935 a) found that CBA was susceptible to induction of tumours by 1-2-5-6-dibenzanthracene, but reacted more slowly than the high spontaneous frequency strains A, C3H, and D.

Strong (1936 c) and Strong and Francis, 1937 showed that mice of strain CBA have a higher volume of blood precipitate than mice of the susceptible strain A.

An important point is the diversity of different kinds of tumours which appear in CBA mice of advanced age. Mammary gland carcinoma is the most frequent, but the list includes hepatoma, carcinoma of the ovary and lungs, sarcoma of the uterus, papilloma of the skin, and osteoid giant-cell sarcoma. In this regard strain CBA is unique among inbred mouse lines.

8. Strain I. Colour genotype aa bb CC dd ss. Tests I-1, etc.

This strain was developed by Strong from a cross made in

1927 of a pink eye dilute brown mouse with a dilute brown piebald one. It is one of the most resistant strains known to mammary gland cancer. Bittner (1936 b) reports eight generations in direct line with no recorded tumour, though a few internal tumours had been observed in collaterals. He used strain I in crosses with C3H (susceptible to mammary gland cancer) and found that the reciprocal hybrids showed a strong maternal influence on breast cancer.

Andervont and Stewart state that mice of strain I have no spontaneous mammary gland cancer and are resistant to tumour transplants. They find, however, that practically all animals 10 months old or more show lesions in the pyloric region of the stomach. The condition is characterized by adenomatous hyperplastic growth of the glandular mucous membrane with some signs of infiltration into the basement membrane. Mice of this strain seldom live more than 14 months. Stomach lesions of this type have not been found by Andervont in other mice, with the exception of three old ones of strain C3H.

9. Strain F. Colour genotype aa bb $c^{ch}c^{ch}$ dd ss. Test F.

These mice were developed by L. C. Strong from a race which he obtained during the winter of 1925-26 from H. W. Feldmann, of the Bussey Institution, Harvard University. They bear five pairs of recessive coat-colour genes, and are characterised by their small size (about two-thirds of the average adult weight of Strong's other strains). In January 1935 they were in their 32nd generation of brother-to-sister inbreeding. The only pathological feature found in these

mice is a group of lymphatic symptoms, characterized by hyperplasy of the lymph nodes, enlargement of the spleen and hypertrophy of the thymus. One epithelioma of the anus, transplantable into other F mice, has been observed (Strong, Smith and Gardner, 1936) and some squamous cell carcinomata of the skin (Strong, unpublished). Bagg and Jacksen (1937) have subjected 23 females to their "functional test" without as yet having found any mammary gland tumours.

10. Strain JK. Colour genotype aa bb CC DD. Also carries $s^e s^e$ and $p_1 p_1$. Test JK.

This strain is derived from a cross made by Dr. L. C. Strong in 1927 of representatives of two strains, one the J, obtained from Dr. H. Feldman of the Bussey Institution, Harvard University, and the other, the K, from Dr. Gregory Pincus of the same Institution. Both the parent strains have since been discontinued. Since this mating the mice have been continued by brother-to-sister mating, with selection towards longevity. Only one animal in the direct line (6th generation) has developed carcinoma; a few have shown small round-cell sarcomas. Mammary gland cancer has never occurred. The age at death of females is regularly about 21 months. JK mice exhibit the highest readings of blood precipitate of any strain examined (Strong 1937 b).

Bagg and Jacksen (1937) have tested eleven JK females by their "functional test" and have so far discovered one spontaneous lymphosarcoma and no carcinomata.

11. Strain N. Colour genotype aa bb CC dd ss. Test N.

Developed by Dr. L. C. Strong as a line highly resistant to cancer. It is a slow-growing race, and is difficult to perpetuate due to the fact that 70-80% of females have cystic ovaries and are sterile (Strong, unpublished). In 1934 Strong had observed no tumours in mice of this strain. It has been inbred since 1921 (Roscoe B. Jackson Laboratory leaflet, 1934).

12. Strain Y. Colour genotypes Yellow: A^{y}a bb CC. Test Y-Yel.

Black: aa BB CC. Test Y-Bl.

This strain was described by the Roscoe B. Jackson Laboratory in 1934 as showing a "relatively high occurrence of spontaneous sarcoma of different types", and more recently (1937) as having a medium incidence of mammary tumours and a medium to high incidence of internal tumours. It has been inbred for more than 15 generations.

The yellow and black segregates of this strain show a slight difference in their rates of development, but apart from secondary effects of this difference, have the same cancer incidence (Little, 1934 a, et supra).

METHODS

1. Preparation of Material

A. Smearing

In making preparations for the study of mammalian chromosomes, only fresh material can be used, and this must be fixed with the utmost possible speed. For this reason, among others, the smear method is particularly suitable since with a very thin layer of tissue on the slide the fixation of all cells is practically instantaneous, and the delayed fixation caused by slow penetration of fixative through a block of tissue, such as is used for sectioning, is avoided.

The mouse to be tested is killed by decapitation, or rendered senseless by cutting the spinal cord in the neck region, or by stunning. The last two methods are probably the best since circulation is not interrupted. Dr. Hearne used the decapitation method. For the smearing of lymph nodes it was found most convenient to cut the spinal cord, thus desensitizing the lower part of the body without interfering with respiration or circulation. This allowed time for the finding and removal of a number of lymph nodes in the lower abdomen, which cannot be done satisfactorily if the animal is instantaneously killed.

Anaesthetics were avoided because of their possible effect on chromosome behaviour. Ether and other fat solvents have been shown in tissue culture to affect spindle formation and anaphase movement of chromosomes (v. supra) and ether anaesthesia has been found by Victor (1934) to decrease the respiratory rate

in normal mouse lymph nodes. Although Ludford (1930) finds no effect of anaesthesia on tissues in situ, and in any case the likelihood is small that variation in different mice in the length or degree of anaesthesis might affect differentially the splitting or pairing behaviour of chromosomes in somatic or germinal tissues, it seemed best to avoid any possible confusion from such a source.

Immediately after killing or stunning the mouse, the testes were removed one at a time, cut into small pieces with scissors, and smeared on a clean glass slide. The slide is at once plunged face downwards into a shallow bath of the fixative. The removal, smearing, and fixing of each testis takes about ten seconds. Ten or twelve smears can be made from the testes of a six weeks old mouse.

The same procedure was used in the preparation of lymph node smears. These can best be obtained from (1) the inside of the abdominal skin, one large lymph node on each side being associated with the mammae, (2) from the intestine, particularly near the appendix, and (3) from the axillary region of the fore-limbs. The node is picked off with fine forceps, cut open with scissors, and smeared on a slide.

B. Fixation.

1. For mitotic divisions, metaphase of meiosis, and general purposes it was found that several osmic acid fixatives used in plant cytology produced excellent results. Osmic acid fixatives, however, are always expensive. The best general purpose medium not contain-

ing osmic was found to be Allen's modification of Bouin's fluid (used by Painter (1924) and other investigators).

The fluid is made up as follows:

Solution A: 8 parts.		Solution B: 2.5 parts.	
Saturated picric acid	500 cc.	Formalin	200 cc.
Glacial acetic acid	30 cc.	Urea	16 gm.
Chromic acid	10 gm.		

The two solutions are kept separate and freshly mixed just before using. Fixation is from 4 to 24 hours, as convenient. Better penetration can be obtained by using the fixative at blood temperature.

2. For prophase of meiosis and chiasma counting Kaiser's solution, followed by Belling's modification of Navashin's fluid is undoubtedly the best fixative tried (Huskins and Hearne, 1936). This method also gives better preparations of early mitotic prophase than does Allen's Bouin or the osmic acid fixatives. The solutions are

Kaiser's: 10-30 minutes.		Navashin's: 12-24 hours. Solution A. One part.		Solution B. One part	
Mercury bichloride	10 gm.	Chromic acid	1 gm.	Formalin	200 cc.
Glacial acetic acid	3 cc.	Glacial acetic	50 cc.	Distilled water	175 cc.
Distilled water	300 cc.	Distilled water	320 cc.		

C. Staining.

In order to obtain the best possible preparations for the study of chromosomes, it is necessary to use karyological, rather than

histological, stains. Throughout this work Newton's gentian violet method of staining for chromosome structure, which is extensively employed in the study of plant chromosomes, has been used. It is purely a chromatin stain, being taken up by chromosomes and nucleoli and leaving the nuclear plasm, nuclear membrane and cytoplasm colourless.

The method of staining has been described by Huskins and Smith (1935). A more intense stain is obtained after chromic acid fixation, and it is sometimes advisable to soak slides for a few minutes in 1% chromic acid solution before hardening. This was usually done with slides fixed in Kaiser-Navashin. Slides are then washed thoroughly in running water, and brought up slowly to 70% alcohol through several stages. Here they are hardened for 12-24 hours. They are then stained according to the following schedule:

(1). Washing in running water to remove the alcohol.

(2). Staining in a 1% aqueous solution of crystal violet for 15 minutes.

(3). Rinsing in tap water.

(4). 95% alcohol for 5 seconds.

(5). Solution of 1% iodine and 1% potassium iodide in 80% alcohol for 30 seconds.

(6). 95% alcohol for 10 seconds.

(7). Absolute alcohol for 10 seconds.

(8). A mixture of half clove oil and half absolute alcohol until cleared (about 2 minutes).

(9). Xylol, five changes with at least two hours' total time.

(10). Mounting in Canada Balsam.

II. Collection of data.

A. Estimation of the amount of precocious chromosome splitting in spermatogonial mitoses.

When testis tissue is lightly smeared on a slide, some sections of the seminiferous tubules remain unbroken, and adhere *in toto* to the glass surface. In the walls of the tubules at points along their length may be found groups of dividing spermatogonial cells. If the tissue be well cleared after staining, such cells are excellent material for the study of mitotic chromosomes.

To determine the amount of pre-anaphase splitting, spermatogonial metaphase stages were selected in which all 40 chromosomes (the normal diploid number in mice) were clearly visible. The cells were drawn by camera lucida (at an initial magnification of 3600 diameters) to verify the chromosome count and to provide a permanent record of the observations. After all the drawings had been made for the complete test, the slides were restained to ensure uniform visibility. Each chromosome was then carefully reexamined to determine whether or not it was split. The criterion used to decide this point was the visibility of four lines representing the edges of the parts of a double structure. The split chromosomes were then recorded on the drawings and counted. The counts on the two strains examined were made on alternate groups from each strain, to avoid any influence on the results of a possible change in standard on the part of the observer during the course of the work.

Fifty cells each of strains A and I were counted in this way. Apart from this, additional counts were made on twenty-five cells each of strains A, JK, CBA, and M-Leaden. These were done

by inspection, without the use of drawings, from freshly-stained preparations.

B. Estimation of chromosome length at metaphase in spermatogonia.

Chromosome length has been measured in fifty cells each from spermatogonia of strains A and I. The measurements were made for each chromosome to the nearest millimeter from the camera lucida drawings mentioned above. The comparison therefore comprises measurements of 4,000 chromosomes.

C. Estimation of chiasma frequency in primary spermatocytes.

For chiasma frequency counts, cells at stages from mid-diplotene until diakinesis were selected. Only cells clearly showing 20 bivalents (the normal haploid number for mice) were used. Observations were made at a magnification of 1260 diameters. A rough sketch was made of each cell, representing diagrammatically the form and position of each bivalent and the number of its chiasmata. In all cases a bivalent was counted as having the minimum number of chiasmata necessary for its configuration. Huskins and Hearne used the same method of counting. In this way comparable values for different groups of cells can be obtained, although the absolute values for chiasma frequency in mice are no doubt somewhat higher than those given in the observations herein presented.

Twenty-five complete nuclei (500 bivalents) were counted for each test.

III. Statistical Analysis of Data.

Huskins and Hearne (1936) analyzed their chiasma frequency results by Fisher's method for the analysis of variance, and derived a single figure as a standard for the comparison of means. This method assumes that the variability is homogeneous for all samples in the population. With the addition of the new results reported here, it has become clear that this method could not be continued. The new test on strain I (test I-2), for example, has an exceptionally high standard deviation, and the addition of this one test to the figures of Huskins and Hearne so increases the total variability that a higher difference between means is at once necessary for significance even when the comparison does not involve test I-2. Much more accurate is a comparison of means based solely on the variability of the tests which they represent. For this reason all the data on chiasma frequency have been analyzed by the standard error method. A difference between two means which is three times its own standard error may be considered significant. It represents a probability of .997 that the samples are from different populations. A difference of twice its standard error has a .955 chance of being significant.

The standard error of the difference is represented by the symbol "S", and is obtained from the formula $S = \sqrt{\sigma_1^2 + \sigma_2^2}$ where σ_1 and σ_2 are the standard errors of the two groups concerned. The standard error of a population is equal to $\frac{\sigma}{\sqrt{N}}$ where "σ" is the standard deviation and "N" the number of observations. Standard deviations were in all cases worked out from ungrouped data.

In the following tables the differences between means have been divided by their "S" values for all comparisons so that significance or non-significance may be readily seen.

Observations on chromosome splitting and on chromosome length were analysed by Fisher's method for the analysis of variance. To determine the proper interpretation of the results the greater mean square is divided by the lesser, giving a figure, "F", whose significance can be judged from Snedecor's table of "F" (Snedecor 1934, table XXXV). This table is based on Fisher's values of "t" and "z" (v. Fisher, 1936, tables IV and VI). In Snedecor's table two values are given (under appropriate columns for degrees of freedom) representing Fisher's 5% and 1% points respectively. The first of these may be considered significant: the second, highly significant.

In the present work only differences which were three times their standard error (or greater than Fisher's 1% point) have been considered significant. In the chiasma frequency counts differences which fall between two and three times their standard error have been treated as borderline cases. This is done because of the relative smallness of the samples and the fact that it is sometimes impossible to determine exactly what the count for a given cell should be.

OBSERVATIONS AND RESULTS

1. The comparability of the results of the present tests of chiasma frequency with those of Huskins and Hearne, and the consistency of several counts on the same strain

Multiple tests have been made on five strains (A, dba, C57 Black, M-Leaden, and I). Of these the first three had already been tested twice by Huskins and Hearne, with consistent results (v. their Table III, and infra, Table III). The additional tests were made as a further examination of consistency within strains, and with the purpose of showing, by their general trend, whether possible differences in the standards of the observers would prejudice the direct comparison of the present results on new strains with the tests of Huskins and Hearne. To provide a more accurate decision on the latter point, a count on C57 Black (test CB1-6) was made from the preparations used by Huskins and Hearne for one of the original tests on this strain (CB1-1).

The numbers of chiasmata observed in additional counts on strains tested by Huskins and Hearne are given in Table I; an analysis of these, together with a similar analysis of Huskins and Hearne's figures for the same strains (see their Table II) are given in Table II, and a comparison of the means in Table III.

The results of the comparison of means within strains show that:

(1). Except in strain C57 Black, there is agreement between different tests within strains. In no other case is the difference between the means greater than about twice its standard error.

All other differences between means within strains are less than twice their standard errors, and are therefore in no way significant. The test (CB1-6) made by the writer from the preparations of Huskins and Hearne is statistically identical with the test made by them from the same preparations (CB1-1). The highest difference occurs in M-Leaden. The test on this strain made by the writer (M-3) gave a result which shows a difference from one of the tests of Huskins and Hearne (m-2) of 2.06 times its standard error, i.e. a difference approaching significance (see Table III). Its difference from the other of Huskins and Hearne's tests is, however, only 0.31 times its standard error. Since test M-3 is not significantly different from tests M-1 and M-2 when these are combined to form a single population, the difference between M-2 and M-3 was not taken to indicate either an alteration in this strain or a difference in the standards of the observers.

(2). In the case of strain C57 Black, there is complete agreement between all the counts made on stock obtained from Little (the Roscoe B. Jackson Laboratory) or its progeny, but mice of this strain obtained from Bagg's colony (tests CB1-3) have a very much higher chiasma frequency. This is clear from the fact that the difference between the mean for Bagg's stock (test CB1-3) and the highest mean obtained among the four tests on Little's stock (test CB1-1) is 6.03 times its standard error (Table III). Actually the chiasma frequency in Bagg's derivative of this strain is higher than any other found in the course of this work.

TABLE NUMBER I

CHIASMA FREQUENCIES IN NEW COUNTS IN UNFOSTERED MICE

TEST NO.	A-3	D-2	M-3	CBL-6	CBL-7	CBL-3	I-2	I-3
	28	25	27	32	32	44	34	34
	29	29	31	29	29	37	32	30
	31	26	31	31	29	33	34	32
	30	24	24	34	30	38	31	35
	26	30	24	30	26	35	39	32
	31	28	30	31	32	40	26	31
	31	29	29	33	28	31	36	29
	30	25	29	29	28	31	31	33
	26	27	30	33	29	35	32	34
	31	25	26	31	28	33	34	37
	27	26	27	32	28	33	40	33
	28	24	30	29	31	35	36	36
	26	25	23	28	28	33	32	31
	33	28	26	32	30	35	32	33
	31	28	26	29	33	35	31	34
	25	28	30	35	33	38	31	34
	29	26	29	31	33	34	34	35
	30	30	28	29	29	37	32	31
	28	29	30	32	29	30	37	38
	28	24	27	28	31	36	30	31
	25	27	24	32	30	34	31	34
	27	32	31	30	31	35	42	31
	24	26	29	30	24	38	38	34
	28	29	28	30	29	32	36	31
	24	28	30	28	32	33	35	33
TOTALS	706	678	699	768	742	875	846	826

TABLE NUMBER II

ANALYSIS OF ALL COUNTS IN UNFOSTERED MICE OF CHIASMA FREQUENCY

TEST	MADE BY	MEAN	STANDARD DEVIATION	(STANDARD ERROR)^2	ORIGIN OF STOCK
A-1	**H/H	28.44	2.58	0.266	Mice received from*RBJ, Nov. 1933.
A-2	H/H	29.24	1.45	0.084	Mice received from RBJ. Nov. 1935.
A-3	**AH	28.24	2.42	0.234	Mice received from RBJ. Feb. 1936.
D-1	H/H	27.40	1.88	0.141	Mice received from RBJ. Jan. 1935.
D-2	AH	27.12	1.80	0.129	Progeny of stock received from RBJ. Nov. 1936
M-1	H/H	27.76	2.20	0.191	Mice received from RBJ. Jan. 1935.
M-2	H/H	26.68	1.95	0.153	Mice received from RBJ. Nov. 1935.
M-3	AH	27.96	2.39	0.229	Progeny of stock received from RBJ. Nov. 1936
CBL-1	H/H	30.32	1.67	0.111	Mice received from RBJ. Nov. 1933.
CBL-2	H/H	29.72	2.01	0.162	Mice received from RBJ. Nov. 1933.
CBL-6	AH	30.72	1.87	0.139	Count made on the slides of H/H.
CBL-7	AH	29.68	2.17	0.188	New stock received from RBJ. August 1937
CBL-3	AH	35.00	3.03	0.368	Progeny of stock received from H. J. Bagg Nov. 1936.
I-1	H/H	33.92	1.57	0.099	Mice received from RBJ. Sept. 1934.
I-2	AH	33.84	3.51	0.492	Mice received from L. C. Strong Nov. 1936.
I-3	AH	33.04	2.14	0.184	Progeny of stock received from L. C. Strong Nov. 1936.

**H/H: Test of Huskins and Hearne (1936).
**AH: Test made by the writer.
*RBJ: In this and other tables refers to the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

TABLE NUMBER III

COMPARISON OF MEANS WITHIN STRAINS

COMPARISON	DIFFERENCE BETWEEN MEANS	S	DIFFERENCE / S
Between A-1 and A-2	0.80	0.59	1.36
Between A-1 and A-3	0.20	0.71	0.28
Between A-2 and A-3	1.00	0.56	1.79
Between D-1 and D-2	0.28	0.52	0.54
Between M-1 and M-2	1.08	0.59	1.83
Between M-1 and M-3	0.20	0.65	0.31
Between M-2 and M-3	1.28	0.62	2.06
Between CBL-1 and CBL-2	0.60	0.52	1.15
Between CBL-1 and CBL-6	0.40	0.50	0.80
Between CBL-1 and CBL-7	0.64	0.55	1.16
Between CBL-2 and CBL-6	1.00	0.55	1.82
Between CBL-2 and CBL-7	0.04	0.59	0.07
Between CBL-6 and CBL-7	1.04	0.57	1.82
Between CBL-6 and CBL-3	4.28	0.71	6.03
Between I-1 and I-2	0.08	0.77	0.10
Between I-1 and I-3	0.88	0.53	1.66
Between I-2 and I-3	0.80	0.82	0.98

2. New data on the correlation between high cancer susceptibility and low chiasma frequency

Huskins and Hearne made counts on ten distinct strains of mice. Two additional resistant strains (F and JK) have been counted in the present work, and the results compared with the results of Huskins and Hearne for the original ten.

The observed numbers of chiasmata in strains F and JK are listed in Table IV. Table V shows an analysis of the new results together with all the counts previously made in all strains. Where more than one test had been made on a strain, all the tests were considered as representing a single sample and the mean and standard deviation of the whole population were taken to represent the strain. This procedure is justifiable since the results of the different tests have been shown to be identical. Table VI shows a comparison (at the intersection of the horizontal and vertical lines through the titles of the strains) between all the means obtained. The test on Bagg's subline of strain C57 Black (see Tables V and VI) is also included, but is listed separately because of its significantly higher chiasma frequency. Carrel's subline of C57 Black, which is lower, is also listed separately.

Table VI shows that the resistant strains F and JK agree with Huskins and Hearne's data showing an inverse relationship between chiasma frequency and mammary cancer incidence. Both F and JK have a chiasma frequency significantly lower than the resistant strain N, but higher than any one of the susceptible strains (Y Yellow and Y Black, C3H, A, C57 Brown,

and dba). When compared with strain I, F shows no difference; JK is lower, with a difference which approaches significance (2.81 times its standard error).

Bagg's subline of strain C57 Black has a chiasma frequency significantly higher than that of all other strains except N, I and F. When compared with the latter two, the difference still approaches significance. Carrel's subline, on the other hand, has the lowest mean chiasma frequency of any strain and is significantly lower than all of them except C57 Brown, M-Leaden and dba. Little's subline occupies an intermediate position, but is significantly lower than five of the resistant strains (N, I, F, CBA and JK; cf. Huskins and Hearne's Table III).

Apart from the exceptional strains already known to exist from the results of Huskins and Hearne (namely, C57 Black, and M-Leaden) the combined results summarized in Tables V and VI show good agreement with their working hypothesis.

TABLE NUMBER IV

CHIASMA FREQUENCY IN TWO ADDITIONAL RESISTANT STRAINS

	F	JK
	30	31
	27	35
	32	37
	28	32
	27	33
	35	33
	34	34
	33	28
	33	32
	33	26
	39	31
	27	28
	35	34
	35	32
	38	30
	31	31
	28	32
	33	29
	30	33
	32	33
	36	35
	34	32
	36	33
	34	35
	32	31
TOTALS	812	800

TABLE NUMBER V

ANALYSIS OF GROUPED TESTS FOR ALL STRAINS

STRAIN	NO. OF TESTS	MEAN	STANDARD DEVIATION	$(\text{STANDARD})^2$ ERROR	ORIGIN OF STOCK
Carrel's C Black	1	26.72	1.61	0.104	Mice received from RBJ. Jan. 1935. *H/H.
dba	2	27.26	2.00	0.080	See Table II.
M	3	27.47	2.26	0.068	See Table II.
C Brown	1	27.88	1.61	0.104	Mice received from RBJ. Jan. 1935. H/H.
A	3	28.64	2.25	0.067	See Table II.
C3H	1	29.16	1.97	0.140	Mice received from RBJ. Jan. 1935. H/H.
Y Black	1	29.16	2.15	0.184	Mice received from RBJ. Oct. 1933. H/H.
Y Yellow	1	30.00	1.77	0.125	Mouse received from RBJ. Oct. 1933. H/H.
C Black (Little's)	4	30.11	1.98	0.059	See Table II.
JK	1	32.00	2.45	0.240	Mice received from L.C. Strong Dec. 1936. **(AH).
CBA	2	32.16	2.01	0.081	See Table II.
F	1	32.48	3.29	0.432	Mice received from L.C. Strong March 1937 (AH).
I	3	33.60	2.57	0.088	See Table II.
N	1	34.92	2.00	0.160	Mice received from RBJ. Prob. Sept. 1934. H/H.
C Black-3 (Bagg's)	1	35.00	3.03	0.368	See Table II.

* H/H: Test of Huskins and Hearne (1935).
** AH: Test made by the writer.

8.28	8.20	6.88	5.76	5.44	5.28	3.39	3.28	2.44	2.44	1.92	1.16	0.75	0.54	
0.69	0.51	0.44	0.73	0.43	0.59	0.38	0.48	0.54	0.49	0.41	0.46	0.41	0.43	C Black
12.00	16.08	15.64	7.89	12.65	8.95	8.92	6.83	4.52	4.92	4.68	2.52	1.83	1.26	Carrel
7.74	7.66	6.34	5.22	4.90	4.74	2.85	2.74	1.90	1.90	1.38	0.62	0.21		
0.67	0.49	0.41	0.72	0.40	0.57	0.34	0.45	0.51	0.47	0.38	0.43	0.38	dba	
11.55	15.63	15.46	7.25	12.25	8.32	8.38	6.09	3.73	4.04	3.63	1.44	0.55		
7.53	7.45	6.13	5.01	4.69	4.53	2.64	2.53	1.69	1.69	1.17	0.41			
0.66	0.48	0.39	0.71	0.39	0.55	0.33	0.44	0.50	0.46	0.37	0.41	M		
11.41	15.52	15.72	7.06	12.03	8.24	8.00	5.75	3.38	3.67	3.16	1.00			
7.12	7.04	5.72	4.60	4.28	4.12	2.23	2.12	1.28	1.28	0.76				
0.69	0.51	0.44	0.73	0.43	0.59	0.38	0.48	0.54	0.49	0.41	C Br			
10.32	13.80	13.00	6.30	9.95	6.98	5.87	4.42	2.37	2.61	1.85				
6.36	6.28	4.96	3.84	3.52	3.36	1.47	1.36	0.52	0.52					
0.66	0.48	0.39	0.71	0.38	0.55	0.33	0.44	0.50	0.45	A				
9.64	13.08	12.72	5.42	9.26	6.11	4.45	3.09	1.04	1.16					
5.34	5.76	4.44	3.32	3.00	2.84	0.95	0.84	0.00						
0.71	0.55	0.48	0.76	0.47	0.62	0.42	0.51	0.57	C3H					
8.23	10.47	9.25	4.37	6.38	4.58	2.36	1.65	0.00						
5.84	5.76	4.44	3.32	3.00	2.84	0.95	0.84							
0.74	0.59	0.52	0.78	0.51	0.65	0.47	0.56	Y Bl						
7.89	9.76	8.54	4.26	5.88	4.37	2.02	1.50							
5.00	4.92	3.60	2.48	2.16	2.00	0.11								
0.70	0.53	0.46	0.75	0.45	0.60	0.40	Y Yel							
7.14	9.28	7.33	3.31	4.80	3.33	0.28								
4.89	4.81	3.49	2.37	2.05	1.89									
0.64	0.45	0.36	0.69	0.35	0.53	C 57								
7.64	10.69	9.69	3.43	5.86	3.57	Bl L								
3.00	2.92	1.60	0.48	0.16										
0.78	0.63	0.57	0.82	0.57	JK									
3.85	4.63	2.81	0.59	0.28										
2.84	2.76	1.44	0.32											
0.67	0.49	0.41	0.72	CBA										
4.24	5.63	3.51	0.44											
2.52	2.44	1.12												
0.89	0.77	0.72	F											
2.83	3.17	1.56												
1.40	1.08													
0.68	0.81	I												
2.06	1.33													
0.08														
0.73	N													
0.11														
C57														
Bl B														

TABLE VI

COMPARISON OF MEANS FOR ALL STRAINS

Three figures are given for each comparison

1st figure - Difference between means

2nd figure - S (Standard error of the difference)

3rd figure - Difference ÷ S

3. Chromosome splitting in normal tissue of resistant and susceptible mice

A. Observations on lymph node tissue

Smears of lymph nodes from several strains of mice (A, M-Leeden, CBA and N) showed that in this tissue the majority of dividing cells are large and, although not plentiful, occur in sufficient numbers to make a study of the mitotic chromosomes possible. In all metaphase cells examined, regardless of strain, longitudinal splitting of the chromosomes is present to some extent. In some cells the split can be seen only at the ends of the chromosomes; in others it is clearly visible in every chromosome of the cell, and in some cases the two halves of the chromosomes are widely separated although their form, and the arrangement of the plate, in no way suggest that this is an anaphase stage. Figure 1 is a drawing of a metaphase cell from the right mammary lymphatic gland of a mouse of strain N. The normal somatic number of chromosomes (40) is present and each chromosome is clearly split, four of them widely so. A photomicrograph of another metaphase split is shown in Figure 2.

The presence of pre-anaphase splitting in all dividing cells of the lymphatic glands was an unexpected occurrence and one which led to the discarding of this tissue for the present investigation. Such splitting has not previously been described in the literature dealing with normal cells, and it was felt that cells of the lymphatic germinative centres may be in some way atypical. Possibly the large dividing cells examined represent the last divisions which these cells will undergo before they reach their final stage of differentiation. Partly owing to this possibility,

and partly because most cytological work on normal mammalian tissue has been carried out on spermatogenic tissue, it was decided to use testis as material for investigating the time of chromosome splitting. Here at least it is known that the mitotic divisions of the spermatogonia will be followed by other cell generations.

B. Observations on Spermatogonia

Examination of a few spermatogonial divisions from strains A, N, M-Leaden, and CBA showed at once that splitting of the chromosomes at metaphase is present in almost every cell, regardless of strain, and that therefore a comparison of strains in this respect must be treated as a statistical problem. Figure 3 shows a group of seven cells drawn by camera lucida, and Figure 4 a photomicrograph of two other cells, which illustrate this point. The possible reasons for the appearance of split chromosomes at metaphase in the present material, when most authors have not seen them, will be discussed later.

An interesting, and perhaps significant, observation which was made in the course of this study is that there are apparently two types of spermatogonial cells. One has longer and thinner chromosomes arranged in a characteristic ring on the metaphase plate with the smaller ones in the centre, while in the other type the chromosomes are thicker, more condensed, and tend to be irregularly scattered over the plate. Figure 5 is a photomicrograph of three spermatogonial cells from a CBA mouse which illustrate the two types. These differences could not be correlated in any way with position in the tubule wall, either radially or longitudinally (as shown by

additional sectioned material used to test this possibility), and hence neither with stage of division nor with possible cell generation. The two types are not distinct, and there are intermediate stages. This is indicated both from direct observation and from the measurements of chromosome length (v. infra) which do not fall into two classes but form a more or less continuous series from the shortest to the longest. There is no appreciable difference in the number of split chromosomes in cells classified arbitrarily according to the degree of condensation of their chromosomes.

Table VII gives the number of split chromosomes observed in fifty spermatogonial metaphase cells (previously drawn by camera lucida) in each of strains A and I. The mean frequency of split chromosomes is higher in strain A, but an analysis of the two populations shows that the difference is not significant.

Observations on strains A, M, JK and CBA made by inspection of smaller numbers of cells are given in Table VIII. Analysis of the results for strain A compared with each of the resistant strains shows that the differences are not significant. These results indicate that there is no difference which can be detected by the methods employed in the amount of pre-anaphase chromosome splitting in spermatogonia of resistant and susceptible strains.

TABLE NUMBER VII
NUMBER OF SPLIT CHROMOSOMES IN SPERMATOGONIAL METAPHASES FROM STRAINS A AND I

STRAIN A	STRAIN I
10	2
4	8
7	4
9	5
6	4
10	7
20	13
4	7
2	3
4	1
18	4
14	7
5	2
15	14
7	7
4	13
11	4
12	8
4	8
7	5
13	3
5	9
6	4
15	9
13	4
13	8
6	13
7	14
12	8
6	4
7	20
9	7
5	12
7	7
15	15
4	2
9	12
11	15
6	6
6	10
5	9
5	12
4	9
13	9
4	6
12	4
5	11
6	5
10	2
15	7
TOTALS 427	382

ANALYSIS OF OBSERVATIONS ON NUMBER OF SPLIT CHROMOSOMES IN SPERMAGONIAL METAPHASES OF STRAINS A AND I.

	A	I	TOTALS
No. cells	50	50	100
No. split chromosomes	427	382	809
Sum (split chromosomes)^2	4541	3780	8321
(No. split chromosomes)^2	182329	145924	328253

ANALYSIS OF VARIANCE

$$\text{Correction factor} = \frac{(809)^2}{100} = 6544.81$$

$$\text{Total variance} = 8321 - 6544.81 = 1686.19$$

$$\text{Variance between strains} = \frac{328253}{50} - 6544.81 = 6565.06 - 6544.81$$

$$= 20.25$$

SOURCE OF VARIANCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE
Total	99	1686.19	17.032
Between strains	1	20.25	20.25
Within strains	98	1665.94	16.999

$$F = \frac{20.250}{16.999} = 1.19$$

Value necessary for significance: 3.94 for Fisher's 5% point.

TABLE NUMBER VIII

OBSERVATIONS ON NUMBER OF SPLIT CHROMOSOMES BY INSPECTION OF SPERMATOGONIAL METAPHASES IN FOUR STRAINS

STRAINS	A	M	JK	CBA
	6	8	8	5
	13	16	7	9
	8	8	2	7
	16	5	7	7
	4	4	8	13
	6	4	4	9
	3	5	5	3
	8	6	2	9
	5	6	10	8
	3	6	8	3
	7	2	5	10
	10	4	7	10
	11	3	10	12
	5	2	5	8
	4	3	6	8
	11	4	4	
	6	9	4	
	5	5	3	
	7	11	10	
	7	9	6	
	10		9	
	3		5	
	10		6	
	15		8	
	8		11	
TOTALS	191	120	160	121
MEAN	7.64	6.00	6.40	8.07

ANALYSIS OF OBSERVATION ON STRAINS A AND M

	A	M	TOTALS
No. of cells	25	20	45
No. of splits	191	120	311
ΣX^2	1773	940	2713
(No. of splits)^2	36481	14400	50881
$\frac{(\text{No. splits})^2}{\text{No. cells in col.}}$	1459.24	720.00	2179.24

Correction factor = $\frac{(311)^2}{45} = \frac{96721}{45} = 2149.36$

Total variance = 2713 - 2149.36 = 563.64

Variance between strains = 2179.24 - 2149.36 = 29.88

SOURCE OF VARIANCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE
Total	44	563.64	12.81
Between strains	1	29.88	29.88
Within strains	43	533.76	12.41

$$F = \frac{29.88}{12.41} = 2.41$$

Value necessary for significance : 4.06 for Fisher's 5% point.

ANALYSIS OF OBSERVATIONS ON STRAINS A AND JK

	A	JK	TOTALS
No. cells	25	25	50
No. splits	191	160	351
ΣX^2	1773	1178	2931
(No. splits)^2	36481	25600	62081

$$\text{Correction Factor} = \frac{(351)^2}{50} = \frac{123201}{50} = 2464.02$$

Total variance = 2951 - 2464.02 = 486.98

$$\text{Variance between strains} = \frac{62081}{25} - 2464.02$$

$$= 2483.24 - 2464.02 = 19.22$$

SOURCE OF VARIANCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE
Total	49	486.98	9.938
Between strains	1	19.22	19.22
Within strains	48	467.76	9.745

$$F = \frac{19.22}{9.745} = 1.97$$

Value necessary for significance : 4.03 for Fisher's 5% point.

ANALYSIS OF OBSERVATIONS ON STRAINS A AND CBA

	A	CBA	TOTALS
No. cells	25	15	40
No. splits	191	121	312
ΣX^2	1773	1089	2862
$(\text{No. splits})^2$	36481	14641	51122
$\frac{(\text{No. splits})^2}{\text{No. cells in col.}}$	1459.24	976.07	2435.31

Correction factor = $\frac{(312)^2}{40} = \frac{97344}{40} = 2433.60$

Total variance = 2862 - 2433.60 = 428.40

Variance between strains = 2435.31 - 2433.60 = 1.71

SOURCE OF VARIANCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE
Total	39	428.40	10.98
Between strains	1	1.71	1.71
Within strains	38	426.69	11.23

$$F = \frac{11.23}{1.71} = 6.57$$

Value necessary for significance : 249.04 for Fisher's 5% point

4. Chromosome Length at Metaphase in Spermatogonia of a Susceptible and a Resistant Strain

Observations on chromosome length at metaphase in strains A and I were made from camera lucida drawings of fifty spermatogonia of each of these strains. Table IX gives the total chromosome length in millimeters for each cell. The analysis which follows shows that there is no difference between the means for the two strains.

TABLE NUMBER 1X

OBSERVATIONS ON TOTAL CHROMOSOME LENGTH IN 50 SPERMATOGONIAL METAPHASES OF STRAINS A AND I

STRAIN A LENGTH	STRAIN I LENGTH
255	358
260	309
335	296
347	297
332	314
333	274
343	282
302	351
310	349
275	349
271	321
288	285
307	302
308	289
282	283
309	313
264	289
279	287
363	286
288	312
269	301
318	286
299	282
306	304
328	327
351	303
299	289
312	307
289	336
323	326
348	297
339	311
335	306
299	293
303	287
290	329
316	307
286	308
301	309
264	314
273	311
321	300
351	297
296	321
310	314
273	290
302	291
270	303
253	294
263	291
TOTALS 15008	15280

ANALYSIS OF OBSERVATIONS ON CHROMOSOME LENGTH IN SPERMATOGONIAL METAPHASES OF STRAINS A AND I

	A	I	TOTALS
No. cells	50	50	100
Chromosome length	15008	15280	30288
Sum (Lengths)^2	4609012	4688054	9297066
(Chromosome length)^2	225240064	233478400	458718464

Variance Correction Factor = $\frac{917362944}{100}$ = 9173629.44

Total Variance = 9297066 - 9173629.44 = 123436.56

Variance between strains = $\frac{458718464}{50}$ - 9173629.44

= 9174369.28 - 9173629.44

= 739.84

Source of Variance	Degrees of Freedom	Sums of Squares	Mean Square
Total	99	123436.56	124.68
Between	1	739.84	739.84
Within	98	122696.72	1252.01

$$F = \frac{1252.01}{739.84} = 1.69$$

Value necessary for significance : 254.32 for Fisher's 5% point.

5. Chiasma frequency in hybrid mice

Chiasma counts have been made in eight F-1 progenies from crosses between strains. Crosses were made of I ♀ X A ♂, I ♀ X dba ♂, and reciprocally between A and C57 Black (Bagg's subline), between A and JK, and between A and dba. The frequencies of chiasmata observed are listed in Table XI, and in the following pages are given an analysis of the figures and a comparison of the means obtained for the hybrids with those of the parent strains (as listed in Table V). These analyses show the following results:

(1). In all crosses of low with high frequency strains, chiasma frequency in the hybrid is like that of the low chiasma frequency parent, and significantly lower than that of the high chiasma frequency parent, regardless of the direction of the cross. The highest difference observed between the mean for the low chiasma frequency parent and the hybrid offspring is only 1.38 times its standard error.

(2). The two progenies of reciprocal crosses between two strains have the same chiasma frequency.

(3). The progeny of reciprocal crosses between two low chiasma frequency strains (A and dba) which themselves show a significant difference (see Table VI) have a lower chiasma frequency than that of the lower parent (dba). The difference between the mean for strain dba and that for the F-1 progeny of the cross A ♀ X dba ♂ is 2.41 times its standard error, and therefore approaches significance. The difference between dba and the progeny of the reciprocal cross (dba ♀ X A ♂) is highly significant, being 4.04 times its standard error.

TABLE X

CHIASMA FREQUENCY IN HYBRID MICE

Progeny of Crosses*

A X C Bl B	C Bl B X A	A X JK	JK X A	I X A	I X dba	A X dba	dba X A
26	26	28	30	29	27	29	25
27	28	32	27	28	28	23	29
29	29	27	29	34	22	28	26
26	24	26	35	29	28	26	24
27	30	26	37	33	24	27	23
26	25	28	28	29	26	26	25
28	27	30	29	25	24	23	27
31	32	29	23	32	28	24	22
23	33	33	32	32	26	30	25
24	28	26	26	32	29	26	23
25	29	32	26	28	27	30	23
26	28	28	31	28	33	30	23
28	28	29	27	25	28	25	24
32	32	30	27	30	24	24	26
32	25	26	32	28	24	24	25
34	27	29	30	28	28	24	31
32	27	24	29	32	30	27	26
28	32	29	29	29	26	23	25
30	28	30	25	26	28	25	23
34	28	30	29	30	32	25	27
27	28	29	28	34	32	27	25
26	30	30	25	29	29	28	26
28	26	27	28	30	27	28	26
25	27	32	24	25	28	24	28
27	28	34	27	31	31	23	24
TOTALS 701	705	724	703	736	689	649	631

* The female parent is named first.

CHIASMA FREQUENCY IN HYBRID MICE

1. Crosses between strains A and C57 Black Bagg.

	MEAN	STANDARD DEVIATION	$(\frac{\text{STANDARD}}{\text{ERROR}})^2$	ORIGIN
A	28.64	2.25	0.067	See Table II
C57 Bl Bagg	35.00	3.03	0.368	See Table II
A X C57 Bl B	28.04	2.95	0.347	Progeny of A♀ X C57 Bl♂
C57 Bl B X A	28.20	2.26	0.205	Progeny of C57 Bl♀ X A♂

COMPARISON OF MEANS

	DIFFERENCE	S	$\frac{\text{DIFFERENCE}}{\text{S}}$
Between A and C57 Bl B	6.36	0.66	9.64
Between A and A X C57 Bl	0.60	0.64	0.94
Between A and C57 Bl X A	0.44	0.52	0.85
Between C57 Bl - A X C57 Bl	6.96	0.85	8.19
Between C57 Bl and C57 Bl X A	6.80	0.76	8.95
Between A X C57 Bl and C57 Bl X A	0.16	0.74	0.22

2. Crosses between strains A and JK

	MEAN	STANDARD DEVIATION	(STANDARD ERROR)^2	ORIGIN
A	28.64	2.25	0.067	See Table II
JK	32.00	2.45	0.240	See Table II
A X JK	28.96	2.41	0.232	Progeny of A♀ X JK♂
JK X A	28.12	2.64	0.279	Progeny of JK♀ X A♂

COMPARISON OF MEANS

	DIFFERENCE	S	DIFFERENCE / S
Between A and JK	3.36	0.55	6.11
Between A and A X JK	0.32	0.55	0.58
Between JK and A X JK	3.04	0.69	4.41
Between A and JK X A	0.52	0.60	0.87
Between JK and JK X A	3.88	0.72	5.39
Between A X JK and JK X A	0.84	0.71	1.18

3. Cross between strains I and A

	MEAN	STANDARD DEVIATION	$(\frac{\text{STANDARD}}{\text{ERROR}})^2$	ORIGIN
I	33.60	2.57	0.088	See Table II
A	28.64	2.25	0.067	See Table II
I X A	29.44	2.58	0.266	Progeny of I♀ X A♂

COMPARISON OF MEANS

	DIFFERENCE	S	$\frac{\text{DIFFERENCE}}{\text{S}}$
Between I and A	4.96	0.39	12.72
Between I and I X A	4.16	0.59	7.05
Between A and I X A	0.80	0.58	1.38

4. Cross between strains I and dba

	MEAN	STANDARD DEVIATION	$(\frac{\text{STANDARD}}{\text{ERROR}})^2$	ORIGIN
I	33.60	2.57	0.088	See Table II
dba	27.26	2.00	0.080	See Table II
I X dba	27.56	2.70	0.291	Progeny of I♀ X dba♂

COMPARISON OF MEANS

	DIFFERENCE	S	$\frac{\text{DIFFERENCE}}{\text{S}}$
Between I and dba	6.34	0.41	15.46
Between I and I X dba	6.04	0.62	9.74
Between dba and I X dba	0.30	0.61	0.49

5. Crosses between strains A and dba

	MEAN	STANDARD DEVIATION	$(\frac{\text{STANDARD}}{\text{ERROR}})^2$	ORIGIN
A	28.64	2.25	0.067	See Table II
dba	27.26	2.00	0.080	See Table II
A X dba	25.96	2.29	0.210	Progeny of A♀ X dba♂
dba X A	25.24	2.04	0.167	Progeny of dba♀ X A♂

COMPARISON OF MEANS

	DIFFERENCE	S	$\frac{\text{DIFFERENCE}}{\text{S}}$
Between A and dba	1.38	0.38	3.63
Between A and A X dba	2.68	0.53	5.06
Between dba and A X dba	1.30	0.54	2.41
Between A and dba X A	3.40	0.48	7.08
Between dba and dba X A	2.02	0.50	4.04
Between A X dba and dba X A	0.72	0.61	1.18

6. Chiasma frequency in fostered mice.

Observations on chiasma frequency in twelve fostered litters comprising strains A, dba, I, JK, and C57 Black (both Little's and Bagg's stocks) are listed in Table XI. In the following pages will be found an analysis of these results and comparisons between each and the mean chiasma frequencies (as listed in Table V) of the strain to which the fostered mouse belongs and of the strain on which it was fostered. The analyses show the following results:

(1). Chiasma frequency in all mice of low frequency strains fostered on mothers of high frequency strains (A on Bagg's C57 Black, A on JK, A on I, dba on I) is the same as in unfostered mice of the same strain. The highest difference observed between the mean obtained for fostered mice and the mean for their own strain is only 1.24 times its standard error.

(2). Chiasma frequency in mice of high frequency strains fostered on mothers of low frequency strains (Bagg's C57 Black on A, I on dba, JK on dba) is lower than in the strain to which the fostered mouse belongs. The smallest difference observed is between Bagg's C57 Black and one test (CB1-4) on Bagg's C57 Black fostered on A, and is 1.92 times its standard error. The next smallest (between A and A fostered on dba) is 2.38 times its standard error. The others are all more than three times their standard errors.

(3). The same results are shown where two strains are used which, although both definitely low, nevertheless are different (A and Little's C57 Black, A and dba).

(4). The means for high frequency mice fostered on low

frequency mothers are not generally as low as those for the foster mother's strain. However, one test (CB1-5) on Bagg's C57 Black fostered on A shows a difference from A of only 2.55 times its standard error; the difference between dba and JK fostered on dba is only 2.80 times its standard error, and in the case of A fostered on dba, the result is identical with dba.

(4). In Bagg's subline of strain C57 Black appears a serious disagreement between counts on two different mice fostered on strain A (tests CB1-4 and CB1-5). Although these counts were made on brothers from the same litter, fostered at the same time and killed only a few days apart, they show a difference between means of nearly six times its standard error.

TABLE XI

CHIASMA FREQUENCY IN FOSTERED MICE

Test

A-5	A-4	A-6	CBl-4	CBl-5	A-8	A-7	I-4	D-3	JK-2	A-9	D-4
27	32	31	35	28	32	30	38	29	29	29	24
29	30	29	35	27	30	32	33	26	29	29	28
29	27	29	36	30	30	30	34	26	33	26	28
32	26	28	36	31	29	28	29	28	27	27	25
28	28	27	36	34	30	26	34	26	29	30	27
27	30	30	34	29	29	28	35	25	27	31	31
31	26	31	32	31	28	29	28	25	31	29	28
28	34	29	29	33	29	33	29	25	28	26	29
30	32	26	33	28	23	33	27	26	28	26	27
26	28	31	34	30	29	31	28	29	28	31	28
32	27	28	35	34	29	27	31	26	24	28	26
29	29	27	26	30	30	29	35	30	27	27	27
26	30	28	30	28	25	23	31	30	25	26	29
33	27	35	31	28	31	30	31	28	31	24	28
25	30	27	30	30	32	30	32	30	24	29	28
29	25	31	29	30	32	29	31	34	33	31	30
30	30	32	34	33	27	28	32	23	30	28	25
28	32	28	36	29	27	27	33	25	31	26	24
31	32	27	36	30	25	29	35	33	28	30	27
27	28	28	34	31	24	32	32	29	31	23	28
29	31	26	32	27	28	29	29	28	30	27	25
26	34	26	31	30	27	28	29	24	28	27	29
29	29	29	33	29	28	27	35	30	31	23	23
25	32	27	37	28	30	27	30	27	28	26	29
26	25	28	34	28	33	26	31	24	30	26	26
TOTALS 712	734	718	838	746	717	721	792	686	720	685	679

CHIASMA FREQUENCY IN FOSTERED MICE

1. A AND C57 BLACK (LITTLE'S)

	MEAN	STANDARD DEVIATION	$(\frac{\text{STANDARD}}{\text{ERROR}})^2$	ORIGIN
A	28.64	2.25	0.067	See Table II
CBL(Little)	30.11	1.98	0.039	See Table II
A-5	28.48	2.19	0.192	Fostered on C57 Black (Little's)

COMPARISON OF MEANS	DIFFERENCE	S	$\frac{\text{DIFFERENCE}}{\text{S}}$
Between A and CBL (Little)	1.47	0.33	4.45
Between A and A-5	0.16	0.51	0.31
Between CBL (Little) and A-5	1.63	0.48	3.40

CHIASMA FREQUENCY IN FOSTERED MICE

2. A AND C57 BLACK (BAGG'S)

	MEAN	STANDARD DEVIATION	$(\frac{\text{STANDARD}}{\text{ERROR}})^2$	ORIGIN
A	28.64	2.25	0.067	See Table II
CB1-3(Bagg's)	35.00	3.03	0.368	See Table II
A-4	29.36	2.57	0.265	Fostered when 3 days old on C57 Black (Bagg's)
A-6	28.72	2.13	0.181	Fostered since birth on C57 Black (Bagg's)
CB1-4	33.52	2.37	0.224	Fostered on A
CB1-5	29.84	1.97	0.156	Fostered on A

Comparison of means

1.20 0.47 2.55	5.16 0.72 7.17	0.48 0.65 0.74	1.12 0.58 1.93	3.68 0.62 5.94	CB1-5
4.88 0.54 9.04	1.48 0.77 1.92	4.16 0.70 5.94	4.80 0.64 7.50	CB1-4	
0.08 0.50 0.16	6.28 0.74 8.49	0.64 0.67 0.96	A-6		
0.72 0.58 1.24	5.64 0.80 7.05	A-4			
6.36 0.66 9.64	CB1-3 (Bagg's)				
A					

Three figures are given for each comparison.

1st figure : difference between means

2nd figure : "S" value

3rd figure : difference ÷ S

3. A AND JK

	MEAN	STANDARD DEVIATION	(STANDARD)^2 ERROR	ORIGIN
A	28.64	2.25	0.067	See Table II
JK	32.00	2.45	0.240	See Table II
A-8	28.68	2.51	0.252	Fostered on JK

COMPARISON OF MEANS	DIFFERENCE	S	DIFFERENCE / S
Between A and JK	3.36	0.55	6.11
Between A and A-8	0.04	0.56	0.07
Between JK and A-8	3.32	0.70	4.74

4. I AND A

	MEAN	STANDARD DEVIATION	$(\frac{\text{STANDARD}}{\text{ERROR}})^2$	ORIGIN
I	33.60	2.57	0.088	See Table II
A	28.64	2.25	0.067	See Table II
A-7	28.84	2.29	0.210	Fostered on I

COMPARISON OF MEANS	DIFFERENCE	S	$\frac{\text{DIFFERENCE}}{\text{S}}$
Between I and A	4.96	0.39	12.72
Between I and A-7	4.76	0.55	8.65
Between A and A-7	0.20	0.53	0.38

5. I AND dba

	MEAN	STANDARD DEVIATION	$(\frac{\text{STANDARD}}{\text{ERROR}})^2$	ORIGIN
I	33.60	2.57	0.088	See Table II
dba	27.26	2.00	0.080	See Table II
I-4	31.68	2.69	0.290	Fostered on dba
D-3	27.44	2.73	0.298	Fostered on I

COMPARISON OF MEANS	DIFFERENCE	S	$\frac{\text{DIFFERENCE}}{\text{S}}$
Between I and dba	6.34	0.41	15.46
Between I and I-4	1.92	0.61	3.15
Between I and D-3	6.16	0.62	9.94
Between dba and I-4	4.42	0.61	7.25
Between dba and D-3	0.18	0.61	0.30
Between I-4 and D-3	4.24	0.77	5.51

6. dba AND JK

	MEAN	STANDARD DEVIATION	$(\frac{\text{STANDARD}}{\text{ERROR}})^2$	ORIGIN
dba	27.26	2.00	0.080	See Table II
JK	32.00	2.45	0.240	See Table II
JK-2	28.80	2.35	0.221	Fostered on dba

COMPARISON OF MEANS

	DIFFERENCE	S	$\frac{\text{DIFFERENCE}}{\text{S}}$
Between dba and JK	4.74	0.57	8.32
Between dba and JK-2	1.54	0.55	2.80
Between JK and JK-2	3.20	0.68	4.71

7. A AND dba

	MEAN	STANDARD DEVIATION	$(\frac{\text{STANDARD}}{\text{ERROR}})^2$	ORIGIN
A	28.64	2.25	0.067	See Table II
dba	27.26	2.00	0.080	See Table II
A-9	27.40	2.26	0.205	Fostered on dba
D-4	27.16	1.95	0.153	Fostered on A

COMPARISON OF MEANS

	DIFFERENCE	S	$\frac{\text{DIFFERENCE}}{\text{S}}$
Between A and dba	1.38	0.38	3.63
Between A and A-9	1.24	0.52	2.38
Between dba and A-9	0.14	0.53	0.26
Between A and D-4	1.48	0.47	3.15
Between dba and D-4	0.10	0.48	0.21
Between A-9 and D-4	0.24	0.60	0.40

CONCLUSIONS AND DISCUSSION OF THE RESULTS.

1. The Consistency of results within strains.

The results shown above (Observations, Part 1) demonstrate that, except in Strain C57 Black, different tests on any given strain are in complete agreement with each other wherever duplicate tests have been made. This conclusion, drawn from eight counts made by Huskins and Hearne and eight additional ones by the writer, is justified since it has been shown that a test made by Huskins and Hearne and one by the writer from the same preparations gave identical results.

Such consistency between different tests on the same strain is to be expected since the mouse lines here investigated are highly inbred and therefore should show a minimum of intra-strain variation. In strain C57 Black, however, the chiasma frequency tests reveal that at least in this respect there is considerable dissimilarity both between sublines from different colonies and within one of them. The dissimilarity between Carrel's and Little's sublines is apparent from the work of Huskins and Hearne (see their Table III) while in the present work a distinct difference appears between each of these and Bagg's subline (see Table VI). Since the mice used for testing these three sublines were from different laboratories, these differences may be due to environmental causes (the effect of diet, for example, on chiasma frequency in mammals has never been investigated). On the other hand they may be under genetic control and may represent mutations which have arisen in the different substrains. Whether the substrains differ in their cancer incidence it is not possible to say because records are not available. Opinions of several investigators regarding

the strain, however, show some disagreement as to its cancer behaviour. Some of these have been outlined in the description of the strain (Material, Part 4).

The difference which appeared between two litter mates of Bagg's subline of C57 Black (see Observations, Part 6), which had received in every respect the same treatment, is particularly worthy of note. This difference indicates very strongly a high degree of variability in this stock. Since the nature of this work demands as a necessary assumption that all members of a strain are uniform, so that a single test may be taken as a reliable representation of its chiasma frequency, the results on Bagg's subline of C57 Black cannot be considered as having any significance for the problems at hand.

2. The correlation between high cancer susceptibility and low chiasma frequency in pure line mice nursed by their own mothers.

The conclusion drawn by Huskins and Hearne from their counts on ten strains of mice was that, with the exception of two related strains, a negative correlation exists between mammary cancer incidence and chiasma frequency. The present work shows that the results of chiasma frequency counts on two additional resistant strains support this conclusion (Observations, Part 2).

As discussed above, this work also provides additional counts which indicate that one of the exceptional strains of Huskins and Hearne (C57 Black) shows within itself marked variations in chiasma

frequency (v. supra) and therefore, taken as a whole, cannot be considered as having any real bearing on the question at hand. However, one subline (Little's) gives consistent results. Since it is classed as resistant, and its chiasma frequency, though lower than in other resistant strains, is nevertheless higher than that of any of the susceptible ones (as now appears from the larger number of cells counted: compare Table VI with Huskins and Hearne's Table III), it cannot be said to constitute an exception to Huskins and Hearne's conclusion but rather to support it.

The other exceptional strain (C57 Leaden) gives a chiasma frequency count consistent with previous ones. It is very significant that this strain is now classed as moderately susceptible rather than as extremely resistant (see Material, Part 6). With its present cancer record it is no longer an exception to Huskins and Hearne's conclusions, but actually gives them additional support. Indeed, in this case the chiasma frequency tests of Huskins and Hearne might be considered as a prediction justified by later developments, since they obtained a low count on this strain at a time when it was still classed as resistant.

Some points have already been noted which cast doubt on the reliability of the C57 family. The different behaviour of the several branches of this stock constitute a clear example of what happens when one subline of a strain, even though this is already highly inbred, is isolated and its progeny inbred inter se. The described cancer incidence of the derivatives C57 Brown and C57 Leaden is now quite different from that of the parent C57 Black stock

from which they arose as coat colour mutants at a time when C57 Black was already inbred. This difference can only be due to mutations occurring in one or more of the lines. That such mutations do occur means that continued close inbreeding is necessary not only to establish a stock but also to keep it relatively homozygous (cf. Haldane, 1937). C57 Black is a fast breeding strain which has been distributed during the last few years to a large number of laboratories throughout America and to some in Europe. It seems possible that in the course of breeding the very large numbers of mice required for this purpose mutations having an effect on cancer or chiasma frequency or both may have occurred which have led to segregation of factors in the members of the current generations and which might be held responsible for the results obtained in this work.

The cancer frequencies of the various inbred mouse lines described here, and of other lines, show that besides those strains with an incidence approaching 100%, and those showing virtually no cancer, there is also a series of intermediate strains having more of less high frequencies. This fact in itself is interesting because it demonstrates the complex genetical nature of the problem. With regard to the question in hand, it is significant since the chiasma frequencies found in the twelve lines examined in this laboratory also form a series, and this series coincides fairly well with the series into which the strains fall if grouped in order of their cancer incidence. The comparison is not very satisfactory since strictly comparable data on tumour incidence is not available for more than

a few strains, and the rather loose verbal descriptions supplied by the Roscoe B. Jackson Laboratory do not permit of accurate classification of the lines. Nevertheless, the similarity of positions on the two scales of the different strains (excepting Carrel's C57 Black, which was unofficially reported to show some internal tumours: see Material, Part 4) is very striking.

The work of Huskins and Hearne originally took into account only the mammary gland cancer incidence of the various lines. Since that time a number of facts have come to light concerning the internal tumour incidence in some of these strains, and the question arises as to whether chiasma frequency is correlated with mammary gland cancer susceptibility or with cancer susceptibility in general. As has already been seen, mammary gland carcinoma and other malignant tumour types seem to be controlled by different factors, both genetic and environmental (using this term in its broadest sense). The records of the frequency of malignant tumours other than mammary gland ones in the strains described here are not sufficiently complete (due, no doubt, to their less frequent occurrence when compared with mammary gland cancer and their greater difficulty of diagnosis) to justify any conclusions with regard to a possible correlation between chiasma frequency and internal tumour frequency, independent of mammary cancer incidence. Strains selected expressly for lung tumour incidence without mammary gland cancer, and strains showing a high percentage of spontaneous leukaemia, although they exist, are at present unfortunately not available. It is interesting to note in this connection that strain I, recently reported by Andervont

and Stewart (1937) as showing a high frequency of stomach lesions which are possibly malignant in nature, has one of the highest chiasma frequencies found.

3. Mitotic chromosome behaviour in resistant and susceptible mice.

The results given above (Observations, Parts 3 and 4) show that in a resistant and a susceptible strain there is no difference which is detectable by the methods employed in the amount of metaphase chromosome splitting or in metaphase chromosome length in spermatogonia.

The observations which were made on dividing cells of the spermatogonial layer and of the lymph nodes reveal, however, two interesting facts. One is that pre-anaphase splitting is present in almost every cell of these tissues. This implies that if a difference in chromosome behaviour exists between two animals, it must be a quantitative, not a qualitative, one. The chromosomes of spermatogonia have been described by a number of authors, some of whom have already been cited. Cox (1926) illustrates three cells from white and house mice in which a thickened appearance of the metaphase chromosomes was attributed to "precocious" splitting, but her figures do not show splitting to a degree comparable with the present material. Bryden (1932) has observed a split at early metaphase in spermatogonial cells of the rat. In other papers there is no indication of the presence of a split before anaphase. The difference between the present observations on spermatogonia and those of almost all other investigators is most readily explained by the difference of

staining technique employed. The staining method used here is one designed specifically to reveal the internal structure of the chromosomes and it is probable that the presence of two chromatids is obvious when stained in this way where it would not appear with the usual "bulk" chromosome stains (for example haematoxylin, or gentian violet not followed by Newton's method of destaining and clearing). The appearance of spermatogonial chromosomes stained in iron-haematoxylin to test this point indicates that the extensiveness of metaphase splitting observed here is largely due to differences in the visibility of the split due to the staining method.

The more rapid fixation and absence of "clumping" secured by the use of the smear method may also contribute to the difference between the present observations and those of others on spermatogonial chromosomes. It was observed that in sectioned material the metaphase chromosomes are less distinct in outline and rarely show a longitudinal split.

In the dividing cells of lymph node tissue, however, the separation of the half chromosomes at metaphase is in many cases so wide that it would be distinct with any method of chromatin staining. The large majority of investigations on mammalian chromosomes in normal cells have been made on testicular or embryonic tissue, since these are the most readily obtainable types in which dividing cells are plentiful. As shown above, precociously split chromosomes have not generally been observed in spermatogonia. In embryonic tissue, on the other hand, they have been seen, especially in the foetal membranes (Painter, 1924). In tumour cells they occur frequently

(v. supra). Descriptive work on chromosome behaviour in different normal mammalian tissues has, however, not advanced far enough to allow any conclusion to be drawn as to the significance of pre-anaphase splitting or separation in any one tissue.

A second observation of general cytological interest made in the course of the studies on spermatogonial divisions is that the amount of metaphase splitting in these cells is very variable. The number of split chromosomes per cell varies from two to twenty in one strain, and the figures obtained show no particular grouping around the mean or any other level of frequency, but form a more or less continuous series. So also do the figures for chromosome length taken from measurements of the chromosomes of the same cells (Observations, Part 4). These facts might be interpreted as indicating that the cells examined represent a series of stages from early to late metaphase, since it is commonly agreed that the metaphase stage is a long one. This possibility can only be tested by using some means of classifying cells according to their stage within the metaphase. The characteristic changes in degree of contraction of the chromosomes during the division cycle makes this a commonly used method of determining stage. In fact in many instances it is the only possible method. No correlation exists, however, between the number of split chromosomes within the cell and the degree of contraction of the chromosomes as indicated by their length. Therefore, unless degree of chromosome contraction is not an indication of earliness of lateness of the metaphase, it must be concluded that the observed variability between cells in relation to both these factors is due to chance (a term which includes, of course, all those factors of whose existence we have no knowledge).

The variability of the counts is sufficiently great to admit the possibility that a difference between strains may still exist in amount of chromosome splitting, but cannot be detected except by the observation of a very much larger number of cells. Since this possibility has not been eliminated, the lack of difference in the results obtained on the two strains cannot be taken as conclusive evidence that the chromosome splitting behaviour in each is the same. It must be interpreted as an indication that if a difference exists it is sufficiently small to be effectively masked in any sample of reasonable size by the inherent variability of the cells at metaphase.

An attack on the problem through the observation of time or amount of chromosome splitting in mitotic prophases has been considered. The split is visible at a relatively early stage in good preparations (see Figure 6), both in spermatogonia and in lymph node tissue, and in both of the strains examined. Here again any difference between strains must therefore be a quantitative one. The difficulty of analyzing prophase nuclei precludes a statistical investigation of the number of split chromosomes per cell at this stage. Furthermore, it is difficult to eliminate variations due to slight differences in the lateness of the prophase. It thus appears that this problem is not one which can be investigated by direct observation of the chromosomes. Possibly some indirect method may suggest itself.

If mitotic chromosome splitting is the same in susceptible and resistant mice, then the antepenultimate hypothesis of Huskins and Hearne, that early chromosome splitting is associated with high cancer frequency, breaks down. So also does their penultimate hypothesis that early somatic splitting is associated with loose

meiotic pairing, at least as tested in mouse spermatogonia and spermatocytes. Their ultimate hypothesis, however, that low chiasma frequency is associated with high cancer frequency, has been justified by their own observations and those of the writer shewn above (Observations, Part 2). Since the present results on somatic chromosome splitting indicate, but do not prove, that chromosome behaviour in this respect is the same in the two strains examined, the only conclusion to be made is that chiasma frequency is correlated with cancer susceptibility, but probably for reasons which are not the same as those envisaged in the original working hypothesis of Huskins and Hearne.

4. Chiasma frequency in hybrid and fostered mice.

The work of Little, Bittner, and their associates, discussed in an earlier part of this work, has shewn that susceptibility to mammary gland cancer in mice is inherited predominantly from the maternal parent, and that the extrachromosomal factor involved is transmitted largely through the milk. The counts made in the present work on chiasma frequency in hybrid and fostered mice (Observations, parts 5 and 6) indicate that this characteristic is not inherited in precisely the same manner.

The first point of difference is, that while the progeny of reciprocal crosses between strains differing in cancer incidence and chiasma frequency shew a cancer incidence approaching that of the

maternal strain, they have a chiasma frequency like that of the strain of the low chiasma frequency parent, regardless of whether this is on the maternal or the paternal side. In other words, as far as first generation hybrids are concerned, low chiasma frequency behaves as a simple genetic dominant over high chiasma frequency.

A second point of difference arises from the fact that, although chiasma frequency, like cancer susceptibility, is influenced by the milk which the young mouse receives, this influence is effective in the case of chiasma frequency in one direction only (Observations, part 6). Chiasma frequency in mice of low frequency strains fostered on mothers of high frequency strains is the same as in unfostered animals of the strain to which the fostered mice belong. The milk of a high chiasma frequency mother can therefore be concluded to have no effect on chiasma formation in mice inheriting factors for low chiasma frequency. But the milk of a foster-mother of a low frequency strain reduces chiasma frequency in mice which have inherited factors for high frequency. Thus the milk received by the young mouse may lower its chiasma frequency but cannot raise it.

If low chiasma frequency be considered as dominant over high chiasma frequency, and the milk of low frequency strains as having a depressing effect on chiasma frequency while that of high frequency strains has no effect, the results on hybrid and fostered mice can readily be explained. On these assumptions the progeny of any cross between a high and a low frequency strain would be expected to have a chiasma count similar to that of the low frequency parent. That

the true explanation is not quite so simple is indicated by the tests made on hybrids between strains A and dba, which, although both low frequency lines, nevertheless shew a difference in their mean chiasma frequency. In this case the progenies of the reciprocal crosses have chiasma counts lower than that of the lower parent strain, one of them significantly so, and one on the border-line of significance (2.41 times its standard error). These results suggest that a cumulative effect due, perhaps, to complementary factors, is present when both parent genotypes are of the kind which leads to low chiasma frequency.

While the results which have been obtained may be explained in this way, it is still entirely possible that chiasma frequency may be under the control of some physiological factor associated with heterosis and influenced also by some constituent of the milk. Bryden (1935 et seq.) demonstrated that in rats chiasma frequency is influenced by changes in the temperature at which the animal is kept and also by local temperature changes in the scrotal region. Many environmental factors, particularly temperature and moisture, affect chromosome pairing in plants, and the work of Oehlkers (1935 et seq.) has shewn that in Oenothera these agents act on the meiotic chromosomes through the physiological changes which they bring about in the plant as a whole. From the present data it is impossible to draw any conclusions as to what physiological factors may have an effect on chiasma frequency in mice. The most plausible possibility is that low chiasma frequency may be correlated with high rate of growth. This would explain

the low frequency observed in hybrids which, due to hybrid vigour, grow faster and mature earlier than inbred mice. Genes controlling growth rate are known to exist and it has been shewn (Goss and Gregory, 1935) that young rabbits which have been nursed have a higher content of glutathione (a substance whose concentration in the tissues is related to cell proliferation) than unnursed animals starved for forty-eight hours. The assumption that mice of high cancer frequency strains transmit through the milk an active agent while the milk of low frequency strains has a neutral effect would explain the chiasma frequency behaviour of the fostered mice. An alternative possibility is that the important factor is merely the amount of milk received by the young mouse.

The literature concerning the strains used in this investigation contains no data on their comparative rates of growth. However, from the relatively small numbers of mice bred in this laboratory during the last year there are indications that in general the low chiasma frequency strains mature earlier and breed faster than the high frequency strains. This certainly applies to strains A and dba as compared with I, F, and JK. Whether or not growth rate is really correlated with chiasma frequency must remain for the present an open question. If it is, then a correlation also exists between growth rate and cancer incidence - a not unreasonable corrolary in view of the influence on the appearance of malignant growths of metabolic and developmental factors such as age, maturity, reproductive activity and possibly diet.

Even if the interdependence of cancer incidence, chiasma frequency and growth rate be assumed, there is still no explanation for the low cancer incidence reported in hybrid mice from crosses between a resistant strain female and a susceptible strain male, unless the additional assumption is made that chiasma frequency can be influenced by chromosomal genes and by milk independently, while in the case of cancer susceptibility the parental genotype has little or no effect except through the milk. It thus appears that in order to reconcile the observed chiasma frequencies in hybrid and fostered mice, a number of postulates are necessary. The possibilities outlined are purely speculative, but seem the most reasonable ones in the light of the present results.

In conclusion it may be said that a correlation exists in mice between low chiasma frequency and high cancer susceptibility, but that neither the observed somatic chromosome behaviour nor the chiasma frequency in mice under experimental conditions known to influence susceptibility give any definite clue as to the reason for this correlation. It may be due to the chain of events involved in the working hypothesis originally used by Huskins and Hearne, but this, as they foresaw was possible, now appears unlikely. It seems more probable that the connection is through some physiological factor having a fundamental relationship to neoplastic growth.

SUMMARY

(1) Duplicate counts on chiasma frequency in certain of the strains of mice examined by Huskins and Hearne (1936) shew that except in one line (C57 Black) chiasma frequency is consistent in different generations of the strain.

(2) Counts on chiasma frequency in two additional resistant strains of mice have confirmed and extended the correlation found to exist by Huskins and Hearne between low chiasma frequency and high susceptibility to spontaneous mammary gland cancer.

(3) The two exceptional strains found by Huskins and Hearne are shewn to be no longer truly exceptional. Strain M-Leaden is now listed as having a cancer incidence consistent with its chiasma frequency in view of Huskins and Hearne's correlation. Strain C57 Black has three sublines each shewing a different chromosome behaviour. One (Bagg's) shews inconsistent results which are therefore of no significance for the general correlations; another (Carrel's) has a low chiasma frequency and is of doubtful resistance; and the third (Little's) has a chiasma count not inconsistent with its reported cancer incidence.

(4) No difference is detectable, by the methods of observation used here, in the time of chromosome splitting in spermatogonia of resistant and susceptible mice. The longitudinal split is visible,

both in spermatogonia and in the dividing cells of the lymph nodes, at a relatively early prophase stage, and at metaphase is present to a variable extent in every cell examined.

(5) In hybrid mice from crosses between high and low frequency strains, the chiasma count is the same as that of the low frequency parent. If two low frequency strains are crossed, the offspring may shew a count significantly lower than that of the lower frequency parent.

(6) Fostering of mice of low frequency strains on mothers of high frequency strains does not influence their chiasma count.

(7) Fostering of mice of high frequency strains on mothers of low frequency strains causes a significant lowering of their chiasma count.

(8) A tentative explanation of these results is put forward, and their possible significance is discussed.

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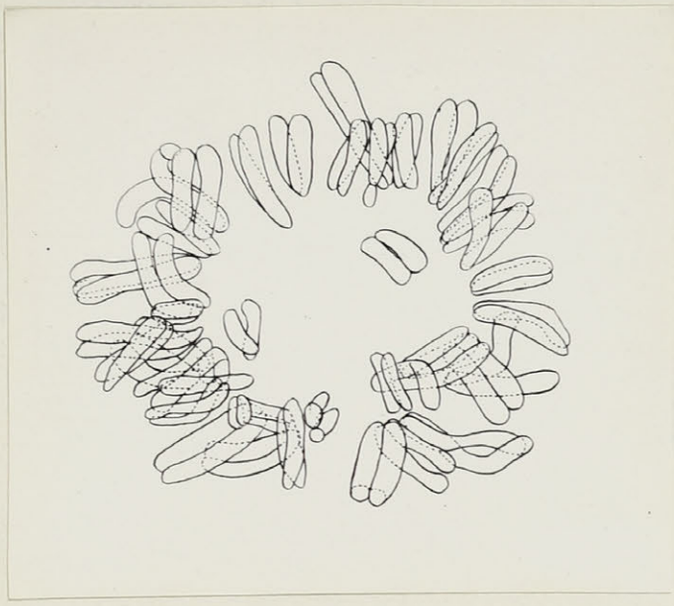

Figure 1

Camera lucida drawing of a cell at metaphase from a lymph-node of a mouse of strain N.

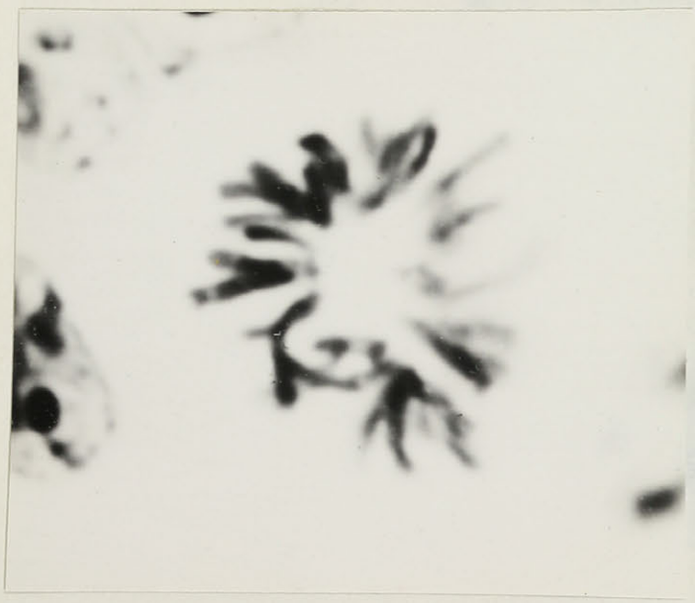

Figure 2

Photomicrograph of a metaphase cell from a lymph-node of a mouse of strain N.

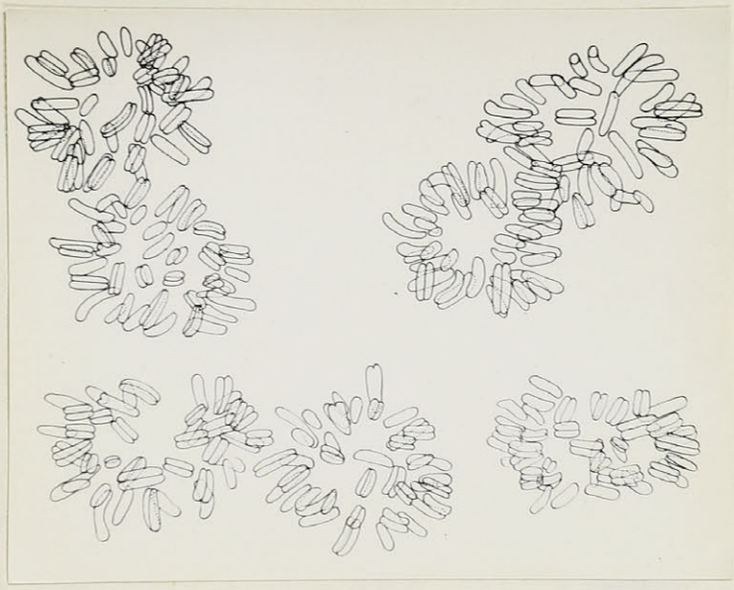

Figure 3

Camera lucida drawing of a group of seven spermatogonial cells at metaphase from a mouse of strain A.

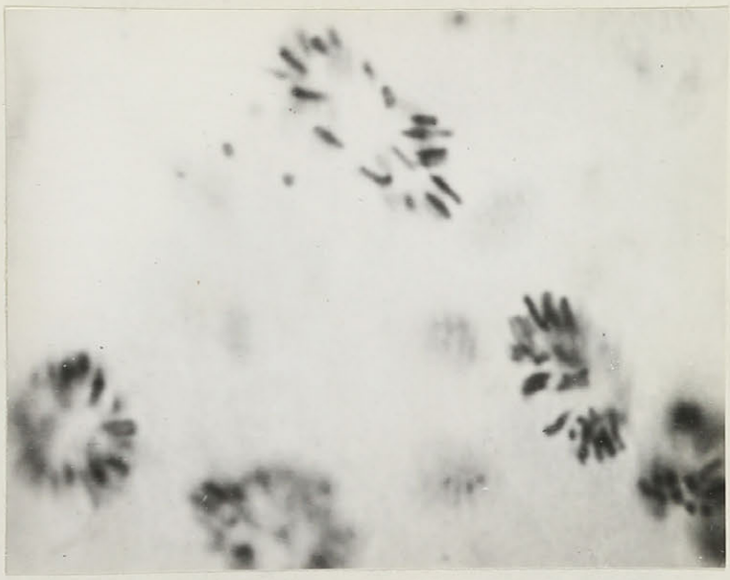

Figure 4

Photomicrograph of a group of spermatogonial cells at metaphase from a mouse of strain A. Splitting is clear in several chromosomes.

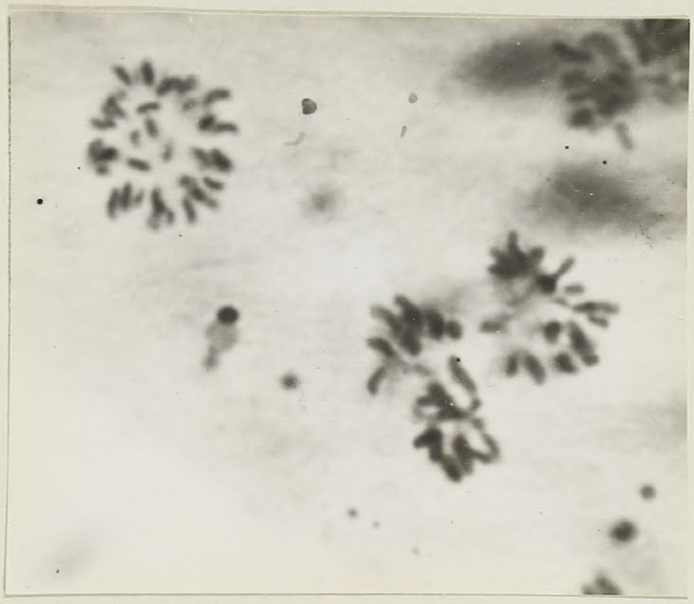

Figure 5

Photomicrograph of a group of spermatogonial cells from a mouse of strain CBA shewing the differences between cells in the thickness of the chromosomes. Splitting is visible in several chromosomes.

Figure 6

Photomicrograph of a mitotic prophase from the testis of a mouse of strain A. Splitting of the chromosomes is visible at several points.