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DEPARTMENT OF GENETICS

Cold Spring Harbor, Long Island, New York

A. F. BLAKESLEE, *Director*

The arrangement this past summer whereby the symposium of the Long Island Biological Association was devoted to a genetic subject gave the Department an opportunity to offer facilities of land for experimental plants to those plant breeders who were in residence in Cold Spring Harbor during this period. The following investigators were thus guests of the Department and grew on Departmental grounds the plants listed in parentheses: Dr. M. M. Rhoades, of Columbia University (maize); Dr. and Mrs. B. R. Nebel, of Geneva, New York, Agricultural Experiment Station (*Tradescantia,* snapdragons, and marigolds); Dr. Barbara McClintock, of the University of Missouri (maize); Dr. Harriet B. Creighton, of Wellesley College (maize); Dr. Edgar Anderson, of Missouri Botanic Garden (maize); Dr. Norman Giles, of Yale University (*Tradescantia*); Professor W. J. Bonisteel, of Fordham University (*Digitalis*). It has been of distinct advantage to the Department to have the association of such outstanding geneticists as were in Cold Spring Harbor during the summer.

A detailed report of the scientific work of the different research groups of the Department for the year ending September 1, 1941, is given in succeeding pages. These reports will be briefly summarized.

The group studying the gene have devoted considerable attention to the relation of genes to breaks in the chromosomes induced by radiation. Analysis of deficiencies in the Notch locus made by Drs. Demerec and Fano indicates that deficiencies up to about 15 salivary-gland chromosome bands in length may be caused by one-event process, whereas larger deficiencies are induced by two events. Thus a single-event process may have a radius of action of about 600 Å. An analysis of the data regarding breaks in chromosomes indicates that in about 10 per cent of cases a break affects the locus in its proximity. From a study of dominant lethals Dr. Fano is led to conclude that they cannot be entirely explained by single breaks in chromosomes, but that they frequently involve some as yet unobserved type of chromosomal rearrangement. Cooperative experiments with monochromatic ultraviolet radiation undertaken by Drs. Demerec and Hollaender show that a difference exists between the effects of the different wave lengths. Since the *Drosophila* males when subjected to treatment are easily injured by shorter wave lengths, these investigators have found it necessary with higher dosages to use the longer wave lengths. Difficulties are encountered in the use of ultraviolet radiations due to difficulties in penetrating the tissues surrounding the germ cells which are treated. When these difficulties are overcome the rate of gene mutations may be very high. Added evidence has been found to show that ultraviolet differs from X rays and radium in being ineffective in inducing breaks in chromosomes although it is effective in inducing gene changes. Drs. Kaufmann and Demerec have found that the amount of sperm transferred by a male of *Drosophila* in a single copulation was sufficient for the fertilization of a high percentage of eggs only during the first few days of egg-laying. When more than a single male had mated with a given female it was

found that differences in percentages of the different types of offspring obtained can be attributed to differential viability of embryos and larvae and not to differential viability of sperms leading to selective fertilization. Dr. Brehme found that a number of eye-color mutants in *Drosophila* affect the color of the larval mouth parts and spiracle sheaths and can be used as larval characters as well.

In the group which devotes especial attention to chromosomes, Miss Satina has found additional evidence that there are three distinct germ layers in *Datura* by means of periclinal chimeras, in which the layers are labeled by differences in chromosome number. By periclinal chimeras she has shown that the stamen is a reduced axis, and not homologous with a leaf as is the classic belief. In a study of chemical regulation of embryo development, Drs. van Overbeek and Conklin have developed a technique whereby they are able to take out proembryos less than 0.2 mm. in length and induce them to grow in vitro on artificial media. Others have grown nearly matured embryos in vitro, but no one before has succeeded with such small embryonic growths. A factor contributing to their success was the use of coconut milk, which supplied certain necessary substances. The technique bids fair to be of use in securing hybrid plants which normally abort at an early stage of embryonic growth. If the attempts being made to grow unfertilized egg cells in vitro are successful, a method of inducing parthenogenesis will have been secured. Miss Satina and Mrs. Sansome have shown that very few viable seeds are secured from the cross between a tetraploid and a diploid because most of the $3n$ embryos abort after about 6 days. Since both the $4n$ and $2n$ parents of the cross were from the same inbred line, individual gene differences seem to be eliminated as a cause of the early abortion. The difficulty in securing a cross between diploids and tetraploids is an isolating mechanism which tends to keep newly arisen tetraploids separate from populations of diploids from which they may have originated. Dr. Bergner has made a summary of her extensive study of the chromosome end arrangements in types from nature of six species of *Datura*. Most of the chromosomes are found to have suffered interchange of segments when all the species of *Datura* are considered. The number of interchanges which have taken place in any given species, however, is seen to be relatively small. A recessive gene type has been found in *Datura* which closely resembles in its external appearance the *quercina* virus disease of this species. The morphological similarities suggest a similarity in nature of the virus and the gene or in their method of bringing about changes in the host plant affected. Dr. Warmke has found new polyploid types in *Melandrium* which are being used in furthering our understanding of the sex mechanism in this dioecious species.

There seems to be increasing agreement as to which particular hormone from the pituitary gland stimulates the different special organs of the body. There is wide disagreement, however, as to which pituitary hormones are involved in generalized metabolic processes. For this reason Dr. Riddle and Mr. Opdyke, aided by Drs. Bates and Miller, of the group interested in the endocrine glands, have made a special study of the metabolism of carbohydrate and fat. They report first on the effect of the six fractions which may be obtained from fractional precipitation of pituitary extracts with ammonium sulphate, regarding which there is considerable though somewhat conflicting material in the literature. The original solution and the five fractions derived from it all contained four of the five hormones looked for, the origi-

nal solution having the greatest potency in increasing blood sugar. The fractionation brought about a concentration of prolactin in two fractions, and these were most effective in increasing liver fat in pigeons. Dried preparations increased acetone bodies in blood of rats, but fresh solutions were more effective in pigeons and rabbits. Only the fraction freest from both thyrotrophin and prolactin failed to increase heat production in pigeons. Fractions which others have found to cause experimental diabetes (the increased production of sugar and acetone in the blood) in the dog were shown in normal pigeons to have only temporary diabetic tendencies. Pigeons will tolerate huge doses of insulin, which soon give rise to symptoms of diabetes for which in mammals insulin is used as a cure. After two or three treatments the increase of blood sugar may amount to as much as 100 per cent. After the first injection blood acetone and liver fat are increased, but within 48 hours these effects are replaced by a large increase of liver glycogen. A single injection of prolactin results in a great increase of liver glycogen, and this increase is not maintained by later injections. From these findings Riddle concludes that no single hormone of the pituitary is responsible for the increased formation of acetone, blood sugar, or liver fat. Rather he believes they show that the normal regulation of metabolism of carbohydrate and fat is more seriously deranged when two or more participating hormones are present in unusual concentration and exert their stimuli simultaneously. Valid evidence seems lacking for any unrecognized pituitary hormone with specific action on the metabolism of either carbohydrate or fat. By injecting a minute quantity of the female sex hormone into a female dove shortly before the release of an ovum from her ovary, Drs. Dunham and Riddle found that male embryos from eggs thus treated are partly feminized. They develop left oviducts, which are normally absent in males, and the trace of ovarian tissue which male dove embryos normally carry temporarily on their left testis is both increased in amount and made to persist much later in life. It is suggested that the bisexuality shown by the trace of ovarian tissue on the left testis of males at hatching is itself the result of sex hormone passed by the mother into her maturing eggs. The present studies confirm the earlier view of Riddle that feminine characteristics shown by male doves are probably caused by excess female sex hormone which the egg receives from the mother.

The group studying mouse genetics reports the discovery of a monogenic mutation, screw tail, that promises to provide valuable material for the analysis of genic control of development in an extremely constant genotype. This group further reports that the apparent inheritance of a tendency to spontaneous leukemia can now be related partly to strictly genetic influence and partly to extrinsic influence depending on the nurse. The significance of this result has been established through the cooperation of Dr. John W. Gowen, who is carrying out statistical analyses of the interrelations among the extensive observations gathered to determine by breeding tests whether Mendelian segregation follows the outcross of a high-leukemia strain. Dr. Gowen's results indicate that genetic segregation does take place, but that the significance of the evidence depends on the strain of the nurse. The surprising feature of these results is that nurses from two strains in which leukemia has rarely been found have different effects on the appearance of leukemia. This observation led to a retabulation of the earlier data from F_1 matings between the strains used in the above outcross. Even greater differences

appeared, related to the same two strains of nurses, and the difference between reciprocal matings, reported earlier, was found to be eliminated when the strain of the nurses was held constant. The incidence of leukemia in this first hybrid generation varied from 74 to 50 to 35 per cent according to the strain of the nurses.

Dr. Steggerda in his studies in anthropology and human genetics has devoted considerable attention during the past year to characteristics of human hair in different racial groups. It has been shown that race, sex, age, and distance from the scalp are all factors in producing different widths in human hair. Steggerda has also made a careful study of the change in color of hair during a 10-year period for more than 400 children ranging in age from 6 to 18. During this age range there is a progressive darkening of the hair amounting to about one unit of the Fischer scale per year. This gives a correlation of 0.53 between hair color and age. Another study con
cerns Maya agronomy. An experimenta
plot, kept in corn for several years, i
gradually losing in productivity. In spit
of careful hand weeding, tough grasses ar
slowly invading the area. These growth
ineradicable by any means available to th
Maya, seem, in addition to the normal im
poverishment of a thin soil, to have force
the Maya to employ the so-called "milpa
system, under which any given piece c
land is left lying fallow for ten or mor
years. This system obviously demands s
large an amount of territory that popul
tions would be forced to move more ofte
into new areas than would be the cas
were fertilization possible, or could th
grasses and weeds be combated by plow
ing. The difficulties of agriculture, there
fore, seem to be a possible explanation
the ancient Maya's apparent inability t
remain in a given locality for more than
relatively short time.

CHROMOSOME INVESTIGATIONS

A. F. Blakeslee, A. G. Avery, A. D. Bergner, S. Satina, H. E. Warmke, J. T. Buchholz, M. E. Conklin, E. R. Sansome, E. W. Sinnott, and J. van Overbeek

Genes in Datura

In the last year's report a classification made by Avery was given of the genes distinguished in *Datura,* which now number approximately 500, with 77 located in particular chromosomes. The number of pollen-abortion genes which have been located has been increased so that we now have at least one pollen-abortion gene for all except two chromosomes and have two such genes for each of four chromosomes. They are of unique value in determining the position of other genes within a given chromosome through linkages, without the difficulties inherent in working with double recessives.

Similar Effects from a Virus and fro a Recessive Gene

One of the recessive gene types recentl
secured through X-ray treatment of seed
is of especial interest since it closely re
sembles in appearance the *quercina* viru
disease. Both the *quercina* virus and th
quercina-like gene cause the elimination o
spines from the capsule, the separation o
the tubular corolla into individual petals
the extension of the stigmatic surface o
the inside of the lobes and down two side
of the style, and the production of narrow
eroded leaves. Miss Satina has shown tha
the separation of petals in the flower b
both the *quercina* disease and the *quer*

cina gene type is brought about by the same mechanism, namely, a more rapid growth of the outer sides of the young petals, which are bent inward in the form of a letter U. The edges are thus prevented from meeting and fusing as they normally do in early stages in the bud. The *quercina* disease can be transmitted to a healthy plant by grafting, but this is not true of the *quercina*-like mutant type. It is apparent that genes and environmental factors may bring about similar end results through their effects on the developmental processes in the plant. The similarity of the effects of certain genes and viruses suggests that they alter the internal environment in similar manner. Both viruses and the determiners of heredity exist as discrete self-perpetuating particles, and both are subject to induced mutations. The similarity of their action supports the suggestion that the chief difference between the two may lie in the fact that the virus particle is free and hence capable of undergoing uncontrolled reproduction, whereas the gene is confined to a specific locus within the chromosome and its propagation is limited by the mechanism of heredity. The facts give support to the suggestion of an evolutionary relationship between viruses and genes. It should be emphasized that similarity of end results is not proof of identity of cause; but the similarity in the present case is so striking to one familiar with the expressions of the virus disease that it would seem profitable to explore the possible similarities of the two forms further. It would be of interest, for example, to test the chemical relations by means of the antigen-antibody reactions. Now that we have both the virus and the gene types under cultivation, it is hoped such further tests will be possible.

Periclinal Chimeras

In last year's report we spoke of the new insight into the manner of development and organization of the *Datura* plant gained by means of periclinal chimeras, in which the individual germ layers are labeled by having a different chromosome number from the adjacent tissues. This work has been extended by Miss Satina with the accumulation of additional evidence for our former conclusions and with new conclusions from new evidence.

In order to get some idea of the prevalence of periclinal chimeras, a random sample of 100 plants grown from seeds heavily treated with colchicine were tested for the constitution of their germ layers. Of the 100 plants, 47 appeared to be normal throughout; 11 were mixo-chimeras; in 33 plants about half the branches were normal and the other half were periclinal chimeras with one, two, or three of the germ layers having duplicated chromosome numbers. In 3 of the plants all the branches appeared to be periclinal chimeras with the germ layers affected in the same way; and in 6 plants there were several types of periclinal chimeras in the different branches. During the past year six new types of periclinal chimeras have been found, which, with those previously identified, make a total of fifteen types. The most useful for detailed studies on the development of various organs are those in which one of the three germ layers is octoploid ($8n$) and the other two normal diploid ($2n$).

Some morphologists who are not favored with an adequate method of labeling the individual germ layers have expressed some skepticism concerning our earlier conclusions regarding the germ-layer constitution of the shoot apex, especially our conclusion that the cells of the central core

are derived from the innermost (third) germ layer. It seemed desirable, therefore, to secure additional evidence regarding the matter. We are now able to report from study of over 200 periclinal chimeras. In all these cases the cells of the central core have the same chromosomal constitution as the third layer, and the evidence seems compelling that they are derived from the third layer. Our conclusion, therefore, stands that at least in *Datura,* the shoot apex has three and only three independent germ layers. The same three independent germ layers are also found in the floral apex, which shows no essential difference in structure from the shoot apex. The initial and early development of the sepal and petal is similar to that of the leaf, but that of the stamen is different and suggests that the stamen is a reduced axis. Detailed studies have been begun by the use of periclinal chimeras to determine the contribution which the different germ layers make to the development of the carpel and ovules. A study has also been started to find out the time and manner of differentiation of the three germ layers in the developing embryo.

Chemical Regulation of Embryo Development

Last year we reported attempts to secure haploid (1*n*) plants in *Datura* through the induction of parthenogenesis (development of the unfertilized egg cell) by means of injections of a wide range of chemical substances. The basis of our attempts was the fact that the *Datura* plant had shown itself capable of producing such 1*n* plants through some unknown but presumably chemical stimuli. Although we failed to find any method of inducing parthenogenesis, we were able to show that a number of succeeding stimuli are necessary for the full development of an embryo.

It seemed best to attack the problem th present year from a different angle, an Drs. van Overbeek and Conklin have thi summer been investigating the possibilit of taking out embryos at various stages o development and growing them on art ficial media. Since no method of growin very young embryos in vitro was know their first step was to find a medium whic would support growth from the proem bryo stage to maturity. An agar mediu containing nutrient salts, dextrose, an nine different vitamins and growth facto was found suitable for growing *Datu* embryos with partly developed cotyledon Younger embryos could not be grown c this medium. A unique method of gro ing young embryos was discovered, ho ever, by adding to the nutrient a natur endosperm in the form of coconut mil By this technique it has been possible secure the growth in vitro of proembry 0.15 mm. long which were taken out the embryo sac only 10 days after pollin tion. With the technique developed, it hoped to be able to remove the egg c from the embryo sac by a microinjecti apparatus and to obtain growth and dev opment on artificial media. If this can accomplished we shall have solved t problem of artificial parthenogenesis *Datura.* The success of coconut milk furnishing some accessory substance substances which stimulates the grow in vitro of excised embryos suggests applicability in other species. A method securing growth of young embryos on ar ficial media might insure the success many wide crosses hitherto impossib Such a technique would be of great th retical as well as practical importance.

Crossability between 4*n* and 2*n* Datu

When the first tetraploid (4*n*) plant *Datura* was discovered, in 1917, it v

labeled "new species" because of the difficulty of getting crosses between it and the normal parental type from which it had arisen. When the tetraploid was used as the male parent crosses were practically never obtained, and when the tetraploid was the female parent the number of viable seeds from the cross was extremely small. Although many hundred attempts were made to secure offspring from the cross $2n \times 4n$, only one seedling has resulted. Buchholz has shown that the primary block to this cross is the fact that the $2n$ pollen tubes from a tetraploid are almost never able to grow through the style of a diploid without bursting. The pollen tubes in the reverse cross, $4n \times 2n$, appear to function perfectly. Avery has summarized the records from the cross and finds that a total of 97 offspring have been recorded. Of these 54 were triploid ($3n$), 33 diploid ($2n$), and 10 tetraploid ($4n$). The diploids were undoubtedly all due to parthenogenesis of unreduced $2n$ egg cells, since in those cases in which the two parents were labeled by genes, the diploids were like the female parent. The tetraploids were possibly due to accidental selfing.

Miss Satina and Mrs. Sansome have been investigating the factors involved in the reduction of number of viable seeds from the cross between tetraploids and diploids. Tetraploids of *Datura stramonium* when selfed gave an average of about 88 seeds per capsule, but the $4n \times 2n$ cross gave an average of only about 3 seeds per comparable capsule. Examination of 291 ovules from an ovary 6 days after pollination showed that 70 per cent had a proembryo and endosperm, and that 18 per cent were degenerating and probably had had both a proembryo and an endosperm. Fertilization may have occurred in some of the remaining 12 per cent, which includes cases more difficult to classify. A high degree of initial fertility is thus evident. Chromosome counts of a dozen endosperm nuclei, each from a different ovule, showed the $5n$ chromosome number (60) expected following fertilization. Indications are present that union of male and female gametes takes place, although actual chromosome counts have not yet been secured in proembryos. It is probable, therefore, that the high majority of the egg cells from the cross are fertilized and start development. Shortly after the sixth day, when the proembryo is 3- to 4-celled and the endosperm 16- to 32-celled, the degeneration of both proembryo and endosperm is initiated. The degeneration is often accompanied by the enlargement and ingrowth of the somatic cells of the endothelium surrounding the embryo sac. Sometimes, however, the endothelial cells themselves break down before enlarging to any great extent. Both the diploid ($2n$) and the tetraploid ($4n$) strains used in the cross have come from the same highly inbred and homozygous line. It is not as yet clear, therefore, why the embryos which start development should degenerate in such a high proportion of the cases. It cannot be due to a difference in genic constitution of the male and female gametes which fuse to form the first cell of the proembryo—which seemed to be a plausible explanation for the failure of the cross between diploid *D. stramonium* and *D. metel,* earlier investigated by Miss Satina. It is suggestive, however, that in this latter species cross she found degeneration of embryo and endosperm frequently accompanied by an ingrowth of the endothelium which set in shortly after the sixth day following pollination. One finds qualitative differences between tetraploids and diploids, such as differences in the shape of leaves and capsules. It might be thought that the failure of the $3n$ embryo to develop in the majority of cases may be in some

way due to a difference in the ratios between the chromosome numbers of the different tissues involved in the development of the embryo.

By the use of periclinal chimeras it is possible to test an alteration of the chromosomal ratios in the opposite direction from those in the $4n \times 2n$ cross just discussed. As a result of the discovery that the tissue of the style through which the in the reciprocal cross $4n \times 2n$. Whereas in the $4n \times 2n$ combination the ratios shown in the accompanying table are less than those of the viable combinations ($2n \times 2n$ and $4n \times 4n$), the ratios of this $2n \times 4n$ combination are seen to be greater. The ratios between chromosome numbers in the different tissues of the ovule, therefore, do not appear to offer a clue to why the $3n$ embryos develop so infrequently into viable

CHROMOSOMAL RATIOS BETWEEN TISSUES OF DIFFERENT TYPES OF OVULES IN DATURA STRAMONIUM
(Plus (+) and minus (−) signs indicate ratios higher and lower respectively than those in the viable combinations.)

Type of ovule	$\frac{\text{Embryo}}{\text{Endosperm}}$	$\frac{\text{Embryo}}{\text{♀ tissue}}$	$\frac{\text{Endosperm}}{\text{♀ tissue}}$
Viable combinations:			
$2n \times 2n$	$\frac{2n}{3n}=0.67$	$\frac{2n}{2n}=1.00$	$\frac{3n}{2n}=1.50$
$4n \times 4n$	$\frac{4n}{6n}=0.67$	$\frac{4n}{4n}=1.00$	$\frac{6n}{4n}=1.50$
Mostly nonviable combinations:			
$4n \times 2n$	$\frac{3n}{5n}=0.60\ (-)$	$\frac{3n}{4n}=0.75\ (-)$	$\frac{5n}{4n}=1.25\ (-)$
$2n \times 4n$	$\frac{3n}{4n}=0.75\ (+)$	$\frac{3n}{2n}=1.50\ (+)$	$\frac{4n}{2n}=2.00\ (+)$
Parthenogenesis:			
$1n$ egg from $2n$ ♀	$\frac{1n}{2n}=0.50\ (-)$	$\frac{1n}{2n}=0.50\ (-)$	$\frac{2n}{2n}=1.00\ (-)$
$2n$ egg from $4n$ ♀	$\frac{2n}{4n}=0.50\ (-)$	$\frac{2n}{4n}=0.50\ (-)$	$\frac{4n}{4n}=1.00\ (-)$

pollen tubes grow is epidermal in nature and derived from what has been called the L−I germ layer, it has been possible to make the equivalent of a $2n \times 4n$ cross without bursting of pollen tubes by pollinating a $4n$, $2n$, $2n$ periclinal chimera with pollen of a tetraploid. The earlier stages in ovule development from this cross are being investigated, but judging from the aborted seeds and the very small number of good seeds in the mature capsules, it is reasonable to conclude that the processes of embryo disintegration are the same as seeds. The relative simplicity of this spe cial problem, with differences betwee genes eliminated, leads to the belief tha its solution may be of fundamental im portance to the more general evolutionar problem of the factors involved in crossa bility between species. Perhaps the studie now under way on the chemical regulatio of embryo development may offer an ex planation. Whatever the cause may tur out to be, the fact that it is difficult t secure a cross in *Datura* between a tetra ploid and a diploid from which it ma

have arisen is of interest to the student of evolution in view of the origin of so many species of plants through tetraploidy. It has been pointed out that the pure-breeding types which we had synthesized with extra chromosomal material should not properly be called "new species," since we had failed to provide them with an isolating mechanism which would keep them from crossing with the normal *2n* individuals in the population from which they were derived. We therefore doubled the chromosomes of these synthesized types and thus furnished the isolating mechanism demanded.

Effect of Tetraploidy on Crossability between Species

In a series of tests of the growth of pollen tubes of each of the 10 herbaceous species of *Datura* in the styles of the other 9, Buchholz has found certain combinations in which the tubes are prevented from reaching the ovary by retardation of growth or even bursting within the style. This past year he has made a similar study of pollen-tube growth of tetraploids of the same species, which had been secured by the use of colchicine. It seemed important to discover what, if any, effect doubling chromosome number might have on crossability, since the belief has been current that tetraploidy increases crossability between species. Buchholz' work gives no support for this belief so far as the growth of the pollen tubes of Daturas is concerned. In fact, in *Datura* the tendency is rather in the opposite direction, with the result that on the average relatively fewer tubes enter the ovary in a cross between *4n* than between *2n* races of a given pair of species. Preliminary tests indicate that formation of viable seed from species crosses is not favored by tetraploidy. If this turns out to be generally true, it will follow that when tetraploids arise in nature they will be no more likely to cross with tetraploids of other species than are the diploids of these species to cross inter se. We have seen from the studies of $4n \times 2n$ and reciprocal crosses discussed above that there is a distinct check to crossability between a tetraploid and a normal diploid from which it has arisen. These facts appear to have evolutionary significance.

Effect of Tetraploidy on Self-Fertility

The effect on fertility of doubling chromosome number differs according to the chromosomal and genic constitution of the plant affected. In general, doubling the chromosome number of a self-fertile plant such as *Datura* to form an autotetraploid results in some reduction in the number of viable seed produced. It is probable that this reduction is due in part at least to nondisjunction in meiotic divisions, leading to chromosomal deficiencies and excesses. An extreme example was furnished this past summer by cultures of *Nicotiana langsdorffii* grown in the garden. The *2n* plants set capsules freely, but the *4n* plants had almost no capsules at all.

One of the problems that a method of doubling chromosome number made it possible to study was the effect of tetraploidy on self-sterility. Much of the work on this problem was done by Dr. Warmke. Doubling chromosome number of a self-sterile plant in some cases may increase slightly the production of seed from selfing. Such an increase was observed in *Petunia.* Of 600 capsules from two *2n* plants grown in a screened greenhouse, 3 per cent had seeds, whereas of about 500 capsules from two *4n* plants, 55 per cent had seeds. In another variety of *Petunia,* 2 per cent of 327 capsules from *2n* branches of a single plant had seeds and 27 per cent of 187 capsules from *4n* branches of the same

plant had seeds. In neither *2n* nor *4n* capsules was there a full set of seed. The condition appears to be somewhat similar in *Nicotiana sanderae*. In other so-called self-sterile forms studied, the effect of tetraploidy was less evident. Thus there was no striking difference in the set of seeds from self-pollinating *2n* and *4n* plants of the following forms which are predominantly self-sterile: *Portulaca parana, Cosmos sulphureus, Rudbeckia hirta*. A difficulty lies in the fact that many, and perhaps most, plants which are classed as self-sterile do set a limited amount of seed from selfing. To discover an effect of tetraploidy on self-fertility in the forms just mentioned, which at best cannot be great, would require a more detailed statistical analysis than we have undertaken. In contrast with the uncertainties of the effect of tetraploidy on fertility of normally self-sterile species, doubling the chromosome number of forms such as species hybrids which are sterile on account of chromosome incompatibility brings about fertility, as is seen in the pure-breeding double diploid types we have synthesized in the genus *Nicotiana*.

Evolution of Chromosomes in Nature

Since the beginning of the *Datura* investigations it has been our belief that the genetic findings in our detailed breeding experiments should be related to the behavior of Daturas in nature. With this idea in mind we early began the study and collection of races from nature, first of *Datura stramonium* and later of other species of the genus *Datura*. Fifteen years ago Belling in his hypothesis of segmental interchange gave the clue to the chromosomal constitution of a type from nature which differed from the highly inbred Line 1 which we had been using as a standard in our cultures. Even before this, however, we had been able to distinguish this type by genetic methods. Belling's hypothesis, which has been amply confirmed by later study, showed that in the formation of two of the chromosomes of this type, now called Prime Type 2 (PT 2), the 1·18 and 2·17 chromosomes could be explained as having been derived by interchange of segments between the 1·2 and the 17·18 chromosomes of PT 1. Our method of testing interchanged chromosomes shows that a difference has occurred in the arrangements of their ends, but generally does not give positive evidence regarding the length of the segments involved. With the idea that the clue to the evolution of a species is to be found in the evolution of its chromosomes, an analysis has been undertaken of the chromosomes of the material in our collections. This analysis has been under the immediate supervision of Dr. Bergner. We have from time to time in past reports recorded the tests with certain individual species. It seems profitable at the present time to review the progress of our investigation of the whole genus.

We have first undertaken to make a study of the phenomenon of interchanged chromosomes in different species. These interchanges we have expressed in terms of the chromosomes of our standard line in *D. stramonium*, which we have listed in the accompanying table as Prime Type 1 (PT 1). Interchanged chromosomes are indicated by boldface type. In testing an unknown race for chromosomal constitution, we cross it with our standard PT 1. If the race in question contains a pair of interchanged chromosomes, such as the chromosomes 1·18 and 2·17 of PT 2, there will be seen at meiosis in the pollen mother cells a circle of 4 chromosomes involving the interchanged chromosomes of PT 2, in addition to 10 bivalents. By adequate tests it can be determined which chromosomes are involved in the circle. The table shows that including PT 1 there

INTERCHANGED CHROMOSOMES IN SIX SPECIES OF DATURA IN TERMS OF CHROMOSOMES OF PRIME TYPE I OF D. STRAMONIUM

Datura stramonium
(680 races)

PT 1 (275 races)	PT 2 (327 races)	PT 3 (62 races)	PT 4 (83 races)	PT 7 (61 races)	PT 87 (1 race)	PT 88 (1 race)
1·2	**1·18**	1·2	1·2	1·2	1·2	1·2
3·4	3·4	3·4	**3·21**	3·4	**3·12**	3·4
5·6	5·6	5·6	5·6	5·6	5·6	5·6
7·8	7·8	7·8	7·8	7·8	7·8	7·8
9·10	9·10	9·10	9·10	**9·10^{20}**	9·10	9·10
11·12	11·12	**11·21**	11·12	11·12	**11·21**	11·12
13·14	13·14	13·14	13·14	13·14	13·14	13·14
15·16	15·16	15·16	15·16	15·16	15·16	**15·23**
17·18	**17·2**	17·18	17·18	17·18	17·18	17·18
19·20	19·20	19·20	19·20	**19·20^{10}**	19·20	19·20
21·22	21·22	**12·22**	**4·22**	21·22	**4·22**	21·22
23·24	23·24	23·24	23·24	23·24	23·24	**16·24**

D. stramonium—continued

PT 94 (1 race)	PT 95 (1 race)	PT 2 + PT 3 (62 races)
1·14	**1·2^{20}**	**1·18**
3·4	3·4	3·4
5·6	5·6	5·6
7·8	7·8	7·8
9·10	9·10	9·10
11·12	11·12	**11·21**
13·18	13·14	13·14
15·16	15·16	15·16
17·2	17·18	**17·2**
19·20	**19·20^{2}**	19·20
21·22	21·22	**12·22**
23·24	23·24	23·24

D. quercifolia
(12 races)

Type 1 (6 races)	Type 2 (3 races)	Type 3 (3 races)
1·18	**1·18**	**1·18**
3·4	3·4	3·4
5·6	5·6	5·6
7·20	**7·20**	7·8
9·10	**9·2**	9·10
11·21	**11·21**	**11·21**
13·14	13·14	13·14
15·16	15·16	15·16
17·2	**17·10**	**17·2**
19·8	**19·8**	19·20
12·22	**12·22**	**12·22**
23·24	23·24	23·24

D. ferox
(16 races)

Type 1 (13 races)	Type 2 (3 races)
1·18	**1·21**
3·4	3·4
5·6	5·6
7·20^{16}	**7·20^{16}**
9·10	9·10
11·21	**11·18**
13·14	13·14
15·16^{20}	**15·16^{20}**
17·2	**17·2**
19·8	**19·8**
12·22	**12·22**
23·24	23·24

D. discolor
(4 races)

1·11
3·4
5·6
7·8
9·10
12·22
13·14
16·18
17·2
19·20
21·15
23·24

D. leichhardtii
(7 races)

Type 1 (1 race)	Type 2 (6 races)
1·18	**1·18**
3·4	3·4
5·6	5·6
7·8	7·8
9·10	9·10
11·16	.....*
13·14	13·14
15·12	.....*
17·2	**17·2**
19·20	19·20
21·22	.....*
23·24	23·24

D. pruinosa
(1 race)

1·18
3·4
5·6
7·8
9·14
.....
13·10
.....
17·2
19·20
.....
23·24

* These chromosomes are identical with those left blank in *D. pruinosa*, but their end arrangements have not yet been determined.

are 5 recurrent types of *D. stramonium* found in nature. There are also 5 prime types which have been found only once and hence may be considered sporadic. The interchanged chromosomes of one of these are not included in the table, since the chromosomes of the interchange have not yet been determined. The other species of *Datura* have been tested in much the same way. For each a tester type has been established as a standard, and with it the number of chromosomal types in the given species can be determined. Later by the use of proper testers from *stramonium* the interchanged chromosomes of these species have been determined. The interchanged chromosomes of *D. quercifolia* and *D. ferox* can be relatively easily determined, since there is no difficulty in getting F_1 hybrids with tester races of *D. stramonium*. *Datura discolor* also crosses readily with testers of *D. stramonium* when it is used as a male. There is considerable difficulty, however, in getting a cross between *D. stramonium* and *D. leichhardtii,* and this is possible only when *D. leichhardtii* is the female parent. *Datura pruinosa* will not cross with *D. stramonium,* but we have succeeded in making a partial analysis of its interchanged chromosomes by using *D. leichhardtii* as a bridging species and getting the chromosomes of *D. pruinosa* into the matrix of the *D. leichhardtii* protoplasm, which will allow crosses with *D. stramonium* testers. It will be noted that in the table there are blanks for 3 chromosomes in *D. pruinosa* and in the second type of *D. leichhardtii*. We know that the chromosomes in these two types are modified in the same way, but it will be necessary to make one further cross in order to be sure of the exact order of the interchanges involved. We have under investigation ten herbaceous species of *Datura*. In addition to those listed in the table, there are 3 chromosomal types among 42 races of *D. innoxia* tested; 3 types among 31 races of *D. meteloides;* and 5 types among 60 races of *D. metel*. *Datura ceratocaula* has only 1 type among 2 races tested. The last species mentioned we have not yet surely succeeded in inducing to hybridize with any other Daturas, although histological study shows that fertilization is initiated and seeds have been secured from crosses. The difficulty may lie in the low percentage of germination of seeds of *D. ceratocaula*. This difficulty has now been overcome for the pure species, and we hope to secure hybrids either by better methods of germination of the hybrid seed or by growth of young proembryos on artificial media by the methods of Drs. van Overbeek and Conklin.

It will be noted from the table that interchanges have been found in all species in which a sufficiently large number of races have been obtained. Not all the 12 chromosomes of our standard PT 1 are involved in interchanges with the same frequency. Thus it will be observed that, in the species in the table, the 5·6 chromosome is the only one not involved in an interchange. There are only 2 modified chromosomes related to the 23·24 chromosome, 3 to the 3·4 chromosome, and 4 to the 13·14 chromosome; yet there are 6 modified chromosomes related to the 17·18 chromosome, 7 to the 1·2 chromosome, 8 to the 21·22 chromosome, 9 to the 11·12 chromosome, and 10 to the 15·16 chromosome.

Our earlier table of the geographical distribution of prime types in *D. stramonium* shows that there is a certain amount of localization of these types throughout the world. Thus, PT 1, which is not duplicated in other species, is the predominant type only in the United States, Brazil, and Japan. Prime Type 2 is the predominant type in other parts of the world. Prime Type 3 is found associated with PT 2

throughout Peru. The 1 · 18 and 2 · 17 chromosomes of PT 2 are found in *D. quercifolia, D. ferox, D. leichhardtii,* and *D. pruinosa;* the 2 · 17 chromosome is found in *D. discolor.* It would appear, therefore, that the PT 2 of *D. stramonium* would have been a better standard in that it is more likely to represent the type to which other races and species are related than does the PT 1 which we chose as a standard. It is of interest to note that so far as the end arrangements of their chromosomes are concerned, the third type of *D. quercifolia* is identical with the races of *D. stramonium* from Peru, which are made up of a combination of PT 2 and PT 3. The modified chromosomes of the Peruvian type are also represented in the end arrangements of the first type and of part of the second type of this same species. They are also represented in *D. ferox.* One of the modified chromosomes of PT 3 and one of PT 2 are also represented in *D. discolor.* It is perhaps of significance that the geographical distribution of the Peruvian type, which runs up also into Mexico, overlaps the distribution of *D. quercifolia, D. ferox,* and *D. discolor.* There is certain suggestive evidence also from the morphological standpoint which would add some weight to the suggestion from the interchanged chromosomes that there is a relationship between these three species and the Peruvian race of *D. stramonium.* The last-two species in the table, *D. leichhardtii* and *D. pruinosa,* are obviously rather closely related in morphological characteristics. The first is endemic in Australia, the second is endemic in Mexico. *Datura pruinosa* seems to be closely related to especially the second type of *D. leichhardtii* in its interchanged chromosomes. Likeness of the end arrangement of their chromosomes cannot be used, however, as a dependable criterion of the taxonomic relationship. If the first type of *D. leichhardtii,* for example, were crossed with PT 2 of *D. stramonium,* there would only be a single circle of 4 chromosomes, indicating that all the chromosomes except 2 in the two species had the same end arrangements. Although segmental interchange has apparently not been the cause of differentiation of species, it has accompanied such differentiation and in some ways appears to be a factor in evolution.

In figure 1 are listed the configurations in a series of crosses involving the species *D. innoxia,* with other species and with testers of *D. stramonium.* These crosses will serve as an example of the extreme difficulty involved in determining the chromosomal end arrangements of the species which do not readily cross with the testers of *D. stramonium.* A single test now under way should determine the chromosomes left blank in the table for *D. pruinosa* and *D. leichhardtii,* which have been tested against two types of *D. innoxia. Datura innoxia* will not cross directly with *D. stramonium* but will cross readily with *D. leichhardtii.* Accordingly we have made repeated backcrosses of the *D. stramonium* testers onto *D. leichhardtii* in order to secure the *stramonium* tester chromosomes in a matrix of *leichhardtii* protoplasm. Our intention had been, after several backcrosses to *leichhardtii,* to isolate these tester races in homozygous condition from the selfed offspring of heterozygous parents. A difficulty has arisen in that we have found it difficult to transmit the modified chromosomes of the majority of these testers through the pollen, although they will come readily through the female parent. The heterozygous plants from repeated backcrosses are near enough like *D. leichhardtii* so that they will cross with *D. innoxia* when used as females. Half of the offspring should carry the tester chromosomes. When the crosses in-

dicated on the right-hand side of the diagram have been made and the chromosomal configurations in the hybrids determined, it should be possible to determine the chromosomal end arrangements of the races of *D. innoxia*. The same method is applicable to the races of *D. meteloides*, for which we have already made a considerable number of tests. A somewhat similar treatment should be possible for *D. metel* after the races of *D. innoxia* and *D. meteloides* have been cleared up.

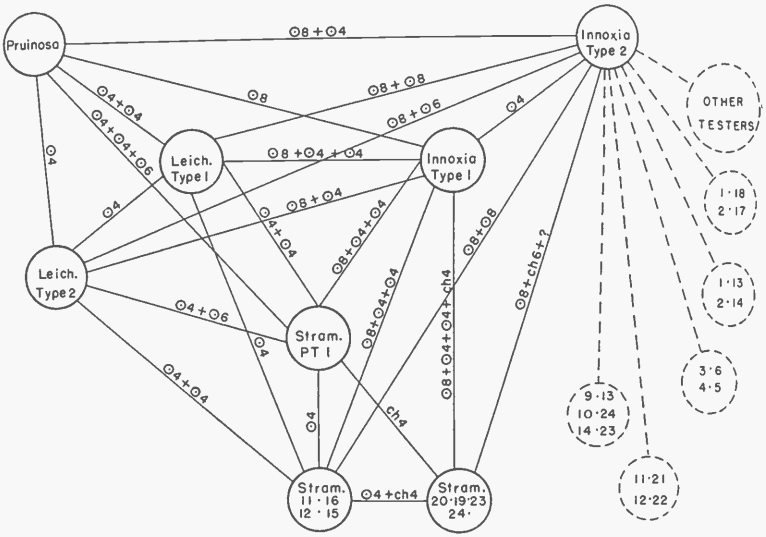

FIG. 1. Tests for interchanged chromosomes in *Datura innoxia*. The *2n* chromosome number in all Daturas is 24. Configurations of 4 or more chromosomes in F_1's are listed on lines connecting parents. Closed circles are represented by the sign ⊙ followed by the number in the circle. *Ch* is abbreviation for open chain. The paired chromosomes are not represented. Dashed lines and circles at right represent projected crosses to selected testers with modified chromosomes, which should complete the determination of the chromosomes in *D. innoxia*.

The study of the occurrence of interchanged chromosomes in species of *Datura* is the only investigation of this scope that has been undertaken with plants. It gives us, however, only a partial picture of the changes in the chromosomes which differentiate one species from another. Already Dr. Bergner has determined by the presence of chromosome bridges at meiosis that inversions are not infrequent both in hybrids between selected races in *D. stramonium* and in hybrids between different species. The extent of inverted segments in chromosomes may be perhaps better determined by two other methods. Dr. Bergner is endeavoring to work out a technique whereby the chromosome may be adequately stained for comparison of paired strands at early prophase divisions. If such a technique is discovered, it should be possible to match up homologous parts of adjacent paired chromosomes through chromomere similarity and to determine the extent of inverted sections as Dr. Mc-

Clintock has been able to do by use of special technique with maize chromosomes. Another method of determining inversion changes which is under way is the reduction of crossing over brought about by inversions, which we trust may be detectable through the use of pollen-abortion genes and viable gene-type testers.

Polyploidy and the Sex Mechanism in Melandrium

Dr. Warmke has continued his studies on sex differentiation in the dioecious species *Melandrium dioicum*. In this he has isolated two new chromosome types: 2A XYY (male) and the type with an extra fragment 2A XXX^{f}Y (hermaphroditic-male). The first of these is important in studying the synaptic relationships of two Y chromosomes in a diploid individual, which has not been possible heretofore. It also provides a means of comparing XY and YY synaptic affinities. The second type, 2A XXX^{f}Y, is important in providing confirmatory evidence for the presence of female genes in the X chromosome by completing the diploid series:

Chromosome constitution	Sex
2A XY	Male
2A XXY	Male
2A XX^{f}Y	(occasional ♀ blossom)
2A XXX^{f}Y	Hermaphrodite (occasional ♂ blossom)

THE GENE

M. Demerec, B. P. Kaufmann, Eileen Sutton, and U. Fano

X-ray and ultraviolet radiations are still the best known agents for inducing changes in genes and chromosomes, and they have been utilized extensively in the researches of the past year. In order to take full advantage of the physical possibilities, we have again cooperated with Dr. A. Hollaender, of the National Institute of Health, Bethesda, Maryland, in experiments with monochromatic ultraviolet radiation; with Dr. P. A. Cole, of the same Institute, in experiments with ultraviolet photometry; and with Mr. L. D. Marinelli, of the Memorial Hospital, New York, in experiments in which refined measurements of X-ray intensity were required.

Correlation of Data on Chromosomal Changes

It was suggested in Year Book No. 38 that the observed deficiencies produced by 2500 to 3000 roentgens, which involve the Notch locus, might arise by either of two different processes. The longer deficiencies would correspond to the usual chromosomal aberrations involving two independent breaks and would be classified as "two-event" processes. The short ones, which are particularly frequent, would arise from some different "single-event" process, and would correspond more closely to the mutations affecting the Notch locus which are not cytologically detectable deficiencies. Since the last report a large number of additional changes at the Notch locus has been accumulated, and these cases have been investigated by Dr. Demerec and Dr. Fano to determine the approximate length of single-event deficiences. Comparison of data by Dr. Kaufmann and Miss Bishop supports the theoretical expectation that the inversion and the deletion of any given chromosome fragment are equally probable. The existing data on the frequency of inversions enable us to evaluate the expected fre-

quency of two-break Notch deficiencies. Comparison with the Notch data shows that only those Notch deficiencies covering more than about 15 salivary-chromosome bands can be attributed to a two-break process. Shorter deficiencies must be due to some other process, whose frequency should depend, to a smaller degree, on the X-ray dosage. Spontaneous Notches should not involve two-break processes since the frequency of two-break chromosome rearrangements is negligible in untreated sperm. In fact, the largest deficiency observed among spontaneous Notches covers 13 bands, which is just below the limit of 15 bands set for single events. The length of 15 bands is about 6 microns in salivary-gland chromosomes and should probably be about 100 times smaller in the chromosomes of the sperm, that is, approximately 600 Å. Thus a single-event process may have a radius of action of about 600 Å.

The sterility induced in the sperms of *Drosophila* by radiation, that is, the production of "dominant lethals," is thought to be mainly due to chromosomal changes. Dr. Fano has considered, on the basis of the available data, whether most of the observed dominant lethals can be attributed to chromosomal rearrangements which are nonviable because they lead to the loss of large fractions of chromosomes, but are otherwise analogous to the observed viable rearrangements. A negative answer must be given to this question. Since, on the other hand, dominant lethals connected with no chromosomal rearrangement must be relatively rare, if they occur at all, one is inclined to conclude that dominant lethals frequently involve some as yet unobserved type of chromosomal rearrangement. Several facts, among which are the rate of production of dominant lethals at low dosage reported in Year Book No. 39 and the very low observed frequency of terminal deficiencies and losses of whole chromosomes, suggest the existence of some effect of single chromosome breaks which is more damaging than the simple loss of a part or the whole of a chromosome.

The correlation of dominant lethals with the observed chromosomal rearrangements requires a preliminary understanding of the production of such rearrangements. A further discussion of the existing data on cytologically detected rearrangements showed that the production of chromosomal rearrangements does not fit well into any simple scheme.

The order of magnitude of several quantities related to chromosomal changes has been evaluated by Dr. Fano on the basis of existing data. A chromosomal break occurring sufficiently close to a genetic locus may affect its phenotypical expression. An appreciable amount of data on the frequency of this process has been collected by Dr. Demerec, especially for the Notch locus. The frequency of breaks along all the chromosomes is approximately known from cytological data. One can then estimate that a fraction of the order of 1/10 of all breaks occurring sufficiently close to a locus affects its phenotypical expression. This fraction seems to be larger for the cut than for the Notch locus, but it appears to be independent of whether the locus is transferred into euchromatin or heterochromatin. In the case of heterochromatin, however, it is not necessary that the break be adjacent to the changed locus. Comparison of cytological data on the frequency of euchromatic X-chromosome breaks with the data on the frequency of sex-linked lethals shows that both of these frequencies are approximately 8 per cent in sperm treated with 3000 r X rays. Since at this dosage approximately one-third of the lethals have been shown to be connected with a chromosomal rearrangement, it appears that about one out of every three

X-chromosome breaks is associated with a lethal change.

Dominant Lethals

The experiments on dominant lethals by Dr. Fano and Dr. Demerec reported in Year Book No. 39 were continued and expanded. Attempts were made to investigate and reduce the sterility of untreated material, with a twofold purpose. On the one hand, any reduction of spontaneous sterility and of its variability affords a more accurate measurement of induced dominant lethals. On the other hand, full understanding of the sterility phenomenon is essential to an evaluation of its selective influence on the genetic material. Spontaneous sterility was not easily brought under control, and appeared to be affected by environmental, individual, maternal, and hereditary factors. Experiments on this subject are still under way. The large variability observed in the sterility phenomena might seriously affect the accuracy of the quantitative discussion of genetic experiments.

The semilogarithmic plot of the residual fertility as a function of X-ray dosage is approximately a straight line in the range of high dosage, between 5000 and 11,000 r, but does not fit a single straight line at all dosages. This seems to indicate that most of the sterility effect, but not all of it, is due to a "single-event" process.

The data of Drs. Demerec and Kaufmann on the sterility induced in mature and immature sperm were extended by further experiments. Young males were treated with X rays at 4000 r. Approximately 4000 sperms used shortly after their irradiation in fertilizing eggs gave only about 20 per cent viable embryos, but 3200 sperms tested 24 days later proved to be about 80 per cent fertile, that is, as fertile as the controls. This indicates that the formation of sperm carrying dominant lethals is selected against during the maturation divisions, as compared with the formation of normal sperms. No restoration of fertility should then be expected when the amount of sperms free from chromosomal changes is so small that it cannot produce any appreciable fertility even when the sperms carrying dominant lethals have been eliminated. This last expectation has been verified by experiments at 20,000 r or more, in which no fertility was detected at any time after irradiation; one would hardly expect that even a single spermatogonial cell would escape chromosomal changes.

High-Dosage Experiments

Since the experiments with dominant lethals showed the existence of an appreciable residual fertility among the sperms of males irradiated with 11,000 r, it was decided to conduct a series of experiments with heavy X-ray treatments up to 12,000 r. This project had a twofold purpose. On the one hand, the occurrence of hereditary changes among the surviving offspring of heavily treated males is expected to be so large that it affords an especially good opportunity to study a large number of individual changes per se. On the other hand, the comparison of all the changes obtained at 12,000 r with those obtained at the usual lower dosages (about 3000 r) might afford a good opportunity to detect any possible shift in the relative frequency of different types of changes. It was originally planned to investigate the following points: (1) the absolute and relative frequency of cytologically detectable changes associated with the phenotypical character Notch; (2) the absolute and relative frequency of chromosomal rearrangements associated with sex-linked lethals; (3) the absolute and relative frequency of different

types of cytologically detectable chromosomal rearrangements. The numerous experiments hitherto completed showed that the occurrence of chromosomal changes among the surviving offspring of heavily treated males is not consistently so high as was expected. This finding required a large amount of preliminary work directed to the investigation of technical factors which might reduce the observed frequency of changes.

A series of tests for sex-linked lethals among the offspring of males irradiated with dosages varying from 4000 to 12,000 r were carried out by Drs. Demerec and Fano. They did not succeed in duplicating the results of previous authors who had reported a frequency of lethals reaching as high as 35 per cent. The tests which were carried out with the standard ClB procedure did not yield a frequency larger than about 10 per cent.

The results obtained by cytological analysis of salivary-gland chromosomes in larvae derived from fathers receiving high X-ray dosages are also somewhat confusing. It was anticipated on two bases, (1) egg mortality induced by dominant lethals, and (2) extrapolation of the curves obtained in the 1000- to 5000-r dosage range, that a very high percentage of all larvae derived from fathers treated with 12,000 r would contain deranged chromosomes. However, the 41 per cent of altered sperm among the first sample of 100 glands analyzed by Dr. Kaufmann is essentially the same as the frequency obtained at the 5000-r level (see Year Book No. 37), although the number of breaks per 100 sperm (161) exceeds somewhat that obtained in the 5000-r material (125 to 144). Before any effort can be made to interpret such findings, a considerably larger amount of data must be collected, not only because of the low values but also because of considerable heterogeneity with respect to the number and types of breaks which were obtained in the three different experiments from which these 100 pairs of glands were obtained.

Comparison of Break Frequency Induced in Swedish-b and Oregon-R Stocks

Experiments by Dr. Demerec reported in Year Book No. 36 showed that when wild-type flies of the Oregon-R stock were treated with X rays, the frequency of X-chromosome lethals was lower than when several other wild-type stocks were treated. As an example, the rate for Oregon-R was about half of that for the Swedish-b. On the contrary, the frequency of dominant lethals induced by X rays, as measured by the death rate during ontogeny, was higher in the Oregon-R stock than in the simultaneously treated Swedish-b material (Year Book No. 37). Since dominant lethals are in general referable to chromosomal derangements, and since some of the sex-linked lethals may also involve gross chromosomal changes, a cytological analysis was undertaken by Dr. Kaufmann of the salivary glands of larvae derived from the two kinds of treated males. Material useful for this purpose was available from several other experiments in which Swedish-b and Oregon-R males were treated simultaneously with about 4000 roentgens and then mated with Swedish-b females. Analysis of about 245 pairs of glands from each of the two crosses yielded approximately equal percentages of altered sperms (35.5 ± 3.06 for Swedish-b and 38.62 ± 3.10 for Oregon-R). But whereas the Swedish-b glands showed 82.04 breaks per 100 pairs of glands, the usual value for 4000 r, the Oregon-R material gave 103.24 breaks per 100 glands. These values indicate that the types of changes observed following irradiation of the Oregon-R sperms are on the average

more complex than those detected following irradiation of the Swedish-b sperm. The reason for these differences remains to be determined. The possibility is now being tested that hybrid vigor in the Swedish-b/Oregon-R larvae is conducive to survival of more individuals containing complex chromosomal rearrangements than obtains in the homozygous Swedish-b larvae.

The *y-sc* Region of the X Chromosome

The cytogenetic analysis by Dr. Sutton of X-ray-induced changes in the *y-sc* region indicates that the *y* and *ac* loci lie within the region 1 A5-8 of the salivary map, and that the *sc* locus is probably associated with the doublet 1 B3-4.

Of 12 changes in the *y* locus, 4 were not accompanied by any detectable chromosome abnormality, 3 were due to deficiency, and 5 were due to "position effects." On the other hand, 14 *sc* changes included only 1 case which appeared cytologically normal and 2 cases of deficiency, the remaining 11 examples being due to "position effect" (gross rearrangements). The *sc* locus thus appears to resemble the *ct* locus, which is found to be very susceptible to changes in position, as opposed to the *y* and *N* loci, which are less frequently affected by such changes.

Recovery of Simple Breaks

In an attempt by Miss Maydelle Bishop to obtain simple terminal chromosome breaks in *Drosophila melanogaster,* advantage was taken of the fact that females hyperploid for the right end of the X chromosome in the Bar-carnation region are as viable as the normal female (Patterson and others, 1935). This allows one to design an experiment such that single breaks in the forked-carnation region have viability equal to or greater than that of double breaks involving this region and some other region of the X chromosome, in so far as their viability is determined by hyperploidy. Wild-type males were irradiated with either 2000 or 4000 roentgens and Bar males with 1000, 2000, or 4000 roentgens, and mated to yellow vermilion forked carnation (*y v f car*) attached X females. In all, 48,081 F_1 females were examined, among which 358 two-break hyperploid females (*v f car, f car, car,* and *v f* females) were found, and 21 apparent one-break hyperploid females (*y v f, y v,* and *y* females). Obviously the known double breaks outnumbered the apparent single breaks, although both the viability and chance expectation would lead one to expect the opposite result. It is impossible to say how many apparently single-hit cases are actually of that type, but some conclusions can be reached by analyzing the data. The probability that the *y v f* hyperploids are derived from deletions having a second break distal to the leftmost marker yellow may be tested by comparing that class with the *v f* hyperploid class. The probability of the right break in the forked-carnation interval is the same in each case, but for the left break there are only 3 or 4 bands to the left of yellow as compared with approximately 300 bands (counting double bands as one) between yellow and vermilion. As the left break approaches vermilion, the viability of the hyperploid *v f* females is decreased. But the interval from white to yellow, which has been shown to have little effect on viability of hyperploid females, has about 100 bands; therefore *v f* hyperploid females with the left break between yellow and vermilion should be many times as frequent as *y v f* hyperploid females with the left break between the tip of X and yellow. Actually they are only slightly more than twice as frequent (36 *v f* as compared with 16 *y v f*). Another possibility is that a second break may

be to the right of the centromere in the small arm of the X. The probability of this type of deletion should be about the same as for a second break in the region distal to yellow, unless the short arm is much longer than has been supposed, or has an exceptionally high break frequency. This comparison leads to the conclusion that some if not most of the apparent single breaks are actual. Supposing that single breaks are responsible for dominant lethals, the data of Fano and Demerec (1941) may be used to compute roughly the expected frequency of any class of single breaks. The calculated single-break frequency in the forked-carnation region is 0.08 per cent at 4000 r. Actually about 0.05 per cent are recovered. In other words, about 1 out of 20 single breaks "heals" and is picked up in the next generation. Calculations of Fano using Sutton's data on tip deficiencies, involving an entirely different region, length of interval, and method of detection from that in the present work, show the same factor of 20. The incidence of non-yellow deletions may be compared with Kaufmann's unpublished data on the occurrence of inversions involving the same region to test the assumption of Demerec and Fano (1941) that deletions and inversions occur with approximately equal frequency. The incidence of deletions and inversions agrees within a factor of about 2, the percentage of deletions being slightly higher.

Experiments with Ultraviolet Radiation

This is a cooperative project with the National Institute of Health, in which Dr. M. Demerec, Dr. A. Hollaender, Mrs. M. Houlahan, and Miss M. Bishop are taking part. During the past year experiments were completed in which *Drosophila melanogaster* males were treated with monochromatic radiation of 3050, 3130, and 3300 Å and the effect on hereditary changes was observed through lethals induced in the X chromosome. In the experiments done earlier and reported in Year Book No. 39, the effect of 2280, 2650, and 2937 Å was investigated. All these wave lengths are effective in producing lethals which may with good reason be assumed to be caused by changes in genes.

Some sterility was induced by all wave lengths tested, shorter wave lengths proving to be more effective than the longer ones. A sharp break in the effectiveness took place between 3050 and 3130 Å. Sterility is apparently due to injury produced in the tissues by radiation which penetrates the abdominal wall. The energy required to produce sterility at 2280 Å is about 2.1×10^6 ergs per cm.^{2}, and the sterility limit is so low that only 1 per cent of lethals can be obtained. At 3130 Å, energy of 83.5×10^6 ergs per cm.^{2} can easily be applied, since it sterilizes only about 50 per cent of the treated males. Such dosage induces about 5.5 per cent lethals. Yellow-white flies showed higher sensitivity to injury than wild-type flies.

For 3130 Å, data are available indicating that the genetic effect increases with the dosage applied, but that the interference of secondary factors induces a great deal of variability in successive experiments. This is probably caused by the absorption of radiation by the tissues which happen to cover the testes during irradiation. In occasional experiments the percentage of lethals obtained was as high as 50, indicating the possibility that ultraviolet may produce mutations at a very high rate.

In one experiment with 2650 Å, one translocation among 116 treated sperm was observed. An additional 762 tests of sperm from males which had been given similar treatment did not disclose any translocation. None was observed among 1073

sperm obtained from males treated at 3130 Å with energy which produced 5.5 per cent lethals. Thus these experiments gave further support to the assumption that ultraviolet radiation is effective in inducing gene changes but is not effective in producing breaks in chromosomes.

Preliminary studies of the absorption of ultraviolet radiation in the salivary-gland chromosomes have been made by Dr. Sutton in collaboration with Dr. P. A. Cole, of the National Institute of Health, Bethesda, Maryland. It has been shown by Caspersson and Schultz that the chromosome bands contain a protein component (indicated by an absorption maximum at 2800 Å) as well as the nucleic acid component (absorption maximum at 2650 Å). Caspersson (1940) has also shown that different types of protein are found in different regions of the chromosomes. The results obtained at Bethesda indicate that the concentration of protein, as well as of nucleic acid, in the bands is high.

Stocks raised at 18° C. show great variability of nucleic acid content, both between homologous bands and within bands.

In order to determine the relation of nucleic acid content to the phenomenon of variegation, it is proposed to measure the absorption of bands in a white-mottled stock carrying modifiers which enhance or decrease the phenotypic variegation.

Mottleds in the Second Chromosome

In the F_1 from *ri* p^p flies irradiated at 4000 r, an individual female was recovered by Mr. T. Hinton which showed an eye containing areas of disarranged facets lighter in color than peach, and within these areas, occurring singly or in groups, facets extremely dark or black in color. Two other similar flies were found in the first 9000 examined, but were not viable. A stock was obtained from the viable mutant, the salivary-gland preparations from which showed an interbrachial inversion in the second chromosome. The break in 2L is immediately proximal to the 26D1 band. The break in 2R is at 41A/B. Since the 41 of 2R is heterochromatic, the inversion causes both the bands which are proximal to and distal to the break at 26D2 to lie adjacent to heterochromatin. The aberration being in the second chromosome and the peach locus on the third, a separation of the peach locus from the mottled effect was undertaken by Mr. K. C. Atwood. The third chromosomes were replaced by wild-type third chromosomes by the use of dominant markers. The jumbled facets and black-spotted appearance were found still to occur with wild-type eye color and with Plum. The mottling effect was more extreme in flies raised at 15° C. than in those raised at room temperature. The aberration is lethal when homozygous. It has already been shown that heterochromatin can cause genes to show mottling. An attempt was therefore made in this case to discover which genes were near enough to the aberration to be thus affected. It is possible that clot (locus 16.5) is responsible for the dark-colored facets, and pied (17.5) for the roughness of the facets. This interpretation implies that the wild-type alleles are producing in the somatic cells a dominant *cl* and a dominant *pi* effect.

Terminal Adhesions in Salivary-Gland Chromosomes

A study has been made by Hinton and Atwood of terminal adhesions in salivary-gland chromosomes. Relative frequencies of the possible combinations differ in the Oregon-R and Swedish-b strains of *Drosophila melanogaster*. Data from the F_1 of Oregon-R males by Swedish-b females agree with those from Oregon-R, indicat-

ing a dominance of Oregon-R chromosomes with respect to terminal adhesions. When males were compared with females of their own stock, the autosomes were found to behave alike in both, but the percentage of adhesions involving the X chromosome was much smaller. When a Swedish-b stock carrying a homozygous deficiency for the tip of X was compared with normal Swedish-b, the relative frequencies of combinations were found to be altered. At least four different self-specific factors at the chromosome tips must be postulated to explain the *melanogaster* data. Chromosome arrangement in the nucleus and the state of chromosome ends in the unsmeared nucleus have both been eliminated as interfering factors. Chromosome length as a factor is being tested. From 200 cases of terminal adhesions found in *D. pseudoobscura,* a nonrandom occurrence of the various combinations was also established for this species.

Utilization of Sperm by the Female Drosophila

The analysis of the biological factors involved in sperm transfer and utilization reported in Year Book No. 39 has been extended. These experiments were undertaken to measure some of the determinable factors which might be responsible for the variability that occurred in different experiments in which males of *Drosophila melanogaster* had received similar treatment. Last year's report considered the time needed for the male to exhaust the supply of mature sperm. The considerable variability in the proportions of fertile and sterile eggs laid by different females following a single insemination by an irradiated male suggested that the production of dominant lethals might not be wholly responsible for the sterility, but that it might depend in part on the deposition of unfertilized eggs. A further study of this point has been made by Drs. Kaufmann and Demerec, using wild type, Swedish-b males which had not been irradiated. Each of these males mated successively with three females, A, B, and C. Following copulation the female was isolated and counts were made of the number of eggs which she deposited and the number of larvae hatching therefrom. It was found that the amount of sperm transferred in a single copulation is only sufficient to guarantee fertilization of a high percentage of the eggs laid during the first few days of laying, that on subsequent days the proportion of sterile eggs increases, and that toward the end of the egg-laying period practically all the eggs deposited are unfertilized. Similar data have been obtained using irradiated males.

An effort has also been made to measure the relative ability of sperms stored in the seminal receptacles to take precedence over sperms subsequently deposited in fertilizing the eggs. For these tests, plexus brown speck females were provided with wild type, Curly/Glazed, and *px bw sp* males in all the possible mating sequences. Among the progeny obtained, Curly and Glazed were slightly more frequent than the wild type, but *px bw sp* represented only about one-sixth of the total. Similar ratios were obtained when three females, one fertilized by each of the three types of males available, were permitted to lay their eggs in the same bottle. Since in these controls there could be no competition of different types of spermatozoa, it seems that the high frequency of wild type and *Cy* or *Gl* and the dearth of *px bw sp* flies hatching from the eggs laid by polyandrous females are to be attributed to differential viability of the different types of embryos and larvae, and not to differences in vigor or mortality among the various types of spermatozoa tested, which might lead to

such selective fertilization as Lobashov believed was occurring in similar experiments which he had conducted.

Larval Pigmentation

Pigmentation of the larval mouth parts and spiracle sheaths has been studied by Dr. K. S. Brehme in the mutants of *Drosophila melanogaster* which affect adult body color. In general, the mutants whose adult body color is lighter than wild type are characterized by lighter pigmentation of the larval mouth parts; the tan alleles, straw[2] and straw[3], and all the yellow alleles except y^{34c} show this effect. The greatest difference from wild type is seen in the cases of y^{35a} and y^{260-28}, although the adults of these genotypes are not markedly lighter in color than the other extreme yellow alleles, such as *y* or y^4. Certain of the mutations which lighten the body or bristle color of the adult do not affect the larval jaws: *svr*, T (1;2)*Bld*, *stw*, *stw*^{4}, and y^{34c}. Larvae whose mouth parts are markedly lighter than wild type are readily classifiable in the third instar, and the difference is already discernible at hatching from the egg. Of the mutants with darkened adult pigmentation, only the ebony alleles increase the pigmentation of the larval jaws. The ebony alleles considerably darken the spiracle sheaths at all stages of development.

The Malpighian-tube color of 33 eye-color mutants has previously been reported by other investigators. Because of the usefulness of this character in classifying larvae for experimental purposes, observations have been made by Drs. Brehme and Demerec of 66 additional mutants, virtually completing the list of factors affecting eye color. No direct relation was found between the amount of pigment in the eye and that in the tubes. Alleles which have a similar effect on eye color, for example the *ras* alleles, appear to have a similar effect on tube color, whereas alleles which have a different effect on eye color (the white series) affect tube color in varying degrees, and in this case dark eye color is not necessarily accompanied by dark tube color. Two useful chromosomal aberrations are readily classifiable in the larva: T(2;3)Pale (white tubes) and In(3L)persimmon (almost white).

Internal Anatomy

The internal anatomy and histology of adult wild type (Swedish-b) *Drosophila melanogaster* were studied by Dr. Albert Miller. As a contribution toward a monograph on the biology of this species, a descriptive account illustrated by drawings and photomicrographs has been prepared for each organ system, namely, the digestive system, circulatory system and associated organs, respiratory system, muscular system, nervous system, and male and female reproductive systems. Manual dissection of living and fixed material and serial microtome sections were employed in this study. Each system has been drawn as a whole as viewed in lateral, dorsal, or ventral dissection to show its component parts and normal topographical relations within the body, and photographs have been prepared to show the histological details as seen in stained sections. The description of each system includes an account of the organs, their location and interrelations, their microscopic anatomy, and the visible developmental or physiological changes that occur during adult life.

In conjunction with the study of the male reproductive system, a special investigation was conducted to determine the position of the testes in the living fly and the effect of compressing the abdomen to secure better exposure of the sperm-containing organs during irradiation with soft rays.

It was found that normally only certain portions of the testes lie near the body wall and the seminal vesicles are surrounded by the testicular coils; also that the degree of expansion of the crop influences the segmental position of the testes. Flattening the abdomen effects a maximal spreading of the testes against the ventral body wall when the crop is undistended by food. Thus optimal exposure of the testes to poorly penetrating rays should be attained by irradiating the ventral surface of undistended flies when the abdomen has been flattened by dorsoventral pressure. Cuticula, epidermis, a single layer of muscle fibers, fat tissue, and the testicular sheaths (totaling 20 to 60 microns in thickness in an uncompressed state) intervene between the sperm and the source of radiation.

Educational Project

Through the initiative of Dr. Bush, *Drosophila* stocks have been made available to laymen and students of biology for simple experimentation on heredity. It has been felt that such an experimental approach to the subject of heredity—at first, in all probability, for the verification of established principles—will help toward a better understanding of the fundamental laws concerned, and may lead subsequently to the collection of data applicable to the solution of current research problems.

A short article was published in the May 1940 issue of the *American Biology Teacher* outlining the new project. This article was abstracted by *Science Service, Science Digest,* and *Science Observer,* and thus made widely known. A 35-page pamphlet was printed by this Institution describing the life history of *Drosophila* and the methods used in breeding and in cytological research. Two sets of mimeographed directions for experiments were also prepared. The first set gave an outline for simple experiments showing monohybrid and dihybrid ratios, and the second set described more advanced experiments demonstrating dominance, linkage, and sex linkage.

In answer to each request, a copy of the pamphlet, the first set of outlines, and cultures with flies were sent, and when the first set of experiments was concluded the material for the advanced set was mailed on request. Extra copies of the pamphlet are on sale at the Office of Publications. A number of colleges used the pamphlet in the laboratory course in genetics, so that the first edition was soon exhausted and the second edition was printed early in the summer of 1941.

During the past year 220 shipments of cultures were made. They were sent to 34 states, the District of Columbia, Puerto Rico, and Canada. The largest number of shipments went to New York state (89), Pennsylvania (17), Illinois (14), Ohio (9), Kansas (8), Massachusetts (7), Missouri (6), and New Jersey (6). High schools and science clubs in high schools received 122 shipments, colleges received 33, and individuals 65.

ENDOCRINE STUDIES

O. Riddle, R. W. Bates, B. B. Wells, R. A. Miller, E. L. Lahr, G. C. Smith, H. H. Dunham, and D. F. Oppyke

Regulation by hormones occurs in perhaps all phases of life, and certainly it is very prominent in development, growth, reproduction, and bodily maintenance. The pituitary gland is known to assist in regulating all these four large aspects of life, though the number of its hormones and some of the effects produced by them

are still unknown. It is especially in the sphere of maintenance that the greatest uncertainty now exists as to the nature and number of participating pituitary hormones, and until these questions are clarified there will be doubt concerning the hormones actually concerned in the regulation of development and growth, and likewise a lack of understanding of the abnormality and disease which attend pituitary failure in the sphere of maintenance. For these reasons the present report, besides noting progress on other studies, gives more than usual attention to results of an examination of the hormones concerned in the metabolism of carbohydrate and fat.

Pituitary Influence on Metabolism of Carbohydrate and Fat

The anterior pituitary fractions obtained by ammonium sulphate precipitation which were found by Young (1939) to be either "diabetogenic" or inactive in the dog, and the similar fractions tested by Campbell and Keenan (1940) for their ability to produce fatty livers and ketosis in mice, have been extensively studied. At this time it seems that an intensive study of these particular fractions is the most promising way to obtain further light on the relation of the pituitary to experimental diabetes. We have therefore repeatedly prepared such protein fractions and carefully assayed them (2-day chicks, pigeons, rats) for prolactin, gonadotrophin, thyrotrophin, adrenotrophin, and posterior-lobe hormones, and also simultaneously tested each fraction for the several following actions: ability to induce (*a*) prompt (at 4 to 10 hours) or delayed (48 to 96 hours) glycemia in pigeons (normal, hypophysectomized, or adrenalectomized) and rabbits; (*b*) ketonemia in rats, pigeons, and rabbits; (*c*) deposition of liver fat and glycogen in pigeons; and (*d*) effects on the heat production and respiratory quotients of pigeons. In an intensive study of these related problems during the year Riddle and Opdyke have been variously aided by Bates, Miller, and Smith. Though parts of the data have not yet been analyzed completely, some of the significant results will be stated here.

Nature and actions of fractions from ammonium sulphate precipitation. Six fractions were obtained and each was studied both in fresh solution and as a dried powder. Four series of such preparations were made. In each case the starting point was, like that of Young, a pH 5.5 soluble fraction (little prolactin) obtained from an extract (pH 8.5) of fresh, quickly frozen beef pituitaries; this fraction, here called *A*, was used in this form for many tests. From an additional supply of this pH 5.5 soluble fraction five additional fractions (*B* to *F*) were prepared. The precipitate at one-third saturation with $(NH_4)_2SO_4$ provided on dialysis a soluble (*B*) and an insoluble (*C*) fraction. The precipitate next obtained from one-half saturation likewise provided on dialysis another pair of fractions, one soluble (*D*) and one insoluble (*E*). Finally, the protein which was precipitated at complete saturation and thereafter was soluble on dialysis ("albumen") was fraction *F*.

Fractions *A* and *D*, the "active" fractions of Young, contain measurable (and, in view of synergisms, probably non-negligible) quantities of gonadotrophin, thyrotrophin, adrenotrophin, prolactin, and posterior-lobe hormone; when freshly prepared they produce a ketonemia (10 hours after injection and 24-hour fast) which is considerable in rats, less in pigeons, and only slight in rabbits; stale and dried preparations lose ability to increase ketone bodies in the blood of pigeons; little loss was observed in rats. The two fractions are not equally potent in producing gly-

cemia in pigeons and rabbits, fraction *A* being more potent than any fraction derived from it. At 4 hours after injection of fresh fraction *A*, the blood sugar of 20 normal nonfasting pigeons was increased 45 per cent, and that of 6 hypophysectomized pigeons 41 per cent; the dry preparations were administered in somewhat larger doses and increased blood sugar by 37 per cent in 23 normal and by 31 per cent in 9 pituitaryless birds. All fractions, *A* to *F*, in both fresh and dried form, produce in nonfasting normal or hypophysectomized pigeons a glycemia at 4 hours after injection, and usually also at 10 hours in fasting birds (larger dosage with *C* and *F* was used to demonstrate this glycemic action); but daily injections of no fraction, in the dosage used by us, were able to produce a fasting hyperglycemia at the end of 4 days. Essentially the same results were obtained from New Zealand White rabbits. Especially at 10 hours the *F* fraction most consistently increased (30 to 52 per cent) the blood sugar of groups of rabbits. Toxicity of the fractions was indicated occasionally by fatal effects in both rabbits and pigeons. Fraction *A* ("diabetogenic" of Young) in particular, like some of our own "adrenotrophic" preparations, produces an early and temporary extreme glycemia—more than 100 per cent—in some (not all) normal and hypophysectomized pigeons, but we have demonstrated the still more significant fact that it does not increase the blood-sugar level of pigeons previously deprived of their adrenals.

It is notable that fractions *B*, *C*, *E*, and *F*, in either the fresh or the dried state, also increase (13 to 50 per cent) the blood sugar of groups of pigeons at 4 and 10 hours after injection; and here also this is not maintained to the third or fourth day. Again, most preparations of fractions *A*, *B*, *C*, and *E*, in the fresh and dried state, are notably ketogenic in rats, whereas dried *D* and *F* are almost free of this action. The more immediate effect of these fractions on the liver glycogen is not yet adequately studied, but in normal pigeons injected for 3 or 4 days (24-hour fast), unmodified values (0.10 to 0.25 per cent) were obtained only with fraction *C*, the others giving values with a range of from 0.45 to 1.95 per cent.

A consideration of the action of these fractions on the liver fat—a subject which has been investigated more widely with the aid of preparations of purified hormones—may be given simultaneously with a statement of the results of our assays of the hormones contained in the several fractions, *A* to *F*. All preparations of fractions *A*, *B*, and *F* contained detectable amounts of posterior pituitary hormones. The latter were also present in two of the four preparations of fraction *C*, and probably smaller amounts in three of four preparations of *D*. Only in the four preparations of fraction *E* did our tests uniformly fail to detect posterior-lobe hormone. Prolactin was present in *A* fractions to the extent of <0.05–0.18 unit per mg.; it was concentrated (0.22–0.50 unit per mg.) only in the *C* fractions, leaving quantities smaller than that found in *A* in all other fractions (except *B*). It is significant that fractions *C*, *B*, and *A*, with the highest prolactin content, were the most potent in the production of fatty livers in pigeons (normal, hypophysectomized, or depancreatized). Fractions *D* and *F*, containing the least prolactin, apparently have the least action on liver fat. On the other hand, fraction *E* contained very little prolactin (and no posterior-lobe hormone detectable by our method), but in both the fresh and the dried state it was fairly potent in increasing liver fat. The gonadotrophin found in fraction *A* (0.06–0.32 unit per mg.) was usually most concentrated in fraction *D* (0.19–0.74 unit per mg.) and least concentrated in fraction *C* (0.02–0.22

unit per mg.). The amount of thyrotrophin in *A* (0.21–0.79 unit per mg.) was always increased in the individual preparations of fractions *B* (0.53–1.44 units per mg.), *D* (0.29–1.18 units per mg.), and in three of the four preparations of *E* (0.14–1.58 units per mg.); *F* fractions (0.12–0.17 unit per mg.) contained least thyrotrophin. Our assays for adrenotrophin were least satisfactory. All fractions stimulated the adrenals of 2-day chicks, but in no case were the four preparations of any fraction consistently high or low, and often there was poor agreement between adrenotrophin values found in the same preparation in the fresh and the dried condition. The data, however, provide no indication that high adrenotrophin values were associated with either glycemic, ketogenic, or liver-fat potency. In this connection it should be noted that our assays of adrenotrophin, made on 2-day chicks and on rats, wholly disagree when extracts of posterior-lobe tissue are subjected to assay by these two methods.

The care given to the assay of these several fractions for five different hormones has resulted in practical certainty that every fraction thus derived from ammonium sulphate precipitation contains four of these hormones in measurable and variable amounts, and that most of them also contain posterior-lobe hormone in addition.

In line with what would be expected from their thyrotrophin and prolactin content, all fractions—except *F*, which contains least of both those hormones—after 1 to 3.5 days of treatment increased the nonfasting respiratory metabolism of the young pigeons on which these various studies were made. This failure of the *F* fraction to increase heat production gains added significance from the fact that it, like other fractions, increased the blood sugar of normal pigeons at 4 hours (40 per cent, nonfasting) and 10 hours (30 per cent, fasting) and of fasting rabbits (28 per cent) at 10 hours after injection. This indicates either (*a*) that thyrotrophin and prolactin are not primarily concerned in the causation of this quick and transitory glycemia in pigeons and rabbits, or (*b*) that one or the other of the two hormones must be present in larger amount or in a different proportion to induce such a glycemia, or (*c*) that the threshold for blood-sugar disturbance by these two hormones is lower than that required to increase the rate of heat production.

Combining tests made with the same fraction but after variable time and dosage, the following average heat-production values were obtained for normal birds: +30 (*A*), +12 (*B*), +77 (*C*), +1.3 (*D*), +41 (*E*), −12 (*F*). For small numbers of hypophysectomized birds these values were: +56 (*A*), +51 (*B*), +56 (*C*), +11 (*D*), +70 (*E*); no test was made with *F*. In general, it seems clear that these fractions increase heat production to a greater extent in hypophysectomized than in normal pigeons. Both fresh and dried preparations were thus active. The average of 47 determinations made with the fresh solutions on normal pigeons was +23.3; on 7 hypophysectomized, +43.8. In 47 tests on normal birds, dried preparations gave an average of +30.6; on 15 hypophysectomized, an average of +43.3.

Though it is evident that some hormone not assayed by us may be responsible for the experimental diabetes produced in certain other animals by fractions *A* and *D*, it is certain that the effective hormone (or hormones) was present in our *A* fraction, and it has been made evident that these fractions do not yield the full picture of such diabetes in the pigeons and rabbits used by us, though in some cases the tests were for 6 or more days. These birds, however, very temporarily yet very clearly show diabetic tendencies—glycemia, keto-

nemia, excess liver fat—after which the blood-sugar level is brought rapidly and completely under control. This control is likewise attained in birds deprived of their pituitaries, but, as in the cats and rats studied by Long and Lukens, a glycemia is not produced in birds deprived of their adrenals.

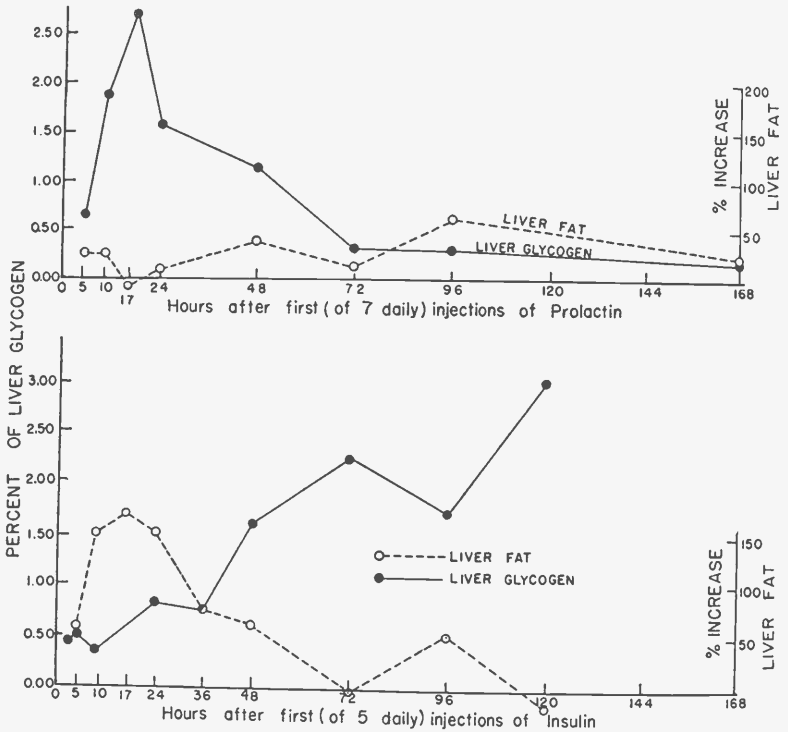

FIG. 2. Diagram showing the nature and extent of changes in liver glycogen and fat which follow the first and subsequent daily doses of 5 mg. prolactin or 30 units of insulin. The normal liver fat is taken as 100 per cent and increases are based on that value. All measurements were made on pigeons fasted 24 hours.

Prolactin and insulin actions on liver glycogen and fat. Much interest attaches to results of a study by Riddle and Opdyke of the effects of prolactin and of high doses of insulin on the processes of glycogenesis and lipogenesis in the livers of pigeons. Since pigeons withstand huge quantities of insulin, and their livers respond by growth to prolactin as to no other hormone, these animals are exceptionally suited to this study. The results obtained are sketched in figure 2. All values shown were obtained in birds at the end of a 24-hour fast, and in birds injected once each 24 hours.

It will be observed that prolactin mod-

erately increases the liver fat at 5 and 10 hours, though apparently not at the end of 17 and 24 hours. Under daily dosage thereafter a variable but considerable percentage increase of fat (the original amount of fat considered as 100 per cent) occurs along with a doubling of the size of the liver itself. The most remarkable effect of prolactin, however, is its swift, powerful, and nonrepeatable effect on the storage of liver glycogen. By the fifth hour after a *first* injection of prolactin the liver glycogen has been slightly increased (to 0.66 per cent) and after 17 hours it reaches a very extraordinary value (2.66 per cent), which is clearly decreased at 24 hours (1.54 per cent). Despite the repeated daily injections of the same dose of prolactin, the glycogen further decreases at hours 48 (1.13 per cent), 72 (0.35 per cent), 96 (0.29 per cent), and 168 (0.18 per cent). Thus the first dosage swiftly piles up available carbohydrate in the liver, but soon thereafter other mechanisms of adjustment come strongly into action and these reinforced mechanisms can prevent a recurrence of this abnormal storage of glycogen during light fasting. It has been shown that when fasting is prolonged to 48 hours, and carbohydrate is less readily available, the same dose of prolactin after 10 hours will only slightly increase (to 0.53 per cent) the amount of liver glycogen. These data for glycogen were obtained from 83 pigeons of the same age and race.

Results of the parallel study made to determine the effects of insulin on the liver fat and glycogen of pigeons, and particularly the time relations for these two effects, are also shown in figure 2. It is notable that the *first* injection of insulin (30 units per bird) is followed by a swift temporary increase in liver fat, and that the curve which describes its changes is essentially parallel with the curve for glycogen (not fat) storage following a first injection of prolactin. This action of insulin is also observed in hypophysectomized pigeons. Likewise a first heavy dose of insulin produces a marked ketonemia in pigeons, and this is not produced by dosage 2 days later. Initially, liver glycogen is unchanged or decreased by insulin, but we have observed a sharp temporary increase at 10 to 17 hours (not shown in the figure) and a durable extraordinary increase after 36 hours.

The "diabetogenic" actions of insulin. Insulin is much more than a cure for diabetes; the cure itself may be used to disrupt the regulation of carbohydrate and fat metabolism. Several years ago it was observed that pigeons tolerate huge doses of insulin and that, in some birds thus treated, the short period of very low blood sugar might be followed by a great excess of blood sugar. Extreme glycemias (increase of more than 100 per cent) have now been shown by Riddle and Opdyke to be the usual result of two, three, or four daily injections of pigeons with huge doses (30 units) of insulin. These glycemias persist during only a few days following a last injection, but in this they equal or excel any glycemias we have observed following the use of pituitary extracts. Again, a marked ketonemia results from the first of such insulin injections (10 hours after injection, 24 hours fast) at a time when the blood sugar is still somewhat reduced by the heavy dosage; after three daily doses, however, when a marked glycemia is present, no ketosis occurs. A similar ketonemia is obtained in rats. The extent to which injections of 30 units of insulin into normal pigeons result in an increase of both liver glycogen and liver fat has been noted in the preceding paragraphs. In hypophysectomized pigeons extreme fatty livers (3.66 times normal) are produced during the first 24 hours, and thereafter this action is progressively diminished; however, in such birds an increase of liver glycogen has been

found at no time between 10 and 120 hours.

Thus in normal pigeons four important changes in the metabolism of carbohydrates and fat—changes which are commonly described as results of administration of anterior pituitary substance—have now been observed to result also from high dosage with insulin.

Hormones which increase liver fat. Still other studies conducted during the past three years have sought to learn the action or lack of action of the various hormones on liver fat as this action may be detected in pigeons and rats. These studies by Riddle, Opdyke, and Senum are still in progress. Comparable results have not always been obtained in the two species chosen for study, and it is still uncertain whether apparent differences are real or whether only time relations and degree of response are different. It has been satisfactorily demonstrated that in pigeons liver fat is increased by estrone, desoxycorticosterone, and insulin; also to an irregular extent by prolactin, and consistently by some pituitary hormone or mixture of hormones other than purified prolactin, adrenotrophin, gonadotrophin, or thyrotrophin. Like Campbell and Keenan we find that liver-fat activity of pituitary extracts is separable from ketogenic activity, but unlike those authors we have been able to concentrate (though not completely to separate) liver-fat activity from a suitable pituitary extract by precipitation at one-third saturation of ammonium sulphate.

The results of the several studies described above seem to make it probable that no single pituitary hormone is responsible for either ketogenic, strongly glycemic (diabetogenic), or excess liver-fat activity. These results are in better accord with the view that normal regulation of carbohydrate and fat metabolism is more seriously deranged when two or more participating hormones are present in unusual or in unfavorable concentration and exert their stimuli simultaneously. Intensification of the activity of a single hormone is probably less disruptive to a very generalized and many-sided process than is intensification of contradictory activities in two or more organ systems. There is little or no valid evidence for the existence of an extra or otherwise unrecognized pituitary hormone with specific action on the metabolism of either carbohydrate or fat.

This study of carbohydrate and fat metabolism was aided by a grant from the Committee on Research in Endocrinology, National Research Council. Much technical assistance in these and other studies was given by Mr. Louis Stillwell, Jr.

Seasonal and Cytological Endocrine Changes

Cyclical response in viscera. It was noted last year that at two periods of the year, namely April-May and October-November, the crop sacs of pigeons give an especially low response to prolactin. Other data obtained by Bates and Riddle in connection with that study have now been summarized. The 239 White Carneau pigeons thus examined at short intervals over a period of two years were all sacrificed at the age of 7 weeks. Their body weight showed an *annual* variation, with a maximum in winter and a minimum in late summer. Weights of heart and adrenals showed no obvious cyclical or seasonal change. Length and weight of intestine at this age were found to be independent of body weight and essentially constant throughout the year. If these values are corrected to constant body weight, they would therefore seem to show annual cycles which are reciprocally related to body weight. When weights of pancreas

and liver are calculated to constant body weight, they, however, tend to show a *semiannual* cyclic variation similar to that found, with or without that calculation, in the crop sacs. The similarity of this seasonal variation in the response of pancreas, liver, and crop sacs to prolactin may therefore be regarded as still another indication that prolactin is associated with the growth or functioning of these three organs. On this view, however, one would expect the intestine to fluctuate similarly; actually, in these data intestinal length and weight show neither semiannual variations in response to prolactin nor dependence on body weight.

A stepwise change in thyroids. Though seasonal changes in thyroid weights of pigeons were reported from this laboratory nearly twenty years ago, and though the establishment of races of doves and pigeons characterized by hereditarily large and small thyroid size was reported five years later, the remarkable changes described below are notable despite the fact that they are not yet understood. Incidentally to making numerous assays of pituitary hormones during the past several years, the thyroid weights of large numbers of untreated (control) pigeons have been obtained by Bates, Riddle, and Lahr. White Carneau pigeons aged 6 weeks were obtained from the same commercial hatchery (Palmetto Pigeon Plant, Sumter, South Carolina) over a period of five and a half years, and thyroid weights from 862 such birds were obtained 7 to 10 days later. During the first two years of observation the average weight of the thyroids was uniformly close to 40 mg. Successive lots of birds received in the autumns of 1938, 1939, and 1940 showed a stepwise increase in thyroid without subsequent complete return to previous summer levels. Early in 1941 an average weight of 179 mg. was attained, and in the summer of 1941 the average was reduced to 60 mg.

The source or cause of these changes is largely unknown, but a combination of hereditary and environmental factors is suspected. The nature of the changes has received some attention. Incomplete histological data indicate that the later and larger thyroids of these young birds were functionally more active. A few old birds in the parent colony have been found recently to have extremely large thyroids, 13 to 14 g., which histologically seem practically inactive. Ninety-one determinations of the basal metabolic rate made by Smith and Riddle on young birds before and after the period of thyroid enlargement indicate an increase of only 5.5 per cent in the group having larger thyroids; eight such measurements made in 1941 on 2 adult goitrous pigeons from the same colony gave basal values 3 to 28 per cent below normal, but with indications that the nonfasting heat production is normal. During the past three years the thyroids of this widely used strain or type of pigeon have been wholly unsuitable for the bioassay of thyrotrophin, though they earlier gave clear evidence of suitability.

Cytological changes in adrenals. We have reported earlier on certain relations between the cytology of the pigeon's adrenal and its physiological activity. In experiments with insulin, continued from last year, Miller and Riddle find that as early as 10 hours after injection cytological changes become apparent in the cortex which are similar to those induced by adrenotrophin, and this stimulation has now been observed in the adrenals of hypophysectomized pigeons. Mitoses and cytological changes observed in medullary cells supply morphological evidence that these cells also are stimulated by insulin. These data apparently provide an explanation for re-

lated observations made in this laboratory. As noted elsewhere, Riddle and Opdyke find that large doses of insulin repeatedly injected into pigeons rapidly lose their ability to depress the blood sugar and indeed soon produce marked hyperglycemias. These facts suggest that both cortical and medullary portions of the adrenal gland actively oppose the hypoglycemic action of insulin. Recent tests have shown that adrenalectomized pigeons cannot tolerate large doses. The notable, and apparently unusual, ability of the pigeon's adrenal gland to respond to insulin may therefore provide an explanation for the resistance of these birds to doses of insulin which would quickly kill a mammal.

Pituitary and Blood Structures

The endocrine control of hemoglobin regeneration and the maintenance of normal distribution of leucocyte types in the blood has been the subject of preliminary investigations by Wells, Miller, and Riddle. Normal and hypophysectomized pigeons have been rendered anemic by bleeding and the hemoglobin concentration observed at intervals of three or four days. In the first of our studies it appeared that nutritional factors were obscuring the influence of the pituitary. To minimize such factors, all subsequent experiments have been conducted upon animals force-fed on 15 g. grain daily. It is found that hypophysectomized pigeons receiving large daily injections of whole anterior pituitary extract show a distinctly lower rate of hemoglobin replacement than do similarly operated animals when left untreated. It is suggested that the normal balance between hemoglobin formation and hemoglobin destruction is subject to pituitary control, but the final validity of this inference must rest on careful exploration of such factors as total blood volume, adequate supply of iron and protein, integrity of hematopoietic tissue in operated animals, etc. Our finding of lower hemoglobin values in birds treated with pituitary extract, and with prolactin, seems paradoxical, but it is in accord with observations made by Querido and Overbeek, who have reported that in rats the mechanism of blood destruction is activated by a pituitary product.

Blood smears from the ringdoves and Carneau pigeons have been studied and the percentage occurrence of the different leucocyte types has been tabulated. We have found it possible to recognize the following distinct forms of white cells: *A,* lymphocytes, small, medium, large; *B,* eosinophiles with rods; *C,* eosinophiles with granules; *D,* basophiles. The distribution of white-cell forms in the blood of pigeons is found so strikingly inconstant that conclusions based on apparent changes in relative occurrence would need the support of inconveniently large numbers of observations. It therefore becomes desirable to search for the factors responsible for the wide variations observed in normal birds and thus determine whether a particular distribution of cells is characteristic of an individual animal or is subject to periodic change. At this time we can only suggest that sex factors play some part in this connection. The male pigeon appears to have a higher percentage of eosinophiles with rods than does the female. A sex difference of this type has been noted by other workers in the leucocytes of chickens.

Hormones and Reproduction

In doves and pigeons the ovum breaks out of its enclosing membranes in the ovary—that is, it ovulates—at a fixed and known hour in the evening. Using this fact, Drs. Dunham and Riddle have studied the ability of several sterol hormones to

hasten, delay, or otherwise affect the process of ovulation in these birds. A single injection of the hormone was made at times varying from 5 to 34 hours before the normal hour for ovulation. From 23 tests of the action of the corpus luteum hormone, progesterone, and from 43 tests of desoxycorticosterone acetate (a synthetic product resembling hormones of the adrenal cortex), it was found that doses of only 0.1 and 0.05 mg. of these substances prevent normal ovulation. Both substances cause the egg membrane and the inner walls of the follicle to break at various places and thus permit the flow of yolk fragments into spaces formed within the follicular wall. Thereafter, the external walls of one or more of these spaces may break and permit the release of more or less yolk into the body cavity. These two substances also decreased the amount of shell which was placed on eggs ovulated prior to injection; the time during which such eggs remained in the shell gland was, however, not diminished. Considerably higher doses of androsterone (6 tests), dehydroandrosterone (42 tests), and estradiol benzoate (102 tests) sometimes produced similar effects on ovulation but had no detectable effect on shell formation. Estradiol benzoate showed a peculiar action in that in 26 of the 102 tests it caused a 24-hour delay in ovulation.

Other investigators have noted earlier that progesterone and testosterone will induce ovulation in certain toads (*Xenopus*) and frogs; and in some mammals hitherto tested the results resemble those observed here in birds. The mammalian studies have been thought to indicate that these sterols exert their action not on the ovary itself, but through suppression of output of gonadotrophin by the pituitary. The present results may be similarly interpreted. This is the more probable in the cases of progesterone and desoxycorticosterone, since studies reported below by Lahr and Riddle show that these substances cause a rapid and extensive atrophy of the testes of adult ringdoves.

In 1937 this laboratory reported that a commercial preparation of testosterone, Erugon, like prolactin, estrone, and progesterone, exercised an anti-gonad action on the testes of adult doves and pigeons. During the past year Lahr and Riddle have made further tests with crystalline hormones which confirm the earlier report for all the above-named hormones except testosterone, and the newer study further adds desoxycorticosterone acetate to the list of anti-gonad sterols. Four series of tests made with pure testosterone or its propionate, at various levels of dosage, have shown little or no anti-gonad action. Three series of tests with crystalline androsterone have all indicated that this hormone tends to increase the weight of the adult bird testis.

Hormonal Modification of Sex

Several years ago Riddle observed the presence of left oviducts in some male doves, and also certain elements of accentuated femaleness in female doves, which were hatched from eggs that matured in rapid succession ("crowded reproduction"). Somewhat later these effects were interpreted as actions of the increased amounts of estrogen which eggs (yolks) formed in rapid succession would probably contain. Others meanwhile have shown that the injection of estrogens into the albumen of eggs of fowl during early stages of incubation is followed by the development of left oviducts and also of ovarian tissue on the testes in genetically normal males. During the past year Dunham and Riddle have subjected Riddle's view to test. This was done by injecting estrogen into female doves at a time when they had in their ovary an ovum (yolk) which would con-

tinue its rapid growth for 26 to 34 hours before breaking from the ovary; thereafter these eggs were hatched, and the young were kept alive for periods varying from 1 day to 9 months, and then sacrificed to observe any modification of the sexual apparatus of male offspring. The tests were limited to the more easily measured changes induced in males.

Only a single injection of 1.0 or 0.5 mg. estradiol benzoate (or of dehydroandrosterone) was given, and this always at 26 to 34 hours before the second ovum of the dove's clutch of two eggs was to be ovulated. During that time the mother bird had opportunity to put portions of the added estradiol into the growing (second) yolk and perhaps later, after ovulation, to put more into the albumen which must form before the egg is laid. It should be noted also that the first egg of the clutch, though already in the oviduct at the time of injection, might have opportunity to take up small amounts of the injected estradiol into its albumen but not into its yolk. Quite in correspondence with the difference in the opportunity for the passage of estradiol into the two types of eggs, the males obtained from "first" eggs showed slight but perhaps significant effects in only 3 of 10 tests, whereas "second" eggs from the highest dosage (1 mg.) all showed (5 cases) significant effects, as did also at least 7 of the 12 cases derived from the lowest dosage (0.5 mg.). Thirty-one males from untreated eggs served as controls.

Effects were observable either in the oviducts or in the left testes, or in both oviduct and testis, in the test males killed at various ages from hatching to 7.6 months. One male at 9.2 months, from treatment with 0.5 mg. estradiol, seemed then to show no effect of the treatment. The left (sometimes also the right) oviducts often showed some degree of persistence, and in several instances oviductal remnants were wholly included in the walls of one or more distended, fluid-filled vesicles; these Müllerian vesicles were wholly characteristic of the treated males and were never found in the controls. In connection with this study we have found that the newly hatched dove, like the sparrow (Witschi) and robin (Unger), normally shows small amounts of ovarian cortical tissue on the left testis, and that this completely disappears within a few days after hatching. A significant effect of the estrogenic hormone on this tissue consisted in extending, both in space and in time, the development of this normal fragment of ovarian tissue in the left testis. Such tissue was not observed in the right testis in any case. It has thus been demonstrated that a female dove may herself pass estrogen from her blood into her maturing eggs, and that males hatched from eggs which receive an unusual amount of such estrogen may show, at least for some weeks or months, developmental effects of that hormone by possessing oviducts or by the persistence of ovarian tissue in their left testis. This new information clearly suggests that the small amount of ovarian tissue (bisexuality) often or usually present on the left testis of the bird embryo is itself the result of estrogen passed by the mother into her maturing eggs.

Genetic Hermaphroditism in Pigeons

The occurrence of genetically determined hermaphroditism in one strain of pigeons in our colony has been reported earlier. The breeding of this strain is of course being continued. During the past year some special study has been made by Riddle and Dunham of hermaphrodite and pseudohermaphrodite offspring of this

strain of birds. Originally a true hermaphrodite was found which proved to be capable of very limited breeding if used as a male. A normal sister of this hermaphrodite, when outcrossed to a male of another race, produced (first generation) one true hermaphrodite which, when bred as a male to a sister, threw 7 true hermaphrodites (second generation). Further breeding is already known to have yielded 3 true hermaphrodites in the third and 3 in the fourth generation. Offspring in all generations are of the following types: normal females, normal males, males with left oviducts, and males (true hermaphrodites) with ovotestis and oviduct on the left side and a testis on the right side. The ovotestes have been found in birds whose ages varied between hatching and 75 months. Biopsy proved the persistence of an ovotestis for more than 40 months. Of special interest is a considerable proportion of males having a left oviduct and two testes without tissue identifiable as ovarian; there is, however, some probability that such tissue was present at an earlier stage of the bird's life. Oviducts have been observed to attain a weight of 1.9 g., which is about double that of the oviduct of a normal female not actively producing eggs. Such development of oviducts in birds otherwise apparently male indicates that these birds are producing large amounts of estrogenic hormone and thus, on the basis of their internal secretions, their gonads also are partly female and bisexual.

MOUSE GENETICS

E. C. MacDowell, J. S. Potter, M. J. Taylor, E. N. Ward, and T. Laanes

Spontaneous Leukemia

In the investigation of the complex interactions of intrinsic and extrinsic influences on the incidence of spontaneous leukemia in mice, a second backcross experiment has been described (Year Book No. 39, 1939-1940), which was designed to determine whether or not the transmitted tendency leading to the spontaneous occurrence of leukemia is subject to Mendelian segregation—that is, under the control of genes in chromosomes. In this experiment the classification of 50 males in the first backcross to the low-leukemia strain was based on the incidence of leukemia among their respective progenies (total 2677 autopsies) in the second backcross to the low-leukemia strain. During the past year the histological diagnoses of these mice have been completed and extensive studies made of the data, which cover 14 criteria for each mouse. The final classifications confirm the preliminary conclusion that no single gene plays an outstanding part and, further, provide evidence of segregation in that the first-backcross animals show genetic diversity in regard to the appearance of leukemia. More than this, indications have been found of the nature of part of this genetic influence and of an important nongenetic influence.

The elementary analysis of these data by means of averages, subclassifications, frequency distributions, scatter diagrams, and other graphic arrangements indicated various heterogeneities and correlations within as well as among the various progenies, but the evaluation of the relative importance of these interrelations and the search for all possible clues to any sort of factor influencing leukemia required far more complex and laborious statistical analyses. This laboratory could provide neither the experience nor the technical facilities to carry out such an analysis with such large numbers. Very fortunately, Professor John

W. Gowen, of Iowa State College, while spending a few weeks here last fall as guest of the Department, became acquainted with these data. His interest in the general problem, as well as his long experience in dealing with parallel statistical analyses, and the organization of statistical machinery and calculators at his disposal, prompted his extremely generous offer to carry through the necessary analyses of these data. Accordingly all the data were coded, sent to Ames, Iowa, and there transferred to punched cards. Though the work has not been completed, certain important results have come to light.

The two inbred strains used in the cross differ in three pairs of genes for color, so that eight color classes appear in the second backcross. Each gene appears in four combinations with the others. The gene for dark eyes shows higher percentages of leukemia than does its mate for pink eyes in three of the four corresponding combinations, but the amounts of these differences do not reach statistical significance, since, with 1 degree of freedom, the X^2 is 0.9 with a probability (P) of 0.40. The gene for black, however (originating in the high-leukemia strain), gives higher percentages for leukemia than does its mate, the gene for brown, in each of the four corresponding combinations, and the gene for dilution (originating in the low-leukemia strain) gives higher percentages for leukemia than does its mate for intensity in each of the four corresponding combinations. These differences are significant since the respective X^2 are 8.9 and 11.6, which, with 1 degree of freedom, indicate probabilities of less than 0.001. In the analysis of the total variance of leukemia, however, the influence of each of these genes in determining whether or not a mouse dies of leukemia is found to be relatively small, in the neighborhood of 4 and 5 per cent.

In the original cross between these high- and low-leukemia strains, the first-generation hybrids with mothers from the high strain gave higher incidence of leukemia than those whose fathers came from the high strain. To observe genetic influences unconfused by such possible maternal influence, the mothers in each generation of the present experiment were taken from the purebred low-leukemia strain; that is, the only ancestor from the inbred high-leukemia strain was the great-grandfather of the mice under consideration. In order to produce the entire second-backcross generation in a short period of time, more than half were nursed by foster mothers from our albino strain, because these mice are exceptionally good nurses. No consideration was given to a possible influence of this foster nursing, for the appearance of leukemia in this strain is rare, as it is in the low-leukemia strain (StoLi) used in the cross. The totals, however, show a higher incidence of leukemia among those nursed by the albino-strain nurses (19.6 per cent) than among those nursed by the StoLi nurses (12.3 per cent). Within 34 of the 50 families the percentage of leukemia is higher for the albino-nursed young; in 12 families it is lower, in 3 it is equal, and in 1 family all the mice were raised by albino nurses. The albino-nursed young gave no leukemia in one family, and the StoLi-nursed young gave no leukemia in 10 families. That this difference is real is indicated by the X^2 of 24.1 with 1 degree of freedom, giving P less than 0.001. Since each family was not divided equally between nurses from the two strains, it is important to see how much the heterogeneity in the distribution of leukemia among the families of the different backcross sires is influenced by the

nurse. Counting only the young nursed by StoLi mice, the X^2 of 72.8 with 49 degrees of freedom gives a probability of 0.015; counting only the albino-nursed young, the X^2 of 134.7 with 49 degrees of freedom gives a probability of 0.00001. Thus the sires are shown to be heterogeneous in their ability to transmit leukemia whichever strain of nurse is used, but the significance of this heterogeneity is considerably greater when the albino-nursed young are considered. In the analysis of variance the F value (12.6) for effect of nurse is highly significant, and in the estimate of proportionate influence the contribution of the nurse seems to be about 20 per cent. Here, then, are different results from nurses from two strains in each of which leukemia is rare. An influence must be sought that is transmitted by nursing and yet ineffective in the animals providing such influence.

With this result in mind, the records of the original first-generation hybrids were retabulated according to the strain of the nurse. When the mother came from the high-leukemia strain the totals showed 61.9 per cent leukemia; when the father came from the high-leukemia strain the total showed 42.5 per cent leukemia. But when these reciprocal hybrids were raised by albino nurses the difference in percentage of leukemia vanished (51.3 and 52.1 per cent). Further, when the hybrids with high-leukemia fathers were raised by StoLi nurses the leukemia was reduced to 35 per cent. This is a greater difference between albino and StoLi nurses than was observed in the second backcross, but it is in the same direction and serves to confirm the result reported above. Hybrids from high-leukemia mothers were also raised by high-leukemia nurses, with a rise in the leukemia to 73.8 per cent. Thus the albino nurses have a more potent influence on the incidence of leukemia than do StoLi nurses, but nurses from the high-leukemia strain have a still more potent influence than the albinos. It appears that the effort to avoid the question of the influence of nursing has resulted in finding strong evidence of its effectiveness in influencing the incidence of spontaneous leukemia in our mice.

Dr. Gowen's analysis has brought out most impressively the influence of age factors. In the analysis of variance of leukemia, age stands out with higher significance than any other known effective variable (F 22.0 with degrees of freedom 1 and 18) and seems to contribute about 51 per cent of the total variance. That the sires differ genetically in their influence on the duration of life of their respective progenies is shown by the highly significant age differences. The ratio of the mean square of ages within families (degrees of freedom 2627) and between families (degrees of freedom 49) is $F=3.2$. Thus, from the point of view of what it is in the inheritance that contributes to the expression of leukemia, the major item appears to be the genetic constitution for duration of life passed on to the second-backcross progenies by their respective sires. But this is not the only effect of the inheritance, for after the duration-of-life effect has been taken out by corrections based on a life-expectancy table constructed from the massed data, there still remain other, as yet unknown, inherited characters which contribute to the appearance of leukemia and become especially effective when the mice are nursed by albinos.

Mitochondria in Leukemic Cells

Numerous investigators have estimated roughly that the mitochondria in individual malignant cells, as compared with normal, are increased in number and decreased in size. As a part of our cytological survey of leukemic cells, the mitochondria in

populations of lymphocytes of spontaneous leukemia, of transplantable leukemia, and of normal animals have been counted. Two observers independently made these counts in random samples of supravitally stained cells on each of ten slides from each source: 1000 cells from each of four lines of transplantable leukemia, 1000 cells from spontaneous leukemia, and 2000 normal cells. The frequency distributions of the counts were characteristically different, with statistically significant differences between the means. The mean number of mitochondria for a population of normal lymphocytes was found to be 21.57 ± 0.21, S.D. 6.95 ± 0.15; for a population from spontaneous leukemia, 27.93 ± 0.26, S.D. 8.40 ± 0.15; and for a population from a transplantable leukemia, 32.47 ± 0.28, S.D. 8.95 ± 0.20. In general, the greater the virulence of the population, the more numerous the mitochondria, but instead of a reduction in size, the increase in number is accompanied by an unquestionable increase in size.

These various populations, however, besides differing in malignant properties, have been shown to differ also in the proportions of cells in the various stages of differentiation. When the number of mitochondria was correlated with the degree of differentiation, it was found that the amount of mitochondrial material increased with the immaturity and the physiological activity of the cells, and that cells of the same degree of differentiation from all populations, whether normal or highly malignant, showed no difference in mitochondria. This cell characteristic therefore does not offer a specific criterion of malignancy.

Analysis of Leukemic Cells

Through the cooperation of Dr. Albert Claude, of the Rockefeller Institute for Medical Research, we have been able to gain information concerning the chemical and physical nature of certain fractions of leukemic tissues.

Dr. Claude has continued his study, mentioned in last year's report, of a fraction of leukemic tissues isolated by means of differential centrifugation. We have made further tests of its antigenic activity. This purified fraction is obtained after repeated high-speed centrifugation, and is composed of small particles which may be suspended in neutral media but will agglutinate in acid media. Microscopically, the variation in size among the granules is not great, and they may be stained with acid dyes when air-dried films are prepared. Dr. Claude has stated in published reports on similar fractions from other tissues that the particle size and proportion of phospholipids and proteins suggest that mitochondrial material is included.

Two sets of animals were injected with this material and tested for immunity reactions. The test dose of leukemic cells was given 10 days after the single injection in the first experiment and 10 days after the third injection in the second experiment. Transplantable line I leukemia was used for the production of the high-speed sediment and also for the test dose. For results see first table on page 249.

Dr. Claude has analyzed chemically the high-speed fraction obtained from two of our transmission lines, one of moderate virulence, line S, and one of high virulence, line I. The high degree of accuracy of the methods makes certain of the differences seem significant. See second table (p. 249).

Screw Tail Mutation

In mice the appearance of a mutation with little variability in somatic expression and in ratios is so rare that special mention is merited. In the present case

interest is attached to the demonstration of simple Mendelian segregation in a highly inbred strain without the conventional outcross. Indeed, the uniformity of the genotypic background after 50 generations of brother-by-sister matings may well be responsible for the value of the mutation and its excellence as a tool for the analysis of developmental processes. However simple the segregation, the effects of the screw-tail gene are far reaching. The tightly coiled tail gives an easy identification of the homozygous screw-tail animals at birth, but besides this, the gene so modifies growth and development that more than two-thirds die within the first four weeks and fertility is exceedingly low. Before birth there is some loss of males; this appears as a deficiency of 5 per cent in the natal sex ratios and as a reduction from the expected 25 per cent of screw tails to 21.1 per cent (of 649 mice) in matings between heterozygous normal parents. In the same matings 24.7 per cent of the 704 females were screw tail. Thus, however early the gene may start its activities, the serious handicaps appear largely after birth, so that the first steps of the study of the developmental physiology may be made with living animals. Indeed, the first problem arises immediately after birth in the consistent failure of the screw tails to fill their stomachs, even with competition removed and without any evidence of maternal discrimination.

Ratios of 3:1 are insufficient to prove genetic segregation, especially when the progenies are selected by the presence of mutants instead of being defined by the experimental procedure. This is the difficulty in interpreting human pedigrees. Accordingly, breeding tests to determine the ratio of heterozygous and homozygous normals become especially important. Of 59 normal females, sibs of screw tails, tested by matings with known heterozygous males, 20 were found to be homozygous normal and 39 heterozygous (expected 19 2/3: 39 1/3). Similar tests of 18 normal females from matings between homozygous and heterozygous normals showed that 7

ANTIGENIC EFFECTS OF HIGH-SPEED SEDIMENT FROM LINE I LEUKEMIA IN STRAIN C58 MICE

	No. mice	No. died	No. survived	Average interval before death (days)	Per cent survived
Experiment 1:					
Sediment, 1 injection	20	19	1	7.26	5
Controls	20	20	0	6.83	0
Experiment 2:					
Sediment, 3 injections	20	13	7	6.88	35
Controls	19	19	0	6.72	0

LEUKEMIA IN MICE (STRAIN C58): CHEMICAL COMPOSITION (IN PERCENTAGES) OF THE "SMALL PARTICLES" ISOLATED BY DIFFERENTIAL CENTRIFUGATION

Element	Line I	Line S
Nitrogen	8.30	8.38
Phosphorus	0.97	1.31
Carbon	52.55	53.10
Hydrogen	8.00	8.57
Ash	10.75	9.10
Sulphur	0.91	1.46
Copper	0.023	0.017
Amount purified fraction in spleen (dry weights)	10.1	10.8

were homozygous and 11 heterozygous (expected 9:9). In spite of the exceedingly low fertility of screw tails, a few litters obtained from screw tails and heterozygous normals included 15 screw tails and 14 normals.

ANTHROPOLOGY AND HUMAN GENETICS

Morris Steggerda and Henri C. Seibert

Anthropology

Corn production in Yucatán. The author's experimental cornfield near Chichen Itzá is now in its eighth year of production. The records of yield and cost of production are published elsewhere, but mention should be made that it is becoming increasingly difficult to raise corn on this plot. At present, weeds and the encroachment of grass, in spite of careful hand weeding, seem to make it impossible to grow corn for any considerable length of time in the Maya fashion at a profit. It is the author's belief that within a few years the weeds and grass will have become so firmly established that no corn can be grown at all on this field. Another factor which probably hinders the growth of corn in Yucatán after continuous production is that the soil is only a few inches deep. Thus, we have shown experimentally possible reasons why the ancient Maya were apparently seldom able to remain in a given locality for a long time before being forced to move to a new location; for if the population increased to such an extent that land was at a premium and old fields could not be left long enough for forest to grow back, thoroughly shade out the weeds and grass, and give the soil a chance to build up, a shift would have to be made to a less crowded area.

Human pedigree studies. This year the senior author has concluded ten consecutive seasons of work in the village of Piste, Yucatán. One project in this village concerned a study of the families and their relatives. The author has now finally checked and has on record complete pedigrees of the four major families in Piste, namely, Ceme, Dzib, Tun, and Mex, as well as one chart covering the families of May, Mis, Ek, Cauich, and others. These pedigrees record the names and relationships of more than a thousand persons, showing which individuals have been measured and described. They have produced data on the demography of the community and are serving in the analyses of hereditary traits which occur in these families; and they will be guides to geneticists and historians who wish to trace family relationships in future studies on the primitive Indians of Yucatán. The pedigrees will be available for future investigations.

Anthropometry

Indians of South America. Considerable time has been spent this year in reviewing the literature on the anthropometry of South American Indians. This was done in connection with a proposed handbook on South American Indians being prepared by the U. S. Bureau of American Ethnology. Our part in this handbook concerns the anthropometry of the living tribes. To date we have reviewed anthropometric data on more than one hundred tribes, including their exact location and linguistic affinities. Charts and maps are being prepared to show the average statures and cephalic indices as well as other body proportions for all tribes which have been scientifically measured.

Growth of children of different races. Each year more data are gathered on the growth of children of four racial groups: Maya and Navajo Indians, Negroes, and Dutch whites. Individual children of these races are measured annually and their growth plotted and recorded. The Maya children measured first in 1931 are now fully grown and for the most part have children of their own. They were measured for the last time this year. The children of the other races are in their ninth and tenth years of measurement and many of them are still growing and attending high schools and colleges. Perhaps the most interesting facts learned this year from the growth data pertained to the eruption time of teeth. We can now say definitely that the order in which teeth appear in the mouth is essentially the same for the four races considered. That order is as follows: first molar, central incisor, lateral incisor, first premolar, canine, second premolar, and then second and third molars. This order has been known for the white race, but it was not known or clearly demonstrated that the Maya Indians of Yucatán and the Navajos of Arizona followed the same pattern. There are, however, significant racial differences in the average age of individuals at the time of eruption of the teeth. Thus, the Navajo have the earliest eruption time of all the races studied, and the Negroes also erupt their teeth consistently earlier than the whites. The Maya erupt all teeth except the central incisors at an earlier time than do the whites.

Female/male index. One hundred Negro males from Tuskegee Institute were measured this year for a comparison with the 100 Negro females measured last year. Each of the 50 dimensions taken on this college group is considered in a ratio, showing the percentage which the female measurement is of the male measurement. Thus, the weight of Negro females averages about 80 per cent of that of Negro males. Such ratios are compared with those derived from the author's previous work on Jamaica Negroes and Dutch whites. The major results of this study are summarized briefly as follows: In stature, the sexes are more alike than they are in weight, having an index of 93.4 per cent. This figure is practically identical with those obtained by the author for other races. For example, Dutch white males and females from Holland, Michigan have a female/male index for stature of 93.5 per cent; Jamaica blacks, browns, and whites have indices of 92.8, 93.3, and 92.9, respectively. In all the measurements considered, the ratios are less than 100. When, however, body indices, composed of a ratio of two body measurements, are considered in female/male ratio, there are many in which the females exceed the males. For example, for the index intercristal breadth divided by biacromial breadth (pelvis width by shoulder width), the female/male ratio is 1.058 for Negroes and 1.145 for Dutch whites. This trunk index shows a marked sexual dimorphism. The index for males averaged 70.64 ± 0.25 and the females 74.76 ± 0.27, a difference which is highly significant, being 11 times its probable error. The Dutch white ratio is higher than the Negro, possibly owing to an age difference in the individuals measured from the two races. The corresponding female/male ratios for trunk index for Jamaica blacks, browns, and whites are 1.102, 1.075, and 1.053, respectively. Similarly in the study of sex ratio each of 50 other dimensions is considered.

A Statistical Study of Human Head Hair

We commonly speak of a "hair's breadth," meaning a very small unit of

measure. A recent advertisement in a scientific journal speaks of "1/5 the width of a human hair." Such statements scientifically mean very little, for studies on human hair show that hairs from different parts of the body differ widely in their diameters. Our studies show that races differ widely in the widths of their head hair, this variation ranging from 10 microns to 200. Similarly the variation in size of hairs of one individual is known to be very great. For example, one Maya Indian had head hairs which varied in maximum diameter from 25 to 120 microns, and in a Negro the range was even greater, namely, from 20 to 130 microns.

Hairs rarely are true cylinders, but are more like an elongated ellipse in cross section and consequently have a maximum and minimum diameter. The greatest difference between maximum and minimum diameter is among Negroes; some Negro hairs may have a minimum diameter of 50 microns and a maximum of 100.

In last year's report preliminary data were given showing racial differences in area, maximum and minimum diameters, and index of cross sections of human hair. In that report we described the technique whereby 100 or more hairs may be sectioned in as little as 10 minutes, as compared with two or three days with the older technique. In this report we shall continue with this analysis of hair "widths" and also with a more general study of hair color.

For last year's report, the area of the cross section was determined with a planimeter and recorded in such units. The means for each dimension were obtained from a relatively small number of cases, with an unequal number of males and females in each distribution. During the year we were able to section hairs from two more racial groups, and for each of the six races a mean was determined based on five males and five females of approximately the same age. Likewise it was determined that the formula

$$A = \left(\frac{D_1 + D_2}{4}\right)^2 \times \pi$$

was as accurate as the planimeter readings, and by applying this formula we were able to express the area of the cross section in square microns, a more satisfactory unit. To avoid cumbersome figures, however, the area is expressed in square microns divided by 100. This change from planimeter units to the metric system will account for the differences between last year's data and those in the table shown herewith.

From the data in the table, as well as subsequent data to be presented, we are now able to discuss briefly the influences

MEANS WITH PROBABLE ERRORS FOR CROSS SECTIONS OF HEAD HAIRS FROM 10 INDIVIDUALS (5 MALE, 5 FEMALE) FROM EACH OF SIX RACIAL GROUPS

Race	No. hairs	Area (μ^2/100)	Maximum diameter (μ)	Minimum diameter (μ)	Index
Maya.....	986	41.05±0.29	79.53±0.34	64.90±0.24	82.94±0.24
Hopi.....	617	45.08±0.50	83.70±0.56	65.31±0.35	80.89±0.36
Navajo.....	1002	40.65±0.38	78.76±0.41	61.96±0.28	79.73±0.27
Zuñi.....	643	43.37±0.49	84.33±0.58	62.81±0.33	77.19±0.36
Dutch.....	858	24.11±0.24	63.93±0.37	47.28±0.22	75.99±0.31
Negro.....	873	40.06±0.35	90.62±0.47	51.70±0.27	57.40±0.26

of race, sex, age, and the region of the shaft sections on size and shape of the hair cross sections.

Race. The Maya have the roundest hair, with an index of 83 per cent, and the Negroes have the most elliptical hair, with an index of 57 per cent. The four Indian groups have hair which averaged together gives an index of around 80 per cent, as compared with 76 per cent for whites and 57 per cent for Negroes. Statistically these differences are significant and show a natural trend for hair shape. Intraracial differences, however, are often greater than interracial differences. For example, the area of the hair of 10 Negro individuals averaged 40.06 ± 0.35 square units, but in this group were individuals whose average hair area ranged from 28.45 to 50.96 units. For the Dutch, with the very low average area of 24.11, the range was between 14.5 and 33.6 square units. The variation in size and shape of individual hairs on one particular head is equally great. Nevertheless, the racial trends do exist.

Sex. Our data show definitely that males of all races have rounder hair than females for the ages from 9 to 19. These differences are significant for all races but the Dutch. In area the differences are not so pronounced, although in four of the races the male hairs are definitely larger than the female.

Female hair in our series seems more variable in its shape than male hair, as indicated by the coefficient of variation. This tendency is not true for hair size.

Age. The change in the size and shape of hair with age is a problem that is now being worked on. Although not all the data have as yet been actually calculated, the trend seems to substantiate that found by other workers, namely, that in young children the hair is small and round. The area rapidly increases after 5 years of age and remains fairly level until the fifties, at which time there is a decrease. The index is high for the first 10 years of age but then drops off slightly. In all cases, however, the variation is very great. For this study only Maya hairs were used, a fact that insures homogeneity of the sample.

Size and shape of head hair along its shaft. In all the work on hair sections so far considered, the hair sample was taken within a few millimeters from the scalp in the occipital region. The section that was measured was made at about 20 mm. from the cut surface to insure uniform and comparable results. In the literature there are suggestions that the hair varies in size and shape along its shaft, but so far no conclusive evidence for this fact has been presented. To add further data on this topic, long hair samples were collected from 16 females: 7 American white, ages 50 to 70, and 9 Maya Indian, ages 10 to 20. In none of these samples has the hair been artificially curled or treated. From 80 to 100 hairs, all averaging 400 to 500 mm. in length, were sectioned at 20, 100, 200, 300, and 400 mm. from the original cut. The results show that the area at 100 mm. is greater than that at 20 mm. for 14 of the 16 samples. This is true irrespective of age or race. At the next 100 mm. the trend is not so obvious, for in only 9 samples does the area increase, and in 7 there is a decrease. In the successive intervals the trend is irregular. Some samples show a remarkable increase in size. In all but 2 cases the hairs at the terminal cut have a greater area than at the beginning.

If the samples are arranged in classes numbered I to VI as described in the table on page 254, we see at a glance the trends which occur in the shafts of samples from 16 individuals. For area, the tendency is to increase as the shaft grows out from the head, but for the index or the ratio of the maximum diameter to the

minimum, the tendency is to decrease as the hair is farther from the scalp. For example, for area there are no cases in class VI, steady decrease, and for index there are no cases in class I, steady increase. The meaning of all this is not yet clear. Undoubtedly long hair is elastic and hygroscopic, and its size and shape may well be influenced by environment. It is doubtful that dressing the hair alone causes the observed changes, for there is no consistent difference between the hair of whites and of Indians, and Maya color was matched with the Fischer-Saller Haarfarbentafel. On this scale the graded samples run from A, a very light blond, to Y, a pure black. Red hair is considered separately on another scale ranging from I to VI.

If the colors are split into three groups—A to G, H to P, Q to Y—and the per cent frequency calculated for each year of age, one finds a steady decrease in the A–G group and a corresponding increase in the Q–Y. If the colors on the Fischer scale are given numbers from 1 to 24, corre-

DIRECTION OF CHANGE IN SIZE AND SHAPE IN HAIR CUT AT VARIOUS DISTANCES FROM THE SCALP

Class	Area	Index
I. Steady increase..	M17+ M19 M45 W73 W66*	.....
II. Increase then decrease.	M17 M13 M11 W68 W35	M17+ M19+
III. Increase then decrease then increase..	M17− W52 W53 W72	M14 W66
IV. Decrease then increase..	M14 M19+	M17 M45 W52
V. Decrease then increase then decrease.	.....	M13 M17− M19 W35 W53 W72
VI. Steady decrease	.....	M11 W68 W73

* M, Maya; W, white; 17, 19, 45, etc., age of individual.

women rarely do anything to their hair except wash it. From these preliminary observations we conclude that the hair flattens out and expands in size as it progresses from the scalp.

Change in hair color with age. It is generally known that hair color darkens with age. The degree or speed of darkening has never been measured quantitatively.

The material used in this study has the advantage over others in that it consists of a longitudinal series of growing children measured and recorded over a 10-year period. The sample consists of 220 male and 194 female children ranging in age from 6 to 18, all of American Dutch stock. Each time they were measured the hair sponding to the letters, a correlation can be made between hair color and age. A coefficient of 0.526 ± 0.009 is obtained. The regression equation indicates that for each year in age the hair color will change almost 1 unit of the Fischer scale. Red hair also darkens, giving a correlation coefficient of 0.351 ± 0.054. The coefficient is negative because on the Fischer scale the darkest red hair is I and the lightest is VI.

It is therefore evident that racial comparisons of hair color must be made with the clear conception that hair color is not a stable physical characteristic during the growth period. Due allowances should be made for hair material that has been analyzed from populations of unknown age.

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PERSONS ENGAGED IN VARIOUS ACTIVITIES

During the Year Ending October 31, 1941

Avery, Amos G., Associate
Bates, R. W., Investigator
Bergner, A. Dorothy, Cytologist
Blakeslee, A. F., Director
Bloxsom, Harry, Laborer
Brehme, Katherine S., Fellow
Buchanan, Jennie S. (Mrs.), Assistant
Bunce, Alma, Assistant
Burks, Barbara S., Research Associate
Burns, Edward, Farmer
Burtch, Ethel P. (Mrs.), Stenographer and Switchboard Operator
Calderon, Mary B. (Mrs.), Assistant
Campbell, Peter M., Carpenter
Carley, Catherine, Computer
Carlson, Frances E. (Mrs.), Assistant
Clarkson, Arthur, Laborer
Conklin, Marie E. (Mrs.), Assistant
Davenport, C. B., Research Associate
Demerec, M., Assistant Director
Donohue, Eunice (Mrs.), Assistant
Dunham, H. Howard, Assistant
Eckert, Julius J., Assistant
Fagan, William J., Animal Caretaker
Fano, Ugo, Fellow
Franklin, Alicelia H. (Mrs.), Assistant
Griffin, J. E. (Mrs.), Assistant
Heaney, James A., Assistant
Hellmer, Alice M., Assistant
Holm, Paul, Caretaker
Holmes, Mary J., Stenographer
Houlahan, Mary B. (Mrs.), Assistant
Kaufmann, B. P., Investigator
Kavanagh, Virgene W. (Mrs.), Assistant
Laanes, Theophil, Assistant
Lahr, E. L., Assistant
Lane, Jean, Assistant
Leler, Robert H., Assistant
Lowe, Jeannette, Assistant
Macarthur, George, Superintendent of Buildings and Grounds
MacDowell, E. C., Investigator
MacFarquhar, Mary, Secretary to Director
Martin, Margaret, Indexer
Marvin, Horace N., Assistant
Miller, R. A., Cytologist
Opdyke, David F., Assistant
Peckham, Leslie, Clerk
Potter, J. S., Investigator
Riddle, Oscar, Investigator
Robinson, Dorothy T., Librarian
Rogers, Claude F., Chief Clerk
Sanders, Mary E., Assistant
Sansome, Eva R. (Mrs.), Cytologist
Satina, Sophia, Cytologist
Schiller, Olga, Assistant
Schmelz, Richard, Assistant
Seibert, Henri C., Assistant
Sepe, Domenico, Greenhouse Man
Shaffer, Catherine, Assistant
Smart, Harriet L. (Mrs.), Assistant
Smith, Guinevere C. (Mrs.), Assistant
Steggerda, Morris, Investigator
Stewart, Maydelle B. (Mrs.), Assistant
Stewartson, Ruby Gay, Artist and Photographer
Stillwell, Louis R., Jr., Assistant
Sutton, Eileen, Assistant
Taylor, Martha J., Assistant
Titcomb, Eunice, Assistant
Van Houten, William B., Janitor
Ward, Elsie N., Assistant
Warmke, H. E., Cytologist
Wells, Benjamin B., Investigator
White, Harry, Painter
Wilson, John, Engineer

Summer 1941 and Temporary

Allen, P. Evelyn, Assistant
Anderson, Edgar, Guest Investigator
Atwood, K. C., Assistant
Ayer, Richard D., Assistant
Banta, A. M., Guest Investigator
Bliven, Floyd, Assistant
Boccia, Josephine, Assistant
Bonisteel, W. J., Guest Investigator
Buchholz, J. T., Guest Investigator
Burkhart, Blanche, Assistant
Chadwick, John B., Assistant
Coverdale, Harriet, Assistant
Creighton, Harriet B., Guest Investigator
Giles, Norman, Guest Investigator
Glabb, Stephen, Laborer
Goldstein, Arthur W., Assistant
Granger, Barbara, Assistant
Hill, Thomas J., Guest Investigator
Hinton, O. Taylor, Assistant
Horan, Martin, Laborer
Johnson, John A., Assistant
Korsch, Barbara, Assistant
Koster, Rudolph, Assistant
McClintock, Barbara, Guest Investigator
Miller, Albert, Guest Investigator
Mitchell, Constance, Assistant
Murphy, Henry R., Laborer
Nebel, B. R., Guest Investigator
Nebel, M. R. (Mrs.), Guest Investigator
Ottolenghi, Lina, Assistant
Rhoades, M. M., Guest Investigator
Risman, George, Assistant
Roosevelt, Clochette, Assistant
Sparrow, Arnold H., Assistant
van Overbeek, J., Guest Investigator
Walser, Margaret, Assistant
Weaver, Anita H. (Mrs.), Assistant
Weisner, Sidney, Assistant
Wolff, George, Guest Investigator