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DEPARTMENT OF GENETICS.[1]

C. B. Davenport, Director.

GENERAL STATEMENT.

In this Department the year ending August 31, 1923, has been productive of a variety of interesting results. On the purely experimental side progress has been made in the induction of mutation, in the further analysis of chromosome variation in relation to somatic variation, in ascertaining the external factors that modify the sex-ratio in *Cladocera*, and in unraveling the complicated interrelation of the internal secretions that influence fecundity.

In the field of human genetics progress has been made in analyzing the hereditary factors that help determine body build and bone defects and in determining relative values in the national population of the different racial elements contributed to it by immigration.

Of all our work, that which seems most fundamental is with the chromosomes. When in 1885 Weismann first used the term germ-plasm to signify the totality of the chromosomal complex characteristic of an organic species, the new term with its implications must have seemed, even to many biologists, unwarranted and speculative. To-day it is perhaps the principal object of investigation in genetical research and the chromosome has been raised from an exceptionally esoteric and technical position to one of acknowledged utmost importance to man and all of his interests. Gradually the conception is forcing its way that we are, under ordinary conditions of environment, "what our chromosomes make us."

This germ-plasm Weismann regarded as singularly protected from environmental influences, and certainly recent experience has fortified this conclusion. Nevertheless, change (evolution) does occur; the chromosomes do change. A minute change in the gene constitution of a chromosome, and still more the addition or subtraction of a single chromosome, will ordinarily be associated with a uniform and definite change in the soma. That change is, obviously, in part due to peculiarities in the constitution of the organism itself, but it is now quite certain that it is possible, by abnormal conditions of temperature, by the X-ray, probably also by alcohol, sera, centrifuging, and other conditions, to modify not only the processes of cell division and chromosome separation, but also the composition of the individual chromosome. This is, certainly, induced or directed evolution.

While the immediate consequence of such induced mutations is not always a new species, with its properties of multiple differentiating characters, inter-sterility, and constancy of traits, there is one class of inducible mutation that meets these conditions, as Dr. Blakeslee has pointed out. That is the class of tetraploidy, in which the number of chromosomes is doubled. Following out this suggestion, Dr. Belling has investigated a number of plant species and finds that in one of them the chromosomes are, indeed, four of a kind, leading to the inference of the origin of this species by tetraploidy—i. e., the failure, somewhere in its history, of the divided chromosomes in some parent cell to get into distinct daughter nuclei. In consequence of this failure

[1] Situated at Cold Spring Harbor, Long Island, New York.

the number of chromosomes in the nucleus is permanently doubled. Furthermore, the resulting new form meets the above-named criteria of a species.

Admitting this to be a real method of species origin, the question of the origin of species is far from being answered. But our experience so far justifies us in concluding that the question of the origin of species is at basis that of the origin of changes in the chromosomes; and the question of evolution is, in part, that of the evolution of the chromosome complex and, in part, that of adjustment to environment, in which the environment acts as the ruthless selector.

Even had we learned the various internal and external influences that control chromosomal changes we should be far from having a complete insight into genetics. The question would still be urgent, How do the determiners or genes of the chromosomes work out their destiny in particular somatic traits? Into this matter we are gaining some insight by studies on the rôle of hormones in development and, indeed, by the various analyses of the genetic elements of adult traits.

Twenty years have passed since this Department or its predecessor (that of Experimental Evolution) was founded. One of the appointees of the first year was a cytologist, a recognition of the importance of the cytological basis of genetics. During this period, which nearly coincides with that of the modern development of genetics, the chromosome has come ever more and more into the foreground. The next new developments are, doubtless, those of biological physical chemistry, a discipline in which little research has yet been done. But until research is pushed in this field we shall not know the relation between the chromosomes and the processes of general growth and differentiation, which are the essence of that development of which heredity is the control.

DETAILED REPORTS ON CURRENT INVESTIGATIONS.

INTERCHROMOSOMAL MUTATION.

The reports of the past few years have recorded in detail the rise and progress of our research on the relation between the extraordinary irregularity of the karyokinetic phenomena in the formation of gametes in the jimson weed (*Datura*) and the equally rare variability in the somas of these plants. To learn all of the somatic changes induced by the chromosomal irregularities requires the cooperation of persons with special technical knowledge in different fields, and this cooperation we have been able to obtain from university men who have come to us for the summer months. The resident group in these experiments is composed of Dr. A. F. Blakeslee, who has directed them from the beginning; Dr. John Belling, who is making cytological studies and has had the temporary assistance of Misses Elizabeth Lord and Rachel Haynes; Mr. M. E. Farnham and his successor, Mr. Gordon Morrison, who have had immediate charge of most of the work in the greenhouse and field. The visiting collaborators have again been Professor E. W. Sinnott, of the Connecticut Agricultural College at Storrs, who has been studying the anatomy and histology of the mutants; Professor John T. Buchholz, of the University of Arkansas, who has continued his studies of the differences in the growth of the pollen-tubes of different daturas in the pistils of *Datura* mutants; and Mr. J. L. Cartledge, of the University of Pittsburgh, who has been engaged in pollen counts and assist-

ing in other ways. Misses Bergner and Watt, of this Department, have assisted in hybridization and counting pollen.

Trisomic Types of Mutants: Primary and Secondary.

Two of the most interesting developments of the work with *Datura* are the discovery of the differentiation of the simple trichromosomic types into primary mutants and their respective varieties and the attempt to determine the causes that lie back of such differentiation.

Dr. Blakeslee reports on this matter as follows:

"It was stated in our last report that, whereas there should be only 12 ($2n+1$) mutants expected if each mutant is caused by the presence of an extra chromosome in a different one of the 12 chromosomal sets, as a matter of fact we have over 20 mutants with a single extra chromosome. It has been possible to arrange the mutants in not over 12 groups. Six of these groups have as yet only a single member, but in the other groups we have a main mutant and one or more varieties. We have sometimes termed these 'apostles' and 'acolytes.' The following is some of the evidence for connecting a main mutant with its variety: (1) Similarity in external appearance; (2) similarity in internal anatomy (Dr. Sinnott has successfully grouped the mutants from study of their anatomical structure without knowing how we had classified them from other evidence); (3) a study of chromosome size in mutants is being made by Dr. Belling and should give further evidence, positive or negative, on the grouping of mutants; (4) the main mutant and its variety in one group (Poinsettia-wiry) give the same trisomic ratios for a Mendelian factor, apparently carried by the extra chromosome; (5) so far as our records have been tabulated, it seems to be true that the variety regularly throws a small percentage of its main mutant in the offspring, while the main mutant does not throw its variety except very rarely.

"As to which in the group is to be considered the main mutant and which the variety is shown by the breeding behavior just mentioned, as well as by the fact that the main mutants occur spontaneously from normals more frequently than their varieties, and especially by the fact that the main mutants are (with only one certain exception) the only ($2n+1$) types so far thrown by triploid ($3n$) plants. Since in triploids each chromosomal set is a trisome and the assortment of its members is at random, we should expect, among the viable combinations of chromosomes in the offspring, the 12 main ($2n+1$) mutants. Out of 784 offspring from triploids, 248 have been normals, 112 double mutants, and 424 ($2n+1$) mutants. Of these latter, 51 were Globe, 39 Poinsettia, 38 Cocklebur, 37 Ilex, 18 Echinus, 24 Rolled, 41 Reduced, 57 Buckling, 32 Glossy, 32 Elongate, 50 Microcarpic, and 2 Spinach. None of the varieties appeared in the triploid offspring, with 1 certain exception and 2 uncertain determinations. We feel confident that we have in the above list at least 11 of the 12 main mutants. The relatively poor viability of Spinach may account for the small number of this type, but the Spinach group is anomalous in certain respects and shows possible relationships to another group.

"For some time our efforts have centered upon the relation between the main mutants and their varieties. Our preliminary hypothesis, that the variety might be a main mutant modified by a Mendelian factor, has been shown untenable by an extensive series of breeding-tests. What may be the clue to the situation was obtained this last winter in the breeding behavior of the group of mutants consisting of Cocklebur and Wedge, the latter by various tests shown to be a variety of the former. Previous evidence indicated

strongly that Cocklebur has its extra chromosome in the set carrying the genes for armed or *inermis* capsules on account of the ratios which it throws when heterozygous for these factors. A selfed heterozygous plant of the variety Wedge threw a 3 : 1 ratio, which is typical of disomic inheritance and seems to indicate that there are in this $(2n+1)$ variety only two chromosomes carrying genes for armed and *inermis* rather than 3 as expected. More data are desirable, but offspring from selfed and back-crossed parents now in the garden seem to confirm the tentative conclusion from the winter's cultures. Our preliminary hypothesis is that the variety may have a part of one of the chromosomes in the trisome deficient (in this group for the armed-*inermis* genes), either by inactivation of this part or by an actual loss of a portion of the chromosome. A tentative scheme of the possible chromosomal behavior has been worked out which squares measurably well with the differences observed between the Cocklebur mutant and its variety Wedge in respect to the inheritance of Mendelian factors and in respect to the fact that the variety regularly throws the main mutant, while the latter rarely throws the variety. The scheme is merely a tentative working hypothesis, however, since it is susceptible of various tests, especially from segregation of Mendelian factors and from the cytological relations. Dr. Belling is undertaking a detailed study of the size relations between the main mutants and their varieties and, if our preliminary hypothesis be correct, should soon determine whether or not there is actually a shortening of the extra chromosome in the varieties instead of a deficiency unrepresented by any morphological difference, as seems to be the condition in reported cases of deficiency in *Drosophila*. The relatively few varieties to each main mutant (generally less than one) speak for a definite region of fragmentation.

"It has been previously mentioned that the Spinach group showed possible relationships to another group. It is also true that the proportion of unrelated mutants thrown by this group is unusually large. The most extreme example is a culture this summer derived from a cross between the two members of the Spinach group. Out of 161 offspring, 95 (nearly 60 per cent) were mutants of various types. The cause of such anomalous behavior is being studied further, both from the cytological and the breeding standpoints. The evidence from the Spinach group, however, indicates that it will be unsafe to apply, without modification, our hypothesis for varieties to all our $(2n+1)$ mutants.

"In an early stage of our study, when we had only 12 $(2n+1)$ mutants, which number was to have been expected on the basis of an addition of a single unaltered chromosome to the constellation of 12 diploid pairs, it seemed that a method was opened up for an analysis of the chromosomal constitution by the unbalancing effects produced when a given extra chromosome was present. The finding of more than 12 $(2n+1)$ mutants made any attempt at an extensive analysis of this kind unwarranted. With the discovery, however, that the main mutants (at least 11 of the 12) can be distinguished from their varieties, the possibility of such an analysis is restored, and, if deficiency, fragmentation, or other alteration of chromosomes proves to be a means of modifying mutants into varieties, we have an additional method of analyzing the chromosomes by studying the unbalancing effect of the assemblage of factors in the fragments. Already, from only a superficial study of the main mutants and their varieties, we can conclude that each of the chromosomes contains factors which affect the somatic characters of habit of growth and of intensity and distribution of purple pigmentation, the various mutants varying in plus or minus direction from the balanced condition in normal diploids. Dr. Sinnott is analyzing the effects of extra chromosomes upon the internal anatomy and Dr. Buchholz the effects upon pollen-tube growth.

"The effects of chromosome differences upon the biochemical product should soon be studied. For such work we are getting together what we believe is the most nearly comparable material ever used in plant breeding. From our first haploid (plant A) in line 1, we have obtained diploids by non-reduction which (barring new mutations) must be absolutely homozygous. It has been our effort ultimately to get all our mutants into this homozygous 1A line and we have already succeeded with the tetraploid ($4n$) and the majority of the main ($2n+1$) mutants. We are now crossing the $4n$ with $2n$ plants in this line and should within a few months have triploids ($3n$) which should throw the main mutants. The latter, with sufficiently large numbers of offspring, should give rise to the mutant varieties.

"We are obviously in need of Mendelian characters with which to tag the individual chromosomes and parts of chromosomes and are trying to locate in their respective chromosomes some 6 or 8 Mendelian factors which we have isolated from our main lines or from new mutations. We have succeeded in hybridizing our *Datura stramonium* with the distinct species *Datura ferox*, and in later generations hope to isolate a number of Mendelian characters from this species cross. In addition, we are planning to sow seed from a large number of selfed parents in line 1A, with the hope of getting new gene mutations recognizable in the seedpan."

Other Mutant Types in Datura.

The occurrence of haploids has been shown to be probably due to a process of true parthenogenesis of the reduced egg. An *inermis* haploid was obtained from a cross between a female parent heterozygous for armed and *inermis* and a pollen parent homozygous for armed. Further, two haploids have arisen from the cross *D. stramonium* × *D. ferox* and resemble in all visible respects the *D. stramonium* parent. In these three cases apparently the male gamete did not enter in any way into the constitution of the haploid offspring. Parthenogenesis has been observed not infrequently in tetraploids when they are crossed with pollen from diploids. By using recessive white tetraploids as female parents and pollen from purple diploids, the white diploids which resulted could be assumed to have originated from the tetraploid parent alone. The purple offspring from this cross have all been triploid.

It has previously been shown that in addition to the usual disomic ratios obtained when the chromosomes are in twos in the set heterozygous for a given factor, distinct trisomic ratios are obtained when these chromosomes are in threes, as in ($2n+1$) mutants; and tetrasomic ratios when they are in fours, as in tetraploids. This season we have obtained the first pentasomic ratio from a ($4n+1$) *Poinsettia* parent heterozygous for purple and white flower-color.

The findings of the past year show that chromosomal aberrations are a not unexpected cause of sectorial chimeras in *Datura* and may account for the reported bud sports in other species. Four plants have been found in our cultures with a branch which bore leaves of a distinct character. In two of these cases, Dr. Belling has found the abnormal branch to be a chromosome deficiency of the type ($2n-1$), so far as the pollen mother-cells is concerned. The two other plants have not as yet been studied cytologically, but from appearance can be provisionally placed in the same category. The deficiency does not appear to be transmitted to the offspring. The deficiencies just mentioned have been parts of a plant otherwise apparently normally diploids. A single plant has been discovered, however, all the branches of which bear

abnormal leaves and almost entirely sterile flowers. Dr. Belling reports the pollen mother-cells of this plant apparently to be $(2n-1)$.

Sectorial chimeras appear to be a not infrequent stage in the production of tetraploid races. In 3 plants otherwise diploid, a branch was found to be tetraploid ($4n$), as indicated by pollen-size. The growth of these $4n$ branches was slow and buds were not secured for chromosome counts, but seeds from the $4n$ branches produced $4n$ offspring, while seeds from the $2n$ branches produced $2n$ seedlings. Tetraploids frequently first appear in a peculiar rough-leaved form in which patches are devoid of palisade parenchyme, and it is possible that, in addition to the sharply defined sectorial chimeras, periclinal chimeras and chimeras with a less orderly mixture of chromosomally distinct tissue may occur in *Datura*.

Rate of Pollen-Tube Growth in Datura Mutants.

Irregularities in some proportions obtained in certain crosses between the varieties of *Datura* have led to a study, by Professor John T. Buchholz, of the relative rate of pollen-tube growth of different varieties, following along the lines of an hypothesis suggested by Correns. This study, made during two summers, has yielded important results described by Dr. Buchholz as follows:

"A study of the germination and growth of normal pollen on the stigmas of a dozen or more simple trisomic mutants of *Datura* on cut flowers under comparable conditions (about 18° to 19° C.) has shown that there is no great difference in the depth of penetration of the pollen-tubes in the different cases, though it is somewhat slower as a rule for most of the mutants. Stigmas of normal plants were used under the same conditions for the germination of the pollen of 18 of these $(2n+1)$ chromosomal mutants, with the result that the pollen differed widely in the percentage of its germination, as well as in the depth of penetration of the pollen-tubes. The pollen-tubes having n chromosomes from each mutant penetrate to about the same distance as normal pollen-tubes, while there seems to be a distinct group of slower pollen-tubes, presumably those having the $(n+1)$ chromosomes, which either fail to germinate or lag behind. In a few of the mutants, such as Sugarloaf, for example, there seems to be a distribution of these pollen-tubes into three modes, suggesting the possibility that there may be more than two classes of pollen produced, while pollen-tubes from normal plants are distributed essentially in a unimodal curve.

"Considerable attention was given to a preliminary study of the physiology of pollen-tube growth in normal plants. Pollinations with second batches of pollen applied to used pistils after intervals of 1, 2, and 3 days show that the second pollen-tubes are retarded to about two-thirds to one-half of the growth-rate of the same pollen on the pistils of fresh normal flowers. A similar application of normal pollen to the pistils of unpollinated flowers collected at the same time and stored beside them under the same conditions shows that this aging through storage also slows down the depth of pollen-tube penetration to some extent, but the effect is much less than that produced by the previous growth of other pollen-tubes. These experiments suggest that pollen-tubes deplete the style of nutrient substance or render it slightly toxic, possibly both.

"From a series of experiments performed last year, it was definitely proved that the fertilization of ovules proceeds downward from the upper part of the ovary, that most of the seeds in the upper half of the seed capsule were fertilized by the first pollen-tubes arriving in the ovary, and most of the seeds in the lower portion of a capsule were the result of fertilization by the slower or later-arriving pollen-tubes."

Irregular Segregation in Chromosomes.

In ordinary diploid plants in the reduction division, one member of each pair of chromosomes goes to each pole, so that each daughter nucleus, and hence each gamete, gets the same number of chromosomes. In triploid plants there is a complication, either two chromosomes of each set go to one pole and one to the other, or else the third chromosome might conceivably be left in the middle and not participate in either nucleus. The former alternative is the one actually realized. The question now arises whether the cell into which the odd chromosome shall go is determined purely by chance, or whether there is any sort of attraction between chromosomes such that an excess or all of the extra chromosomes go into one of the daughter nuclei. To decide this matter, Dr. Belling has made counts on the number of chromosomes going to each pole of the spindle in pollen mother-cells, in addition to those reported previously. Over 100 double counts have been made in the regular triploid. The frequency with which each of the series of *extra* chromosome Nos. 0 to 12 occurs in this 100 agrees with the coefficients in the binomial series $(a+b)^{12}$; the middle numbers of the chromosome series are those most commonly found, but there is a slight excess over expectation of the more unequal assortments.

In haploid plants the first division has now been examined in several cases; it consists usually in a segregation of the chromosomes into 2 centers, but sometimes into 3. In the division into 2 centers the frequency of occurrence in any center of the number of chromosomes in the series 1 to 12 is, so far as observed, in close agreement with the coefficients of the binomial $(a+b)^{12}$.

Two plants from the cross of tetraploid by diploid had 35 chromosomes. These plants provided nearly 100 cases for counting the distribution of chromosomes in the dividing pollen mother-cells. The frequency with which the excess over the normal 12 at any pole was represented in the series of Nos. 0 to 11 is represented by the corresponding series of coefficients of the binomial $(a+b)^{11}$. There was a slight excess over expectation in the more unequal segregations.

One plant from the cross tetraploid by diploid had 37 chromosomes, or 24+13. Of these 13 extra chromosomes, the frequency with which the extra numbers in the series 0 to 13 went to either pole at mitosis agrees with the series of coefficients of the binomial $(a+b)^{13}$, with a slight deficit in frequency of the more unequal segregations.

These results are concerned only with total numbers of chromosomes, and do not regard the distribution of the sets of 2, 3, or 4 homologous chromosomes. It will be possible to follow the fate of such sets in the future by means of their size differences. In the cultivated hyacinth it has for some time been known (de Mol) that three size classes are easily recognized among its 8 pairs of chromosomes. These size classes have been followed in the pollen-grains of a "hexaploid" hyacinth and gave results corresponding closely with the distribution expected in each case if the chromosomes of the three different sizes assorted without mutual attraction or repulsion. *Hemerocallis fulva*, growing escaped from cultivation near the station, has been found by Dr. Belling to be a triploid; and in this case, apparently, to have the distribution of the segregation of the extra 11 chromosomes which follows the law of chance.

Non-disjunction in Tetraploid Plants.

The occurrence of non-disjunction in tetraploid plants offers an opportunity for great variation in chromosome number in the progeny. On this matter Dr. Belling reports as follows:

"The chromosome distribution has now been examined in over 100 true tetraploid *Daturas*, including special studies of 55 plants of one sibship and 17 plants of another. No plants give a constant distribution of 24+24 chromosomes. All show more or less of a segregation into 23+25, etc. The proportions in which this apparent non-disjunction takes place conform, in different individuals, more or less to the laws of random sampling varying around a mean of approximately 25 per cent of the 23+25 distribution and about 2 per cent of the 22+26. The most reliable results agree with a ratio of regular disjunction (2+2) to non-disjunction (1+3) of 35 : 1, for each quadrivalent or set of four.

"Hence a regular tetraploid, with 12 sets of 4 homologous chromosomes each, should give a certain proportion of 47 and 49 chromosome plants in its progeny. The two sibships above mentioned gave 73 plants with 48 chromosomes, 6 plants with 49 chromosomes, and 3 plants with 47. This is considerably less than the numbers of 47 and 49 chromosome plants which would be produced if all the 23 and 25 chromosome gametes were functional, and points to an abortion or failure in competition of those gametes or zygotes with excesses or deficiencies of chromosomes.

"If two gametes with 23 and 25 chromosomes, respectively, combine, the resulting 48-chromosome plant will usually have 10 sets of 4, 1 set of 3, and 1 set of 5 homologous chromosomes. Two such plants have been tested by growing their progeny. By calculation they should give 7 pollen mother-cells dividing 24+24, to 8 dividing 23+25, to 1 dividing 22+26, etc. This they did approximately, giving the proportion 6 : 9 : 1. The progeny, supposing only 24-chromosome pollen functional, and all egg-cells viable, should give plants with 46, 47, ... 50 chromosomes in the proportion 1 : 8 : 14 : 8 : 1. The proportion found was 1 : 12 : 20 : 3 : 1.

"In the progeny of such a pseudo-tetraploid, there should be some cases where two gametes meet whose sum amounts to 48, but which produce a plant having 8 sets of 4 chromosomes, 2 sets of 3 chromosomes, and 2 sets of 5 chromosomes. It may be calculated that such a plant would have a distribution of chromosomes in the pollen mother-cells of about 14 cases of 24+24, 17 of 23+25, 8 of 22+26, 1 of 21+27, etc. Two such plants have been found. In one case the chromosomes have been investigated, and 2 sets of 3 and 2 sets of 5 were found. These two plants gave the expected high ratios of the 22+26 and 21+27 distribution.

"Plants with 47 chromosomes that have originated from selfing a pure tetraploid should have usually 11 sets of 4 homologous chromosomes, and 1 set of 3. These plants should give a distribution of chromosomes, at the first division, of about 7 cases of 23+24, to 1 case of 22+25, etc. The progeny of one such plant was studied. It gave among its gametes the proportion of 7.5 cases of 23+24 to 1 case of 22+25. The progeny, if the parent's 24-chromosome pollen is solely or mainly functional, should give 1 plant with 46, 7 plants with 47, 7 plants with 48, and 1 plant with 49 chromosomes, out of 16 progeny. The actual ratio was 1 : 7 : 9 : 0.

"A plant with 50 chromosomes, in which, there was reason to think from appearance, breeding, and microscopical evidence, there were 11 sets of 4

chromosomes each and 1 set of 6 homologous chromosomes, gave the following in its selfed progeny:

Numbers of chromosomes	48	49	50	51	52
Numbers of plants	1	9	10	1	0
Calculated, 24-chromosome pollen only	3	16	3	..	..
Calculated, all pollen functional	4	4	13	0.3	0.3

"The results agree with the view that some pollen with extra chromosomes is functional here."

Diploid Plants with One Extra Chromosome.

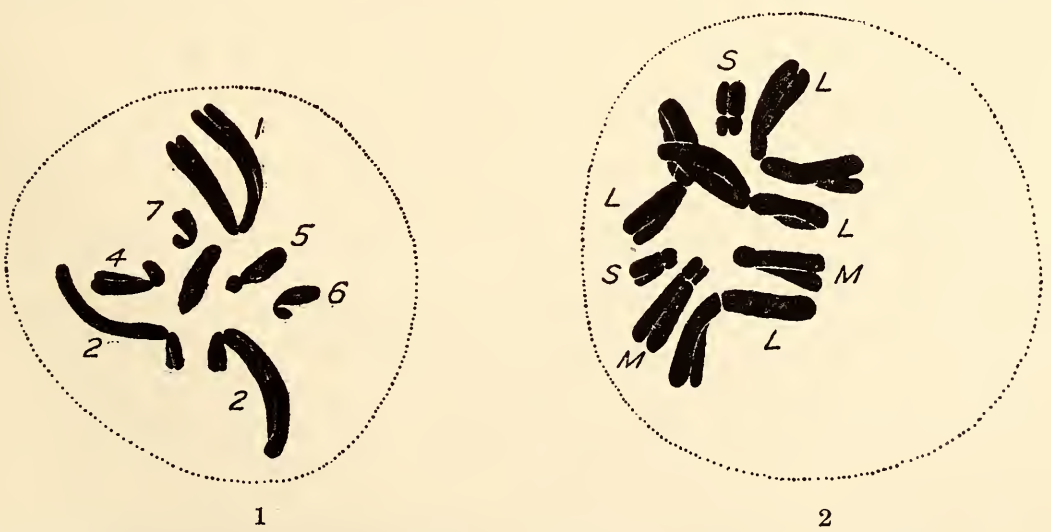
Investigation *ad hoc* is now being made of the chromosomes of the plants with 25 of them. The following methods are being used: (1) Camera drawings are made of several trivalents from each kind of plant; with a view to obtain accurate size measurements and determine the relative frequencies of different methods of combination of the 3 chromosomes; (2) camera drawings are made of complete groups of chromosomes at the first metaphase, to determine with certainty the different sizes; (3) size estimations are taken at the second metaphase, among the group of 13 chromosomes; (4) observation is made of the size of the detached chromosomes found in the 12+1+12 distributions.

Diploid Plants with One Chromosome Missing.

Portions of two plants and one whole plant were found by Doctors Blakeslee and Belling in this condition. Apparently in two (or in all three) of the cases it was the largest chromosome which was missing. There were 11 bivalents and 1 univalent at the late prophase and first metaphase. At the second metaphase the unpaired chromosome appeared larger than in the regular diploid.

Tetraploidy and Evolution.

If in any species the chromosome group in the soma is divisible into sets of 4 chromosomes of each size, it is probable that the species is either a tetraploid or has come from tetraploid ancestry. It has been found that



1 2

Fig. 1.—Pollen-grain of *Uvularia grandiflora*, showing 7 chromosomes, of 6 different sizes.
Fig. 2.—Pollen-grain of hyacinth (Yellowhammer), showing pairs of similar chromosomes.

often, in monocotyledons especially, an estimation of the sizes of the chromosomes is best made in the pollen-grains themselves at their first division, instead of in the pollen mother-cells or in the root tips. Plants which

show two chromosomes of each size, appearing otherwise identical, in the pollen-grains (fig. 2) are probably tetraploids or descended from tetraploids. On the other hand, if all the chromosomes in the pollen-grain are of different sizes or shapes (fig. 1), the plant is not tetraploid or double diploid.

A number of species, especially monocotyledons, have been studied in this respect, such as *Cypripedium pubescens*, *C. acaule*, *Narcissus*, *Hyacinthus*, *Iris*, *Uvularia*, *Funkia*, and *Hemerocallis*. Of these, the ordinary hyacinth, styled diploid by de Mol, who counted the number of somatic chromosomes, shows in the pollen-grain (fig. 2) 2 pairs of V's, each with a clear median constriction; 1 pair of large J's, shorter than the V's; and 1 pair of short, often straight, chromosomes. The members of each pair are apparently identical. It is to be inferred that these hyacinths are either tetraploid or lately descended from tetraploids.

EVOLUTION OF THE GERM-PLASM.

Drosophila.

As pointed out in earlier reports, the genus *Drosophila*, with its numerous species, affords a unique opportunity to study the differences in the chromosomal complex of these species and to obtain the history of the changes by which these differences have been produced. Dr. C. W. Metz and associates have published (Carnegie Inst. Wash. Pub. No. 328, July 1923) a report on their genetic studies on *Drosophila virilis*. This research, of which the progress has been noted in earlier annual reports, shows that the variation from 3 to 6 pairs of chromosomes in the different species could perhaps be explained on the hypothesis that the larger number of chromosomes has been derived from the smaller by fission, or vice versa. If the 6 pairs of chromosomes of *Drosophila virilis* have been derived from the 4 of *D. melanogaster*, then the hypothesis might be tested that at least 2 of these chromosomes should be strictly homologous between the two species, and accordingly should have similar genes arranged in the same order. This hypothesis is being tested and some evidence supports it for the sex-chromosome in the two species. Here, yellow, crossveinless, singed, and forked are found in the sex-chromosome maps of the two species in about the same relation to each other. Evidence is also being obtained that minor changes, in the nature of rearrangements in the order of the genes in one small portion of a chromosome, may occur. The accumulation of evidence on these matters involves the finding of new mutant characters and the location of their genes on the chromosome map—a laborious process.

During the past year, Dr. Metz's group have found about 20 new mutant characters in *D. virilis*, making 60 in all in this species. Also, several new characters have been obtained in *D. willistoni*. Some of these new characters are of particular interest, because they appear to duplicate characters known in other species of *Drosophila*. Most of them have not been studied sufficiently to warrant conclusions at this time, but in the case of others the evidence warrants a report here. Dr. Metz reports in detail as follows:

"In *D. willistoni* the sex-linked characters scute, yellow, vermilion, and forked have already been shown to resemble characters in certain other species and to correspond to these in sequence on the chromosome map. Recently we have obtained the additional 'parallel' crossveinless, making the

series *scute–yellow–crossveinless–vermilion–forked.* In *D. virilis* the series yellow, crossveinless, vermilion, and singed has been noted in previous reports. To this we have now added the parallel scute. In this case the order seems to be *yellow–scute–crossveinless–vermilion–singed.* (*Forked* in the *willistoni* series resembles *singed* in the other series.)

The sequence of yellow and scute, in relation to the other three characters, appears to be just the opposite in these two species. The relations are shown graphically in the accompanying diagrams or chromosome maps, in which are included also the X-chromosome maps of *Drosophila melanogaster* (after Bridges) and *D. obscura* (after Lancefield). Only the loci considered here are included on the maps (Fig. 3).

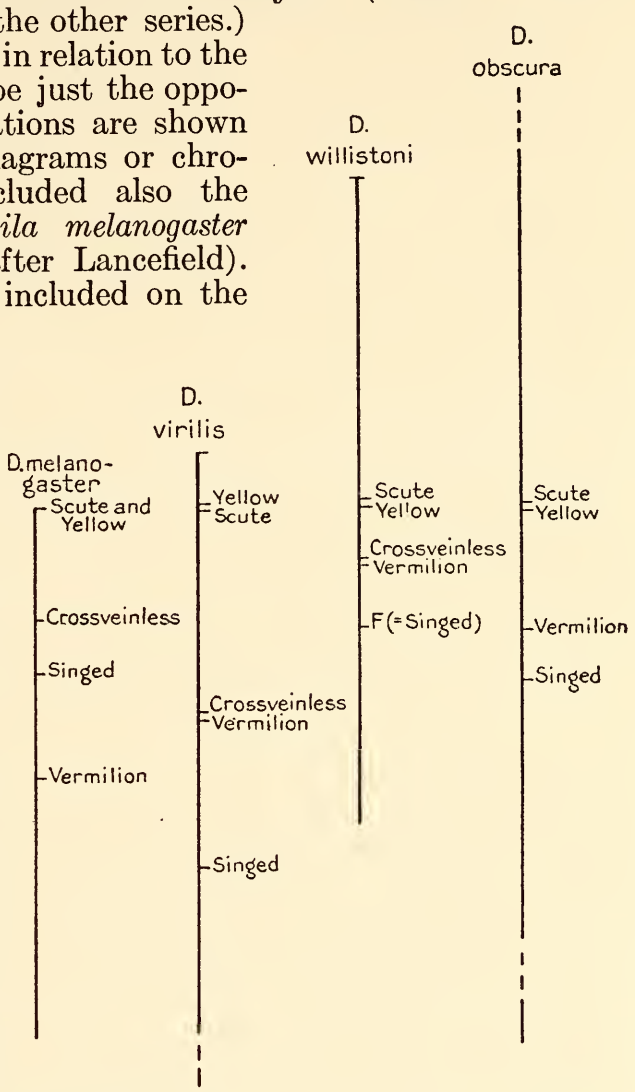


FIG. 3.—Comparison of sex chromosome. Maps in 4 species of Drosophila.

"The characters scute and yellow are emphasized because they are very closely linked in all four species. This parallelism, both in appearance and in degree of linkage of the characters, makes it seem probable that we are dealing with actual homologues. It therefore becomes of particular interest to compare their linkage relations with those of the other parallels in the species concerned.

"In *D. melanogaster* (according to Doctors Sturtevant and Bridges) scute and yellow are so closely linked that not enough crossovers have been obtained to establish their sequence; hence they are given the same locus. In *D. virilis* our evidence, just obtained, is based on experiments involving yellow, scute, and vermilion, in which three crossovers were secured, all of the same type and indicating the order given here. This number of crossovers is not large, but it is believed to be reliable in indicating the sequence.

"The two maps on the left in the accompanying figure are from species possessing rod-like X-chromosomes; the other two represent long V-shaped chromosomes. It will be noted that the sequence of scute and yellow agrees in the two species having V-shaped X-chromosomes, but is reversed in *virilis*, which has the rod-like X.

"The presence of so many 'parallels' indicated that a homology exists between the X-chromosomes of the four species, but the altered sequence suggests that a rearrangement of genes may have occurred. This is also suggested by the map position of vermilion in *D. melanogaster*, which differs from that of vermilion in the other three species. It agrees, too, with the demonstrated case of map-displacement noted by Sturtevant in the third chromosome of *D. simulans* as compared with *D. melanogaster*.

"In both *D. virilis* and *D. willistoni* the number of groups of linked genes now equals that of the haploid number of chromosomes (6 in *virilis* and 3 in

willistoni) and the chromosome maps (based on amount of crossing over) correspond roughly to the chromosomes in size, except in the case of one map in *D. virilis*. In this species there are 5 long chromosomes and 1 very short one, and there should be a corresponding series of crossover maps; but instead the data thus far obtained give 4 long and 2 very short maps, which suggests that crossing over may be reduced in one of the 5 large chromosomes.

"One of the two linkage groups showing little or no crossing over included the characters bent and net, which resemble the characters bent and diminished in *D. melanogaster*. The latter have been shown by Bridges to be due to genes in the small chromosome of *D. melanogaster*. This suggests a homology between the small chromosomes of the two species, but we have not yet been able to make sure of the point, because, with two small linkage groups, we can not tell which represents the small chromosome. Further studies ought to settle this point soon, however.

"The character bent in *D. virilis* involves several different parts of the fly and is readily modified by environmental conditions. For this reason it has been used in an attempt to learn something of the action of the gene during development. In preliminary experiments we have been able to show that the character reacts to low temperature at a definite time, just preceding the pupation of the larva. Exposure to cold at other times has no effect. We have also found that different parts of the fly appear to differ from one another in the time at which they are affected (within the limits just mentioned), which seems to indicate that the gene acts successively in different regions, or else that it sets up reactions which give that effect."

Other Diptera.

The cytological studies on chromosome behavior, chromosome relationships, and gametogenesis, in other Diptera, particularly in the Drosophilinæ, have been continued during the year by Dr. Metz.

EXPERIMENTAL MODIFICATION OF THE GERM-PLASM.

Effect of Cold in Inducing Datura Mutants.

Since the last report, additional data have been secured on the effect of cold in inducing mutations in *Datura*. No doubt the lowered temperature induces abnormalities in the distribution of chromosomes during the formation of pollen and probably also during the formation of egg-cells. When the new constant-temperature chamber is installed it should be possible to discover with greater precision the stage at which the stimulus should be applied to bring the production of chromosomal mutations under a greater control than has hitherto been possible.

Genetics of X-rayed Mice.

This investigation, begun by Drs. C. C. Little and H. J. Bagg in 1921 is being continued by Dr. Little at the University of Maine. He reports that during the past year about 8,000 mice have been recorded. A lethal head and jaw abnormality in mice (*j*) has been found to behave as a Mendelian recessive. The eye abnormality (*h*) which occurred among the descendants of the X-rayed mice has been found to be a Mendelian recessive, overlapping normal. Abnormal feet and hair length in these X-rayed mice have proved to be hereditary, and the exact method of inheritance is being investigated.

CONTROL OF THE SEX-RATIO.

Attempts to control the sex-ratio in the little "water fleas," or Cladocera, have been continued by Dr. A. M. Banta, with the efficient collaboration of Mr. L. A. Brown, of the University of Pittsburgh. This group of organisms seems especially well fitted to throw light on the ages-old problem of how the sex of offspring is controlled, but the final solution is not yet in sight, though we are obviously approaching nearer to it.

Species Studied and Method Employed.

The main work has been done on *Moina macrocopa*. Since the beginning, three years ago, more than 800 experiments have been made with this species, and approximately 160,000 individual young have been sexed by the aid of the microscope, more than 75,000 of them this summer. Upon another species, *Simocephalus exspinosus*, a few additional experiments upon male production have been made, with very gratifying results. As pointed out in previous reports, the method of producing male offspring in these typically female-producing species is to crowd the mothers in small bottles of water.

The Critical Period of Sex Differentiation.

As a preliminary to the determination of the period at which sex of offspring is fixed, it was necessary to study the developmental cycle of the organism. Development in Cladocera does not proceed uniformly, but by a series of molts, each accompanied by a sudden increase in size. Between molts the organism is at a standstill in its size; the standstill stage is called an instar. The first brood is released at the end of the fourth instar, and a molt occurs after each brood is set free. The total number of broods may be 14 or more. The normal individuals of any instar are very similar in size and differ in size from those of any other instar. Hence the number of the instar can be determined by measuring the individual. As a result of over 1,200 measurements of lengths of females, a table of sizes for each instar has been prepared. Considering the increases in length between instars, it appears that the percentage increase in length is almost constant during the young and adolescent instars and the first two adult instars, but following this, as the animals grow older, there is a marked decrease in the percentage increase in size. These results were used also in studies on the relative metabolism of the sexes.

Experiments reported on last year indicated that sex is fixed in the ovarian egg of *Moina macrocopa* during the latter half of the instar preceding its expulsion from the ovary into the brood-chamber, i. e., in the latter half of the third instar. Additional experiments have verified the earlier conclusions and, in addition, limit the critical period during which sex of the young is subject to experimental control to approximately 4 hours previous to the passage of the egg into the brood-chamber. During this short and very definite period, sex is subject to control; following it sex is absolutely fixed.

Analysis of Sex-control Factors.

As stated in previous Year Books, the simple expedient of crowding *Moina macrocopa* and other Cladocera species causes the production of males. Most of the experiments to be reported now consisted of such crowded or semi-crowded control bottles in experiments in which the tests were modified in one way or another. Every experiment has a control and a test bottle and

each control and test bottle contains equal numbers of young from the same brood and handled precisely alike, except the specific treatment given the test.

"Some of the main points of attack and accomplishment upon this problem may best be set forth by the use of a table showing some of the data. (These tabulated data are subject to subsequent verification.) The treatments may be divided into four groups: (1) aeration, to change the content of volatile substances in the culture-water or in the animals themselves; (2) change in the food directly; (3) treatment with excretory products or related substances; and (4) treatment with other substances. The aeration experiments were conducted on the supposition that the accumulation of excretory products and possibly a reduction of oxygen supply in the crowded bottles might be factors in bringing about the production of males. The aeration of the animals themselves was accomplished by exposing them to the air for a time during their 'critical period' in a thin film of water on a microscopic slide or in a Syracuse dish. Such aeration reduced but did not eliminate the production of males, inasmuch as one-third as many males were produced by the aerated mothers as by those not so treated. In other series of experiments, the test animals were reared in the same quantity of culture-water as the controls, but in a wide covered dish, so that the culture-water was only 3 mm. in depth, thus providing much greater aeration than in the control bottles. Such treated mothers produced about half as many males as the control mothers. In other large series of experiments air and, in still others, oxygen was bubbled through the test bottles. Approximately three-fifths as many males were produced in such treated bottles as in the controls. On the other hand, bubbling with nitrogen, in which case the bubbling was much less extensive, reduced the male production, but to a less extent than bubbling with air or oxygen. This suggests that scarcity of oxygen is not a factor, since the bubbling of nitrogen materially reduces the oxygen content of the treated bottles, and further suggests that, since the bubbling was less extensive and consequently removed less carbon dioxide or other volatile excretory products, volatile excretory products are factors in male production.

"Scarcity of food as a possible factor in male production is eliminated by experiments in which crowded mothers in dilute food are shown to produce only two-fifths as many males as those in the regular food solution, notwithstanding the fact that average size of the broods of young was only 9 for the dilute food and nearly 20 for the control. However, variation in the culture medium is shown to be a factor in male production, since animals reared in water containing algæ as food for the animals (instead of bacteria as in our manure culture solution) produce fewer males than those reared in the controls and fed upon bacteria (in the manure solution). Further, the age of the culture medium or of the manure used in making it up is a factor in male production, as shown by the data in line 8 of the table, the newer material producing many times more males than old material. These last experiments are of interest as accounting for the varied male percentages shown in the controls of the different series tabulated.

"Treatment with various amounts of excretory products, including ammonium hydrate, urea, urine, and chicken manure, reduced the percentage of males, though treatment with uric acid and carbon dioxide showed a somewhat greater percentage of males in the test bottles. The other agencies tabulated here (pressure excepted) reduced the percentage of males. The few experiments conducted in which the animals were subjected to pressure produced many more males in the tests than in the controls. Further tests of this are necessary, but it would seem that pressure treatment may be added to uric acid and carbon dioxide treatment as agencies increasing male production.

"In general, the experiments point to excretory products as probable causative factors in male production. Pressure may really serve merely to prevent volatilization of certain excretory products. But lacking ready means for determining the various excretory products and their relative amounts, produced in such small animals as Cladocera, it is obviously difficult to imitate and thus artificially bring about a situation duplicating that which

Percentage of males produced in broods of Moina macrocopa subjected to various special conditions before sex is determined.

Type of special conditions.	No. of exps.	Total young sexed.	Control per cent ♂	Test per cent ♂	Difference.	Per ct. total ♂ prod. by controls.	Per cent total ♂ prod. by tests.	Effect of treatment on male production.
Changing the volatile constituents:								
1. Aeration, animals treated	16	4,828	55.9	19.5	36.4	74.2	25.8	Reduced.
2. Shallow dishes	13	2,679	38.7	24.6	14.1	61.2	38.8	Do.
3. Aeration, culture-water treated	21	6,108	36.6	23.8	12.8	60.6	39.4	Do.
4. Oxygen, bubbled and shaken	9	2,126	44.7	27.7	17.0	61.8	38.2	Do.
5. Nitrogen, bubbled and shaken	21	4,308	35.7	26.7	9.0	57.3	42.7	Do.
Changing the food directly:								
6. Dilute food	2	486	40.9	25.9	15.0	61.3	38.7	Do.
7. Algal food	3	633	56.1	19.2	36.9	74.6	25.4	Do.
8. Old food	16	2,839	29.9	2.2	27.7	93.1	6.9	Do.
Excretory or related substances:								
9. Carbon dioxide	29	5,487	19.4	21.9	2.5	47.0	53.0	Increased.
10. Ammonium hydrate	19	4,283	34.6	17.5	17.1	66.5	33.5	Reduced.
11. Uric acid	19	4,091	10.1	11.4	1.3	47.0	53.0	Increased.
12. Urea	35	6,131	6.9	4.7	2.2	59.5	40.5	Reduced.
13. Urine	41	8,552	19.1	7.2	11.9	72.7	27.3	Do.
14. Chicken manure	8	1,570	11.6	2.2	9.4	84.1	15.9	Do.
Other agencies:								
15. Sodium hydrate	8	1,026	12.4	11.1	1.3	52.3	47.7	Do.
16. Sulphuric acid	21	3,530	10.5	7.0	3.5	60.0	40.0	Do.
17. Alcohol	10	2,247	37.2	0.2	37.0	98.5	1.5	Do.
18. Pressure	4	669	2.4	12.6	10.2	16.0	84.0	Increased.
19. Dried daphnids	5	1,053	8.5	2.2	6.3	79.5	20.5	Reduced.

develops in a 'crowded' bottle of these animals. Hence, if we are to formulate the hypothesis that the accumulation of excretory products is a large factor in male production, it is exceedingly difficult to bring about a treatment which will test the hypothesis. We have tested many combinations (though by no means all the possible combinations) of the presumed excretory products with only indifferent and inconclusive results so far.

"The conclusion would seem to be that the conditions favoring male production are quite complicated, that excretory products probably constitute a large factor, that there is a delicate balance which, if much disturbed, as by most of our treatments, results in a reduction in male production, but that any treatment which materially affects male production throws light on the situation involved and promotes the analysis correspondingly.

"To summarize the treatments catalogued in the table, aeration by whatever method, changes of whatever nature in the food, treatment with many

excretory products or related substances—ammonium hydrate, urea, urine, chicken manure, and treatment with alcohol, sulphuric acid, and sodium hydrate—have resulted in reduced male production. On the other hand, animals treated with carbon dioxide, or uric acid, or reared under pressure, have produced increased percentages of males."

Control of Production and Hatching of Sexual Eggs.

Previous knowledge of production of sexual eggs in Cladocera (in which only parthenogenetic eggs are ordinarily produced) has been extended. Control measures are somewhat difficult, but means of an entirely practicable sort have been developed for *Moina macrocopa* and *Daphnia longispina* and one of our types of *Daphnia pulex*. There is reason to think that these measures may be readily modified so as to apply to most, if not all, the other Cladocera under cultivation. Hatching the fertilized sexual eggs is more difficult than their production, but those of two of our forms may now readily be hatched and some of those of *Daphnia longispina* have been successfully hatched into viable offspring. The occurrence of sex intergrades in two of our laboratory stocks and of two other clear-cut mutations in *Daphnia longispina* makes hybrid matings a matter of much interest. Our attempts at hybridization have so far been unsuccessful, but will be continued.

Cytological Work on Cladocera.

The occurrence of sex intergrades in two species of our Cladocera stocks, and the ability to control through environmental conditions the sex of Cladocera, make the chromosome situation in these animals a matter of much theoretical interest. Cytologically, Cladocera are very difficult, and the services of a good cytologist seemed essential in attacking this problem. Dr. Ezra Allen, of Ursinus College, whose improvements in cytological technique have made accurate studies in mammalian cytology possible, spent two months with us this summer and made a good start upon the study of the cytology of two species of Cladocera. He reports as follows:

"Fixation was made in Fleming's fluid, 10 per cent formalin, Dantchakov's, and B-15 (Allen's modification of Bouin). Fleming proved of no value for the large eggs; its effect upon the chromosomes in the younger tissues has not yet been studied. The fixation of the testis proved quite satisfactory in B–15.

"As to stages studied, since the chief interest in this form lies in the sex differentiation by the parthenogenetic female, the period of, and immediately following, egg-laying was studied at first in hope of determining the maturation phenomena. The large quantity in the yolk of coloring matter that takes both hematoxylin and safranin made this an undesirable and difficult stage to do the first work upon. Consequently embryonic and post-embryonic stages were studied, in females only at first. These consisted of embryos fixed at 13.5, 16, and 20 hours after egg-laying, and of young about ready to be released from the brood-pouch. The young were fixed at about 2, 8, 16, 18, 24 to 26, and 36 hours after release from the brood-pouch. Young males were fixed at 15, 18, 24, 12 to 36, 30, 24, 48, 60, and 72 hours after release.

"The results were that no dividing oogonia were found. Cell division was active in the testes of the 24 and 60 hour stages. The older males have not yet been studied. The cells are very small and not favorable to study. Apparently the haploid number is in the neighborhood of 8 chromosomes,

agreeing with Kühn's estimate of *Daphnia pulex*. The material, while difficult of observation, appears capable of count determination in both spermatocytes and spermatogonia.

"The dividing cells in embryonic tissue are exceedingly small, and it may be impossible to determine the number in the ordinary cells. The oogonia are readily differentiated in embryos 16 hours after discharge from the brood-pouch, and under favorable conditions their chromosomes should be readily studied. The number of oogonia laid down at the 16-hour stage is about 14 to 16. This number more than doubles by the time the embryos are ready to be released. While no counts have been made of the number of oogonia and eggs when the growth stages of the eggs have begun, there seems to be a considerable increase over that of the stage when ready to be released.

"On account of the inherent difficulties in the material of *Daphnia longispina*, it seemed best to work out the cytology of *Moina* before continuing the studies of *Daphnia longispina*. In *Moina* the sex-ratio is influenced by crowding mothers in bottles, much as in *D. longispina*. There is much less yolk and practically no coloring matter in the eggs, conditions which favor the study of maturation stages."

Comparison of Metabolism in the Sexes.

In past years this Department has contributed to the knowledge of the relative metabolism in the production of male and female eggs of pigeons. The existence not merely of males and females, but also sex intergrades in Cladocera, added interest to an investigation of the metabolism of the sexes in this group of aquatic organisms. Dr. V. Obreshkove, of Syracuse University, spent the summer at Cold Spring Harbor determining the rate of carbon-dioxide production in some of Dr. Banta's stock. The rate of carbon-dioxide production serves as an index of the rate of respiration and metabolism in general—the life processes of the organism. To measure the amount of carbon dioxide produced in a given time, a modified Osterhout apparatus was used. Dr. Obreshkove reports as follows:

"The principle involved in the simple apparatus is the colorimetric method of hydrogen-ion determination. Carbon dioxide affects hydrogen-ion concentration. A measure of the time required for the animals to change a constant quantity of indicator solution from one standard hydrogen-ion concentration to a second standard hydrogen-ion concentration is a measure of their rate of carbon-dioxide production. Animals to be tested are placed in a closed tube the color of which accurately matches that of a similar tube which is a standard, containing buffer solution of the same hydrogen-ion concentration, 7.76, and containing the same indicator. The tubes are then gently shaken by tipping them by an apparatus driven by an electric motor. When the color in the tube containing the animals is changed by these animals so that this tube matches in color a third tube containing indicator and buffer solution of a hydrogen-ion concentration of 7.36, the time is recorded (by means of a stop-watch). Successive readings of different animals (and on different days and by different observers) of the same developmental stage are remarkably consistent. With this apparatus a fine series of determinations was obtained for (1) four stages in the developmental cycle of normal *Simocephalus exspinosus* females, (2) mature males of the same species, and (3) mature females of *Moina macrocopa*.

"The very small young just released from their mother's brood-chamber were tested 10 at a time, the time required to produce the standard color averaging 93 minutes and 46 seconds for 10 individuals. Hence for one individual the mean reaction-time is 937 minutes. For mothers which had just

released their first brood the mean for one individual is 9 minutes 51 seconds. Other data are given in the table. The first-brood mother is approximately 44 times as large in actual bulk as a newly released young. Hence, if the rate of carbon-dioxide production were the same, size considered, in young and first-brood mothers, the first-brood mothers should have a reaction time one forty-fourth that for the young, or 21 minutes 19 seconds. Since the actual mean for first-brood mothers is 9 minutes 57 seconds, it is evident that the rate of respiration, and hence the metabolic rate, is greater, size considered, for first-brood mothers than for young females. In fact, it is 219 per cent greater. For fifth-brood mothers (see table), 84 times as large as the young, the rate is 149 per cent that for the young. For ninth-brood mothers, approximately 121 times the bulk of the young, the rate is 66 per cent that of the young. The table gives figures for these comparisons.

Data on the rate of carbon-dioxide production in developmental stages of Simocephalus exspinosus.

	Average length of individuals tested in millimeters.	Approximate number of times the individual surpasses the young in size.	Mean reaction time per individual in seconds.	Rate of carbon-dioxide production, size considered, compared with young (per cent).
Newly released young.....	0.63	.....	56,263	.....
First-brood mothers.....	2.23	44.4	591	219
Fifth-brood mothers.....	2.76	84.2	457	149
Ninth-brood mothers.....	3.12	120.7	711	66
Mature males.....	1.09	5.2	4,589	243

"These data indicate that metabolic rate is relatively high in the first-brood mothers, decreases in the fifth-brood mothers, and shows a still greater decrease in the already senescent ninth-brood mothers. The mature males may be fairly compared with the first-brood mothers. Their rate is, bulk considered, approximately 111 per cent that for the females at the same stage of development. The data are consistent with data of other workers for higher (and much larger) animals, including man, in which the metabolic rate decreases later in life in both sexes and is higher for the male. This first attempt to obtain a measure of metabolic rate in Cladocera opens up a large field, and it is hoped will be extended and continued to the sex-intergrade stock and to several other Cladocera problems."

Selection with Sex-Intergrade Strain of Daphnia longispina.

Experiments on the effect of selection upon the degree of sex intergradeness in various strains of *Daphnia longispina* (referred to in previous Year Books) have been completed. Sex intergradeness appears due to a factor (or factors) which is subject to genetic changes, so that selection and return selection are effective, success depending of course upon our utilizing mutants in our selections. Intergrade production in the lines selected toward reduction of the character has in some cases become restricted to the scanty production of slightly intergrade individuals, and in one line no intergrades of any sort have appeared for eight successive generations. On the other hand, the high trains produce mostly intergrades of fairly high rank.

Selection with Sex-Intergrades of Simocephalus exspinosus.

An effect of "selection" in modifying the sex-intergrade stock of *Simocephalus exspinosus* was not obtained for a time, but has subsequently been realized to a gratifying degree. Of the two "low" lines, one has produced only normal females for the last 8 successive generations, i. e., selection has apparently eliminated the production of intergrades. The other low line has produced no intergrades for the last 23 successive generations. On the other hand, the two "high" lines continue to maintain their usual production of intergrades.

Sex in Relation to Metabolism in Pigeons.

During the year Dr. Riddle has completed some studies on certain aspects of sex and on the relation of "reproduction overwork" to sex in pigeons. Most of these current studies have also been prepared for early publication in various journals. Other results are being incorporated into a complete account of his sex studies.

Determination of Sex in Date-Palm Seedlings.

During the past year considerable progress has been made in the date-palm problem, the most important element of which has been the determination of the chromosome number. The problem was formulated and the material collected by Dr. H. H. Laughlin, the cytological work done by Misses Elizabeth Lord and Rachel Haynes. The haploid count has given 18 chromosomes. Material taken from plants, the sex of which is definitely known, is being prepared for comparative studies in an effort to make an early diagnosis of sex. Practically, the sex of seedling date-palms can not be determined until the plant blooms at the age of 3 or 4 years. The purpose of these studies is to seek a sex-diagnosis in the sprouting seedling, without destroying the seedling itself.

GERMINAL AND SOMATIC VARIATIONS.

Mutations and their Selection in Cladocera.

Sex intergrades occur abundantly in certain lines of *Daphnia longispina*, and the effectiveness of experiments on selection proves that mutations frequently occur in sex-intergrade stock. One of the other mutant stocks, the "excavated head" stock, has been similarly subjected to selection as a method of analyzing the constancy or mutability of this character. Of six strains (derived from sisters and at the start producing the mutant character to an equal extent), three were taken for "high" strains and three for "low" strains. Selection has proceeded for 65 generations. Of the low strains, one showed in the second generation a lowering of the curve of "degree of excavation," and the drop increased materially after the fifty-ninth generation. Its present average grade of manifestation of the character is approximately one-eighth of its original average. Another low strain showed an effect of selection after 10 generations and a further drop after 19 generations of selection. It now averages about one-fourth of its original average manifestation. The third low strain showed no decided decrease in manifestation of the character until after the fifty-fourth generation of selection, and has shown no further drop. It now averages approximately one-sixth of the original manifestation. Of the three high strains, two have shown no decided increase in the manifestation of the character. The other

showed slight increase after 7 generations and a marked increase after 12 generations of selection. It now averages about double its original manifestation of the character, an average approximately 15 times as great as the present average for the low strains. The appearance of the polygons showing the course of this selection experiment suggests that changes in manifestation of the character are of the nature of mutations of smaller or larger degree.

The studies in selection with Cladocera, the first of which was published by the Institution (Carnegie Inst. Wash. Pub. No. 305), demonstrate that in these parthenogenetic organisms genetic changes of the nature of mutations may occur with relative frequency and, as regards a particular character, with probably as great frequency as in most organisms reproducing bisexually.

INHERITANCE OF SPECIAL TRAITS.

Flowering Plants.

The physico-chemical properties of the leaf-tissue fluids of Egyptian and Upland cotton and of their hybrids.—Investigations have been continued by Dr. J. A. Harris and his collaborators along the lines discussed in earlier reports. A comparison of Pima Egyptian and Meade and Acala Upland cotton and the F_1 hybrid has been completed for publication. A study of several newly-imported Egyptian types with respect to osmotic concentration, specific electrical conductivity, hydrogen-ion concentration, and chloride content has been begun.

As at present conducted, these studies involve not merely the comparison of genetically distinct types, but an investigation of the differences in their relationship to the substratum. In addition to the Egyptian cottons, Sea Island has been included in the work carried out in 1923, and some observations made on variant types of both Egyptian and Upland cotton. From the standpoint of methods, the most important advance has been the addition of the determination of the sulphate ion, improvements in the method of determining the chlorine ion, and the rapid determination of reducing sugars. These determinations make possible a more analytical consideration of the variables to which osmotic concentration and electrical conductivity are due.

The studies made in 1923 were limited to the Gila River Indian Reservation, where facilities were afforded by the Cooperative Testing Station. In this work Dr. Harris was assisted by Professor John V. Lawrence, Dr. G. O. Burr, Mrs. John V. Lawrence, Mr. W. B. Sinclair, and Mr. Charles W. Crane. The work in 1923 was mainly devoted to Pima Egyptian and Lone Star Upland cotton and their F_1 hybrids. Preparations for the investigation of the F_2 hybrid are under way.

Genetical analysis of white seedlings of maize.—Dr. M. Demerec is continuing here, in cooperation with Professor R. A. Emerson, of Cornell University, certain experiments on the genetical analysis of white seedlings in maize which he began while in Ithaca, New York.

Variation and Correlation of Fecundity in the Domestic Fowl.

These studies have gone forward steadily during the year, under the direction of Dr. J. A. Harris. Further reports are in press, and considerable progress has been made towards completing a volume summarizing the results of all the work.

Heredity of Learning Capacity in Mice.

Miss E. Vicari was a guest of the Department during the summer and continued her investigation into the rate of learning a maze shown by mice of distinct races and hybrids between them, reported upon last year. She has bred the F_1 and F_2 generations of the hybrids between X-rayed mice and the dilute brown race which have been bred in this Department and which show different reaction times.

Susceptibility to Inoculable Tumors.

The experiments on the genetical factors responsible for susceptibility to inoculable tumors, described in earlier Year Books (1921, pp. 122–127; 1922, pp. 113–114), have been carried out by Dr. L. C. Strong, partly in association with this Department, partly with the aid of a grant from the Rockefeller Institute for Medical Research. Last year was reported the discovery of a tumor dbrB, susceptibility to which depended, apparently, upon the presence of at least two independent factors, since a typical 9: 7 ratio was obtained in the F_2 generation produced from a cross between a susceptible (dilute[2] brown) and a non-susceptible (Bagg albino) strain of mice. Dr. Strong has isolated several genetic families from the F_2 generation and continued them by brother-sister matings to the F_6 generation. One of these families (known as the E VII) has been selected for high percentage of susceptible individuals. In the F_4 generation of this family the following result was obtained: Adding together all of the F_4 progeny derived from E VII, F_3 matings, in which both susceptible and non-susceptible occurred in the individual matings, the ratio of 94 susceptible to 38 non-susceptible individuals is obtained. This ratio is probably a Mendelian 3: 1 ratio, for an ideal 3: 1 ratio would give 99 susceptible to 33 non-susceptible. This result indicates that the F_3 parents differed in only one Mendelian factor. Thus Dr. Strong seems to have achieved the long-looked for result of isolating a family in which the difference between susceptible and non-susceptible individuals depends upon only a single gene. The gene has been called the A^{st} factor. It was already shown (Year Book 1922, p. 113) that the dbrB strain carried two demonstrable factors, A^{st} and B^{st}. The genetic constitution of individuals of the E VII family, as far as their reaction to the dbrB tumor is concerned, is as follows: Non-susceptible individuals have the genetic constitution a^{st} a^{st} B^{st} $B^{st}+$; susceptible individuals have the genetic constitution A^{st} a^{st} B^{st} $B^{st}+$, or A^{st} A^{st} B^{st} $B^{st}+$, the difference being the single factor A^{st}. This family E VII has been continued to the F_6 generation.

Dr. Strong has also made an analysis of the rate of growth of tumors and has demonstrated several important facts which indicate that the difference between two individuals in the growth-rate of the transplanted tumor is caused by genetic differences. Only a part of the evidence may here be cited:

"(1) Within limits, the growth of the individual tumor is constant. By plotting the logarithms of the observed mass of the tumor at uniform lengths of time after inoculation there is obtained a straight line. When the growth rate is slow, the straight line is evident for 7 or 8 weeks after inoculation, whereas when the growth is rapid, the straight line is evident for only about 4 to 6 weeks (fig. 4).

"(2) Individuals belonging to the different hybrid generations grow the same dbrB tumor at significantly different rates. For instance, an indi-

vidual of the homozygous dilute-brown (susceptible) race grows the tumor very slowly. An F_1 hybrid individual (produced by crossing the dilute-browns to the Bagg albinos) grows the same tumor (dbrB) much faster. By continued back-crossing to the original dilute-brown race, the various back-cross individuals grow the same tumor progressively slower and slower until in the advanced back-cross generations only individuals that grow the tumor at the same slow rate as the original dilute-brown individuals are obtained.

"(3) The range of variability for the absolute mass of the tumor at any given interval after inoculation is greater in the F_2 and F_3 generations than it is in the original susceptible dilute-brown strain as well as the F_1 hybrid generation. It is apparent, therefore, that multiple genetic factors are concerned in the growth of the transplantable tumor.

"(4) Families containing individuals growing the same transplantable tumor (dbrB) at different average growth rates can be isolated and continued by selected matings. Figure 4 contains three families, all individuals belonging, however, to the F_4 generation. These families were separated from the F_2 generation and kept distinct up to the present time. Brother-to-sister matings have been used exclusively."

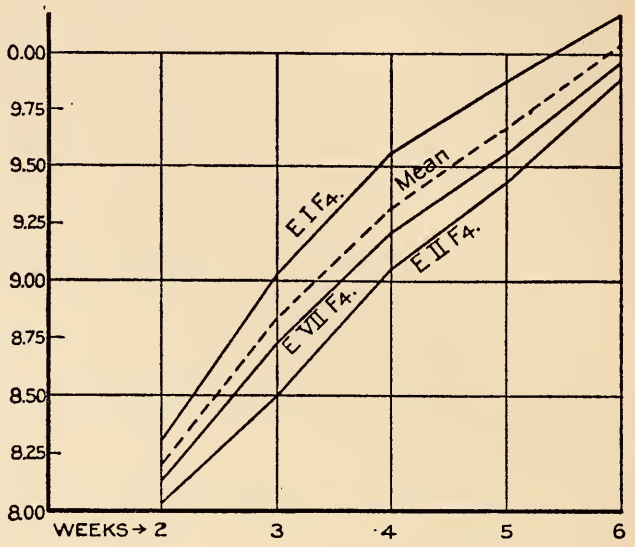


FIG. 4.—Curves of average weight development of tumor in each of three families. Ordinates, logarithm of weight in grams.

In the lines of the dBrA and dBrB, which Dr. Little is continuing at Orono, Maine, five spontaneous tumors have arisen in closely related individuals. One of these is being investigated genetically by Miss B. W. Johnson, who has inoculated over 150 mice of F_1, F_2, and back-cross generations. Miss E. E. Jones also is engaged in isolating new single-factor lines for these tumors, by methods so successfully used by Dr. Strong.

SUSCEPTIBILITY TO ALCOHOLIC INTOXICATION.

The idea is commonly held that certain groups of men are especially immune to alcoholic intoxication; but whether this immunity is due to individual accommodation or is a consequence of a selective process extending through generations is not known. During the progress of the alcoholization of white rats it was found that certain ones always required more than others to yield complete intoxication, but no special records were made of such facts. To attempt to analyze genetically such a subtle character as the susceptibility to alcoholic intoxication might seem too difficult were it not for the successful work of Little and Strong in studying the genetic basis of an even more subtle character, namely, susceptibility to implanted tumors. A series of experiments directed toward the formulation of criteria for comparing the behavior of mice under alcoholic anesthesia has been completed and certain preliminary results have been obtained by Dr. E. C. MacDowell. Over 100 mice have been used in these experiments; of these, 60 have been

tested daily for one to four months. The detailed account of methods may be deferred to the final report. Dr. MacDowell reports as follows:

"The most important result so far obtained from the dose of 3 c.c. for 45 minutes is that the criteria used bring out characteristically different behavior in different mice. Some mice, week after week, give the same reactions; many will regularly turn over at once on coming out of the anesthetizing bottle, run along, and hold back from the edge of the table; others, quite as regularly, will turn over and run along, but fall off the edge three times in succession; again, a mouse will always sit still for a while after turning over at once, and others will lie on their sides for an hour or two before turning over at all. Besides these cases of uniform behavior, all degrees of variable behavior are found, but in the latter cases as well individual characteristics are evident; a mouse will usually turn right over, but every four or five days it will lie on its side for a while; another will never lie on its side, but will usually fall off the edge, with once in a while a day of holding back from the edge. A curious type of incidental behavior is shown by members of one litter, which, after partial recovery, frequently raise their heads, pointing their noses straight up in the air, and then slowly wave one front paw up and down. Nothing approaching this behavior has been seen outside this litter.

"When the mice are left in their bottles every day until they are drunk, the criteria of comparison become reduced to the time necessary to produce motionlessness and the time for recovery. Records have been taken from 22 mice so treated. By this treatment the individual differences are less strikingly brought out, although certain ones clearly take more time to become drunk and others as clearly take more time to regain control.

"The existence of individual differences opens the way for the discovery of strain differences. Although the experiments thus far carried out have been primarily devoted to the study of methods, one case may be cited which suggests that a genetic difference may be involved. From two different lines two litters, of 5 and 6 mice, respectively, all of the same age, were started on the same day with the 45-minute alcohol treatment, and thereafter they were dosed simultaneously. All the males lived in one box and the females in another. Both males and females of one litter regularly turned over, ran along, and held back from the edge of the table, while all of the other litter either lay on their sides, sat still, or fell off the edge.

"Of interest is the rapid accommodation made by the majority of mice to the light dose. They generally lay on their sides after the first treatment at a month old; the time varied from 30 minutes to 2 hours. After 5 or 6 days the mice would usually turn over at once and go away. There is little evidence of further accommodation after the first week, even though the treatment be continued for 4 months. These specially susceptible ones that continue to lie on their sides throughout the treatments do not show a gradual shortening of the time spent on their sides. In the case of the heavy treatment there is an initial period of lengthening treatments, which soon ends, and in some cases the period of treatment then remains fairly constant, while in others it declines. Where there is a decline it is evident that the mouse is losing rather than gaining resistance.

"The effects of temperature on alcoholization and recovery are being studied; but so far are not found to be marked."

New Mouse Mutations.

Both at Cold Spring Harbor and at Orono, Maine, eye abnormalities have been discovered in non x-rayed lines. The Orono mutant, discovered by

Miss E. E. Jones, arose in the Bagg albino races. A white ventral patch mutation was discovered by Miss Johnson and is being genetically investigated by her. It appears to be a recessive. A mutation involving a white tip or band on the tail is also being investigated and its relation to the ventral patch is being considered.

Heredity in Sheep.

The experiments on heredity of twinning in sheep were continued during 1922 and 1923, using as ram, in place of the member of a triplet set, a new ram, the only lamb borne by his mother in 1920. In 1922, 11 lambs were born—3 pairs of twins and 5 as single births—or an average of 1.38 per fertile ewe. In 1923, 28 lambs were born, from the same sire, an average of 1.75 lambs per fertile ewe. Thus using the same sire the proportion of multiple births was greatly increased in the second year. This result can not be ascribed to the greater fecundity of new ewes bred from in the second year; it indicates rather improved vigor of the ram in his second year (a fairly obvious fact to the casual observer). The return to a fairly high fecundity, with nearly the same stock, in the second as contrasted with the first year, shows the importance for multiple births of a vigorous male.

Genetics of the Thoroughbred Horse.

At the request and expense of Mr. Walter J. Salmon, of New York City, Dr. Laughlin has organized studies which have for their purpose the coordination of the performance and pedigree records of the Thoroughbred horse, and the current breeding practices, by the aid of modern genetical knowledge and methods of analysis. A library of very rare and valuable early pedigree and performance records, purchased by Mr. Salmon, is used for this study. There is perhaps no other collection of pedigree records extant for any plant or animal which gives so complete and satisfactory performance data as the speed-records of the Thoroughbred horse. This fact makes the work particularly promising, both for the elucidation of the practical breeding principles of this particular breed of horse, and also (using the methods of pedigree analysis) for the prediction of performance in offspring. The detailed statistical work is being done by Messrs. Clyde E. Keeler, Pierre Hernandez, and Misses Luella A. Smith and Alice Hellmer.

As one of these special studies in horse genetics, Dr. Theophilus S. Painter, of the University of Texas, made investigations at Cold Spring Harbor during the summer of 1923, which resulted in a satisfactory and conclusive count of the chromosome number in the horse. The diploid number is demonstrated to be about 60, including a clean-cut X Y type of sex-chromosome.

Rabbits.

Professor H. D. Fish, of the University of Pittsburgh, as associate of this Department, has continued his researches on the extremely valuable strains of rabbits which he is breeding. The maintenance of these strains encounters certain difficulties arising from parasites, which at times have killed 95 per cent of the offspring. Professor Fish has devised and installed partially self-cleaning cages, and the mortality is now much reduced.

New and improved grading scales have been introduced both for Dutch and English rabbits, to indicate quantitatively the proportion of white pelage

in these piebald races. The lines of self, tan Dutch, white Dutch, dark Dutch, and others are being continued, although high mortality has threatened some of them. Professor Fish reports that he now has two different stocks, each carrying 5 or more recessive factors, and, between them, all of the recessive factors that have been carefully worked out in rabbits are represented. An attempt has been made to repeat Professor Guyer's experiments on the effect of specific sera upon the germ-plasm, but no positive results have been so far gained.

STUDIES ON HUMAN GENETICS.

Heredity in Aristogenic Families.

Dr. H. J. Banker has continued his compilation of the genealogy of a New England family from the eugenical standpoint, following the plan of his paper entitled "The ideal family history," presented before the International Congress of Eugenics. The selected family is one which has shown considerable incidence of eminent scholarship. The plan involves the study of the propositus, his consort, and their ancestry for three generations; also each of their posterity, with the ancestry of each consort. The total number of descendants issued from the propositus is now 235, of whom 63 have had issue. The completed manuscript would include several thousand typewritten pages.

Dr. Banker has continued his collection of scholarship records of coeducational schools, with the aim of studying inheritance of special scholarship. Fortunately he has found, in the adjacent village of Huntington, high school records dating from 1859. They are well preserved and complete from the time of its foundation, and contain a number of instances of not only children but also grandchildren of the earliest students. On account of the stable character of its population and the prevalence of local intermarriage, there appears to be an unusual number of instances of children and both parents educated in the school. Dr. Banker believes the material to be the most valuable in quantity, if not in quality, of any that has yet been obtained.

Heredity in Cacogenic Families.

Dr. A. H. Estabrook has completed his report on the Tribe of Ishmael and submitted it for publication. All original data on the tribe have been filed in the archives of the Eugenics Record Office. Also, there was deposited in the archives a duplicate copy of all data gathered by the Indiana Committee on Mental Defectives during the years 1916 to 1923. This includes some family histories and surveys of mental ability.

Since the middle of November 1922, Dr. Estabrook's time has been chiefly spent in the field on the problem of the South Appalachian population. In seeking to find the best place in which to start, he had conferences at social agencies in New York City, made a tour of the Appalachians through the Virginias, Carolinas, and eastern Kentucky and Tennessee. On this trip men and women who had information about the population of the mountains were visited and consulted as to the proper locations for the first field work. Particularly valuable contacts were made at Berea College, Lincoln Memorial University, University of Tennessee, Asheville Normal School, University of North Carolina, State Board of Public Welfare, Raleigh, North Carolina, and University of South Carolina. Opportunity was gained to learn of

movements of the population to cotton mills and coal-mining fields, and conferences were held with mill superintendents as to source of supply of their workers. The need for certain special studies was urged, such as the Indian-negro-white crosses in the Blue Ridge of Virginia and the Croatans of the coast of the Carolinas.

The first field work was carried out with an Indian-negro-white cross in Amherst County, Virginia, in collaboration with the Department of Sociology of Sweet Briar College, whose students carried on field work under the immediate supervision of Professor I. E. McDougle. About 500 members of this Amherst group have been located and more or less studied.

The major field operation was started in February 1923, in the mountains of eastern Kentucky, in Leslie County, and is being continued. This area was selected because of its inaccessibility; no railroads enter the county and no industrial development has taken place in it. The county is without improved roads, so that all travel is on horse or mule back, mainly in creek beds. A saddle horse has been purchased by the Department for use of Dr. Estabrook. There are about 25 main families in the county, nearly all dating back to 1800 and coming of hunting stock. Much intermarriage has occurred, little immigration since 1800, but considerable emigration in consequence of the Civil War and the call of the coal mines. A study is being made of these old families.

Heredity of Body-Build.

The Director's paper on this subject is being printed by the Institution. In it two types of variation in build are distinguished, the ontogenetic and the adult. The ratio of chest-girth to stature is regarded as the best index of build; and, where chest-girth is unknown, for adults, weight $\div$ stature^2. Racial, geographical, and ontogenetic differences in build are discussed. Changes in adult build with age are relatively greater in families characterized by obesity. The mass polygon of distribution of build shows two modes at all ages, evidence of at least two biotypes of build. Slenderness is associated with tendency toward tuberculosis, pneumonia, nervousness, melancholia. Slender parents have smaller families than fleshy ones, and their children show less regression toward mediocrity than those of fleshy parents, and they are less variable, suggesting that there are recessive genes for slenderness carried by the fleshy. There is a marked tendency for persons of similar build to intermarry. Not all persons of the same build are gametically alike. Some slender parents carry 1, others 2, zygotic factors for build. Some parents of medium build are heterozygotes; others belong to a medium-build biotype. The F_1 generation derived from a slender $\times$ fleshy mating is quite variable, as is commonly found where multiple factors are concerned. Its mode is fleshy, indicating partial dominance of that condition. The F_2 generation is still more variable than the F_1. The back-cross of a heterozygous parent with either parental type tends to fall into heterozygous build and the build of the particular parental type to which the back-cross is made.

It is considered that variations in build are not to be accounted for merely by variations in the relation of intake and outgo of calories, but also by the endogenous factors that determine "economy of nutrition" or the cost in energy of adding an additional kilogram of weight to the body. Hereditary factors are involved in producing such differences and probably work through

the intermediacy of special organs that influence metabolism, notably the endocrine glands. In some cases, at least, three independent factors are involved in very fleshy build, but the number is not the same in all biotypes; in some there is only one, in others 4 or more.

Further collections of family data on body-build are being made by Miss Louise A. Nelson under the Lawrence Memorial Research fund of the Playground Athletic League, of which Mr. Robert Garrett is president and Dr. William Burdick is director.

Heredity of Radio-Ulnar Synostosis.

In cooperation with Dr. Henry Ling Taylor, the New York pædiatrician, a study was made on the heredity of a relatively rare condition by which radius and ulna are congenitally fused at their proximal end. With the assistance of Miss Louise A. Nelson, who did the field work, 15 families were studied; 23 new cases are listed and described; they fall into two types. A review of the mammals shows that proximal radio-ulnar synostosis is not rare. In man the condition is often associated with other bony defects. In most families the inheritance is that of a 2-gene trait, in others of a 3-gene trait. In one family the single gene appears to be in the Y-chromosome. Always synostosis is dominant. Males are twice as apt to be affected as females, taking all families together. Consanguineous matings are frequent in synostotic families. A review of the literature as compiled by Miss Mabel L. Earle accompanies the paper.

Correlation Between Physical and Mental Development in Man.

At intervals, further measurements of idiot boys have been made by the Director at Letchworth Village, with the kind cooperation of Dr. Charles S. Little, superintendent. One paper by Dr. Bertha E. Martin and myself, embodying the earlier studies, is in press, and further analyses of the data were made by Dr. Martin in the summer of 1923. Miss Louise A. Nelson made field studies in central New York State upon the families of these boys.

Variation, Correlation, and Racial Differentiation in the New-Born Infant.

The statistical investigation of the data for new-born infants, abstracted from the records of the Sloane Hospital for Women, New York City, has been continued by Dr. Harris.

GENETIC CONSTITUTION OF THE AMERICAN POPULATION.

Eugenical Sterilization.

In December 1922 the volume on "Eugenical Sterilization in the United States," prepared by Dr. H. H. Laughlin, was published by the Municipal Court of Chicago. Chapter headings are as follows:

Chapter I. Chronological list of laws, amendments, executive vetoes, repeals, official legal opinions, board orders, and court decisions relating to eugenical sterilization previous to January 1, 1922.
Chapter II. Analysis, by States, of sterilization laws enacted prior to January 1, 1922.
Chapter III. Texts and legislative records of the eugenical sterilization laws.
Chapter IV. Statistical and descriptive summary of eugenical sterilization in the several States.
Chapter V. Analysis of the eugenical sterilization laws by subject.
Chapter VI. Analytical outline of litigation growing out of the several eugenical sterilization statutes previous to January 1, 1922.
Chapter VII. Detailed review of litigation growing out of the several eugenical sterilization statutes.

Chapter VIII. Case and family histories of individual subjects of litigation growing out of the several eugenical sterilization laws.
Chapter IX. Legal opinion.
Chapter X. The right of the State to limit human reproduction in the interests of race betterment.
Chapter XI. Eugenical diagnosis.
Chapter XII. The anatomical and surgical aspects of eugenical sterilization.
Chapter XIII. The physiological and mental effects of sexual sterilization.
Chapter XIV. The legal, biological, and practical requirements for an effective eugenica sterilization law.
Chapter XV. Model eugenical sterilization law.
Chapter XVI. Explanatory comments on the model sterilization law.
Chapter XVII. Set of forms suggested for the use of the State eugenicist, the courts, private citizens, and custodial institutions in administering the model eugenical sterilization law.

An attempt was made to make a thorough historical, legal, and statistical review and analysis of the subject in order to determine, from first-hand knowledge, the practice and tendency in eugenical sterilization, to examine these practices in the light of constitutional law, biological principles, and eugenical practice. The principal scientific results of the investigation are summarized in Chapter XV, entitled "Model eugenical sterilization law," in which, according to the opinions of the jurists who have examined the work, the legal requirements of an effective sterilization law have been met. Similarly, analyses of State practices in complying with the several laws were used in developing and incorporating in the law the essential biological and eugenical requirements.

In analyzing the matter of physiological and mental effects of eugenical sterilization (Chapter XV), records of 694 cases of sexual sterilization were studied. Analyses of these cases show that vasectomy in the male and salpingectomy in the female, when performed by skilled surgeons, exercise little or no influence either upon secondary sexual traits or upon the sexual impulse; nor could any severe mental or temperamental effects, especially among the socially inadequate classes, be found which logically could be ascribed to sexual sterilization, by either of the two methods just named.

From the purely eugenical point of view, the analysis was based upon actual pedigree records of families of social inadequates. From this analysis it was concluded that—

"With due heed to the legal, biological, and practical considerations . . . . there is every reason to believe that the greatest benefit would accrue to the natural hereditary qualities of future generations from a law providing for the eugenical sterilization of certain hereditary degenerates and defectives" (p. 444).

The analysis of the legal situation justifies the following conclusion:

"A State may, in the proper and constitutional exercise of its police power, enact practical and enforceable eugenical sterilization laws, devoid of punitive features, but which may include criminals, and which have for their sole purpose the improvement of the natural hereditary physical, mental, and moral endowment of future generations: *Provided*, (a) that such laws are not so unduly discriminatory in their application as to constitute the denial of equal protection of the laws guaranteed by Section 1, Article XIV, of the Constitution of the United States, to all of the citizens of all the States, and (*b*) that such statutes provide for due process of law in their administration" (p. 147).

National Immigration.

Studies on the eugenical aspect of immigration were continued in collaboration with the Committee on Immigration and Naturalization of the House of Representatives and the Department of Labor of the United States Government. On November 21, 1922, the results of the studies up to that date, with particular reference to the relation between social inadequacy and modern immigration, classified according to race and specific type of disorder, were laid before the House Committee on Immigration. These were published under the title, "Analysis of America's modern melting pot" (pp. 725 to 830 of the hearings before the Committee on Immigration and Naturalization, House of Representatives, sixty-seventh Congress, third session, serial 7–C). These hearings comprise an analysis of the detailed diagnostic and racial returns from 445 State and Federal institutions for the socially inadequate classes. In these institutions which collaborated in the study were found a total of 210,835 inmates (date of returns being from January 1, 1921, to March 31, 1922). The particular classes studied were as follows:

The feeble-minded, all types; the feeble-minded, the moron group; the insane, all types; also the dementia præcox group; the manic depressive group; the senile psychosis group; the criminalistic, all types; the criminalistic juvenile group and adult group; the epileptic; the inebriate; the tuberculous; the blind (including the "blind and deaf"); the deaf; the deformed; the dependent, all types, children and adults; all classes of the socially inadequate as a unit; the feeble-minded (census of 1920, survey of 1921) and the insane, all types (census of 1920, survey of 1921).

The analysis was made on the basis of quota fulfillment; each nation and nativity group was allotted an expected quota for each type of degeneracy, based upon the total relative numbers of persons of the particular racial or nativity group in the whole country according to the census of 1910. The quota fulfillment shows a comparison between the number of persons expected and the number found in State and Federal custodial institutions.

The results logically deducible from this particular investigation show that in reference to nervous stability and social adjustment the immigrants who represent the present foreign-born population of the United States are much more poorly equipped, mentally and physiologically, than the immigrants whose blood is to-day represented by the group "Native White, both parents native-born." Further, the immigrant himself is better individually than the germ-plasm which he carries and from which his children develop. This shows particularly in the case of the feeble-minded, who themselves, while legally being entirely excludable, showed a quota fulfillment of 21.56 per cent $\pm$ 1.25, while the native white, both parents foreign-born, show a quota fulfillment of 165.39 per cent $\pm$ 1.34. The native white, one parent native, one foreign-born, show a quota fulfillment of 190.27 per cent $\pm$ 2.05.

Representing the House Committee on Immigration as expert eugenics agent, and the United States Department of Labor as special immigration agent to Europe, Dr. Laughlin sailed for Europe August 8 to make studies in several of the European countries on the possibility of determining, in the home towns and the ports of departure, the facts concerning the hereditary qualities and social values of would-be immigrants, and to determine, further, the probable cost to the United States Government, per would-be immigrant, of such practice, should it be enacted into law.

During the spring and early summer of 1923, Mr. A. E. Hamilton, formerly associated with the Eugenics Record Office, made studies on immigration in

collaboration with the Eugenics Record Office and Hon. James J. Davis, Secretary of Labor, at the expense of the Department of Labor. These studies were based upon the records which we have accumulated, and were on the subject of the several aspects of selective immigration.

THE PHYSIOLOGY OF REPRODUCTION AND DEVELOPMENT.

Modification of Length of Œstrus Cycle by Means of Alcohol.

As described in the Year Book for 1921, Dr. E. C. MacDowell found that when both parent rats were given alcohol before and during mating they produced nearly 11 per cent fewer progeny in a litter than their full brother and sister controls. Moreover, in an equal period of time the alcoholized parents produced 0.72 litter per pair, while the unalcoholized mice produced 2.07 litters per pair. Thus the alcoholized parents had about one-third as many litters and seven-eighths as many young to the litter as the unalcoholized. Consequently, in a struggle between the two kinds of parents, the former would tend to succumb through insufficient numbers. An approach to the problem of the influence of alcohol upon the prolongation of the interval between litters, hence probably of the œstrus cycle, was provided by the notable work of Long and Evans on the œstrus cycle of the rat and of Edgar Allen on that of the mouse. These workers show that the method of vaginal smears is adequate in measuring the œstrous cycle. Dr. MacDowell is continuing work on the causes of reduced fecundity of the alcoholic mice. He reports as follows:

"Mice have been used in the present study on account of the larger number that can be raised in the same space and on the same food as rats. Daily vaginal smears are taken by means of a very small swab and examined microscopically after staining in hemotoxylin and eosin. The alcohol has been given by the inhalation method as before, but instead of tanks holding numerous animals, a pint milk-bottle has been used for each mouse; a measured amount of alcohol is poured over a standard-sized piece of paper toweling which is then slipped into the bottle after the mouse, and a regular milk-bottle stopper is inserted and the bottle inverted. The controls, which live in the same boxes with the corresponding treated mice, are handled in the same way, with the omission of the alcohol. Three series of experiments have been in progress: (1) Starting when the mice are a month old, they have been placed in bottles with 3 c.c. alcohol on the paper every day for 45 minutes, called the light dose; (2) month-old mice have been given the 3 c.c. dose every day until they were motionless and did not move when the bottle was rotated, called the heavy dose; (3) starting after a series of normal cycles had been obtained from the mice as adults, the dose of 3 c.c. for 45 minutes was given, and later in some cases the heavy dose was given.

"So far the following numbers of mice in the respective series have been studied: 50, 13, and 11. The average number of observed cycles per mouse is 5. When the treatment is started early and given for 45 minutes per day, the summaries of the results obtained show *no general modification of the length* of the œstrous cycle in the treated mice compared with their untreated sisters. The time of the original opening of the vagina and the time before the first observed œstrous seem to be equally unmodified by the light treatment. The number of cycles so far obtained from the young ones given the heavy dose is too small to venture a preliminary conclusion. Although there may be no general effect shown by the alcohol treatment, there are marked individual cases that appear to indicate marked effects; giving records of

66 or 69 days, records unapproached by any of the control mice, whose modal cycle-length was 6 days. Similar individual differences are shown by the records of the mice treated after their normal cycle-length was tested. Some appear to show that the treatment changed the length of the cycle, and in others there was no change. This is true of both light and heavy doses.

"Although under the conditions of the experiment alcohol does not seem to have a general or specific influence upon the length of the œstrous cycle, these preliminary findings seem to support the proposition that alcohol influences the length of the œstrous cycle in certain females. This is the situation called for by the data from the rats, a selective action on the mothers themselves. It is to be noted, however, that the rats were given a daily intoxicating dose, and the greater part of the observations so far made upon the mice have been from relatively light dosage."

Recognizing the possibility that different strains of mice may give different characteristic cycle-lengths, an investigation has been begun into the normal length of the œstrus cycle in 10 of the different strains and families at hand. Already, in preliminary fashion, it may be announced that the different lines do, indeed, show differences in the frequency distributions of their cycle lengths. Combining all distribution curves, the mode is found to lie at 5 to 7 days.

A separate study was made of the vaginal smears of 5 yellow mice, a race characterized by an enormous deposition of fat. Yellow mice are also slow breeders. Whether or not the failure of the fat yellows to breed is due to the cessation of œstrus is uncertain. Of the 5 mice, 4 gave fairly regular cycles, averaging 10 days; 1 was examined for 80 days and showed only 3 questionable œstrus periods.

The analysis of the effects of alcohol upon reproduction is being carried further by counts made upon the corpora lutea of pregnancy in living animals. In the case of the rats, formerly studied, the number of young per litter was reduced in the treated animals; but it was not determined whether this was due to a reduction of eggs ovulated or to prenatal mortality. Since the number of corpora lutea formed at one ovulation closely approximates the number of eggs set free, the difference between the number of corpora lutea of any pregnancy and that of young born gives the amount of prenatal mortality. Dr. MacDowell has developed a special technique for finding this difference. During any pregnancy the number of copora lutea belonging to this pregnancy can be readily counted by exposing the ovaries to view, for at this time the corpora lutea of pregnancy are very large, red, and prominent, while all of the older corpora are small, of an opaque cream-color, and sunken into the body of the ovary. The effects of the operation of exposing the ovary are very slight; the young are born normally, even on the following day, with no increase in natal mortality. The operation may be performed repeatedly on the same female without interfering with her subsequent reproductive functions. In normal pregnant mice, 49 operations gave satisfactory counts of the recent corpora lutea in both ovaries; they averaged 8.7 per female. The number of young born to these females after operation averaged 6.4, which may be compared with 5.8, the average given by 1,509 litters in the whole colony. The seven operations on alcoholized mice so far performed indicate that the light dosage does not modify the number of ova liberated.

In connection with the observations on the number of corpora lutea in alcoholized and unalcoholized mothers, certain studies have been made on

modification of the number of eggs ovulated in any œstrus cycle. One of these concerned the influence of removal of one ovary on the number of eggs subsequently ovulated. The left ovary was removed by Dr. MacDowell from 50 adult female mice; subsequently the animals were mated and counts made of the corpora lutea. The question has been investigated before, and it had been found that the single ovary compensates for the loss of its mate. In our experiments the number of corpora lutea in the remaining ovary average about 1 greater than the average produced by two ovaries in our normal females. Thus the single ovary fully compensates and, perhaps, even overcompensates. However, the number of young born from semi-spayed mothers (4.6 per litter) was less than from normal females of the same strain (5.6 per litter). This reduction in the number of young which come to birth is probably an adjustment to the limitations of space in the single horn of the uterus in which all of the young must develop, since passage of embryos to the opposite horn is mechanically almost impossible.

Blood-Sugar Values in Generic Crosses.

As was stated in our report for the last year (Year Book for 1922), Doctors Oscar Riddle and H. E. Honeywell have cooperated in studying the inheritance of blood-sugar values in hybrids between the Japanese turtle-dove (*Turtur orientalis*), which has on the average about 188 mgm. of sugar per 100 c.c. of blood, and the ring-dove (*Streptopelia alba*), which has an average of 149 mgm. of sugar per 100 c.c. of blood. The F_1 hybrids have an intermediate amount of blood sugar. This year further progress has been made on this topic. F_1 hybrids have been obtained between a *Spelopelia* and *Streptopelia*, and these, likewise, show a blood-sugar value that is intermediate between those of the parent genera.

The F_1 hybrids of *Turtur*×*Streptopelia* have been back-crossed to *Streptopelia*, and the hybrids of this generation give an average blood-sugar value that is intermediate between that of the F_1 and *Streptopelia*. The blood-sugar value is apparently inherited in the same way as body-size. It is a complex of several factors, and, it may be predicted, will follow the rules of multiple-factor inheritance. Dr. Riddle points out that the new sugar values found in the hybrids are maintained throughout life, and that such a different value requires new adjustments of equilibrium on the part of all tissue components throughout the developmental period of the hybrid; also, that these adjustments supply one possible basis for various irregularities in the behavior of characters in hybrids derived from wide crosses.

Relation of Reproductive Overwork to Carbohydrate Metabolism.

Last year this Department reported new light upon the mechanism of "reproductive overwork" through the discovery that the suprarenal glands of female pigeons undergo marked and prolonged hypertrophy in very exact coincidence with each ovulation period, and by the further observation that the carbohydrate metabolism of the bird undergoes a simultaneous change, as shown by an increased concentration of the sugar of the blood. These results have been extended, confirmed, and published by Dr. Riddle. The conclusions drawn from the studies on suprarenal hypertrophy are as follows:

"In four kinds of healthy doves and pigeons the weight of the suprarenal glands has been found to increase at each ovulation cycle and the point of

greatest increase is coincident with the act of ovulation. The mean size increase found in the four groups was approximately 40 per cent. The diseases commonly met with in our birds usually produce very marked suprarenal hypertrophy; and all birds with disease recognizable to us have been separately classified and tabulated as diseased birds. Without this separation of healthy and diseased birds a definite relation of suprarenal hypertrophy to ovulation could scarcely be obtained from any reasonable number of birds by our method of study, since the glands of diseased birds are extraordinarily variable in size.

"Previous studies on suprarenal hypertrophy during pregnancy and lactation have apparently overlooked hypertrophy in connection with ovulation; if the latter occurs also in mammals, this is a possible source of error in many results reported. The changes in the suprarenals of mammals at ovulation should be investigated. The suprarenals of male pigeons are probably slightly larger than those of females, even after fully correcting for differences in body-weight.

"When doves are provided with heated quarters and are made to reproduce throughout the year, as were the birds used in this study, it does not appear from the considerable data at hand that season is in itself associated with any change in suprarenal size. If such seasonal changes do occur they do not affect the results reported. Adequate histological studies have not yet been made, but it is probable that both medulla and cortex share in hypertrophy at ovulation.

"These results constitute a contribution to our definite knowledge of the conditions associated with previously observed changes in size of ova, of sex-ratios, and of sexual development in the offspring of pigeons made to reproduce rapidly and continuously throughout the year. They demonstrate that some elements of a new and intimate environment are provided for ova thus made to develop under prolonged and nearly continuous suprarenal hypertrophy."

The studies on the changes in blood-sugar concentration which accompany the suprarenal hypertrophy described above have been made in collaboration with Dr. H. E. Honeywell. These studies have led to the following conclusions:

"Each ovulation period in the pigeon is accompanied by an increase of approximately 20 per cent in the amount of sugar in the blood. The sugar-content of the blood begins to increase at about 108 hours preceding the ovulation of the first egg; it is then maintained at the highest level until the second ovulation 44 hours later; following this period it probably gradually declines to the normal non-ovulation level in an additional 108 hours—unless new ova meanwhile begin their final period of rapid growth and a new ovulation cycle is begun. The curve which thus describes blood-sugar values as related to ovulation is essentially similar to the curve for suprarenal hypertrophy at ovulation earlier described by Riddle. The above results apply to various species of doves, pigeons, and their hybrids. Female pigeons forced to rapid and continuous ovulation throughout the year are thus forced to an almost continuous maintenance of their bodily functions—and to the production of their eggs—under altered and unusual conditions as regards their carbohydrate metabolism."

Relation of Storage Metabolism to Insulin.

Dr. Riddle has made further experiments on this matter with the thyroid secretion referred to in my last report and gets only confirmation of the results reported previously. Insulin, the incretion of the pancreas which

increases the rate of carbohydrate metabolism, has also been found to decrease the size or storage metabolism of the ova. Mr. Walter Fisher has collaborated in this study. It was found that a dosage compatible with continued ovulation, and strictly limited to twice-daily injections during the last half of the growth period of the ovum, reduced the size of ova of 9 birds during a season by 2.8 to 5.7 per cent; and that the portion (one half) of these eggs which was subjected to the effects of insulin during 40 hours longer than the other half showed a further reduction of 4.1 per cent.

From the first of his studies Dr. Riddle has been led by other kinds of evidence to interpret high storage values of the yolk to mean low oxidizing capacity. Still further confirmation of this interpretation has, therefore, been obtained from both of the above studies. Other earlier work has shown a correlation of large yolk-size with femaleness and of small yolk-size with maleness in the embryos arising from such yolks.

Origin of Single-Yolk Twins.

Out of approximately 20,000 incubated eggs of doves and pigeons there have arisen 8 cases of twin embryos, all apparently well formed. These twins arose out of stock so closely watched and intensively studied as to afford very exceptional information concerning the conditions attending their origin. A control is afforded by the simultaneous determinations of yolk-weights in 20,000 cases, yolk-analyses in 1,300 cases, and bomb-calorimeter measurements of energy storage in 500 other eggs. Compared with the control, 5 of the yolks that gave rise to twins were of extremely large size and 3 were of extraordinarily small size. There is no reason for thinking that the twin production was caused by an accidental cooling of the blastoderm, a condition suggested by Stockard as the real cause of twin production in the fowl. Indeed, the experimental subjection of 231 embryos obtained at from 4 to 24 hours of prematurity (Year Book, 1922, p. 121) to low temperatures before and during gastrulation yielded no case of double embryos, though it did produce other kinds of abnormalities. Indeed, not only retardation but also acceleration of the developmental rate produces such abnormalities. The large twin-producing ova were doubtless examples of an extremely low metabolic rate in the unsegmented ova, and the small ova were examples of an extremely high metabolic rate. Twins arose in these eggs as a result of this retardation or acceleration already present in the ovum at the time it left the ovary.

Among agents that induce abnormalities and monsters in early embryos of the pigeon, high concentration of carbon dioxide comes first, then increased concentration of oxygen, decreased oxygen, and low temperature last. During the first 2 to 4 days of development after laying it is the youngest stages that are most affected by increase of carbon dioxide and of oxygen. The later stages are relatively more effected by low temperature. Evidence is supplied that a temperature of 39.4° C. (103° F.) is higher than the optimum and produces a stimulating or accelerating effect during the first 12 to 24 hours of development after the egg is laid. Probably in consequence of this accelerated development, the subjection of embryos during this first 24 hours to exceptionally lowox ygen pressure results in a low survival rate, and exceptionally high oxygen pressure results in a high survival rate.

The results of these studies support and extend the conception that both abnormalities and twins owe their origin to alterations of the developmental rate, or perhaps the metabolic rate, at various and unequally susceptible developmental stages. But whether a stage is "critical" or "indifferent" in Stockard's sense depends in part, at least, upon its modifiability by a retarding or an accelerating agent, and the stage of development at which twins or multiple embryos actually arise extends all the way from the unsegmented egg to gastrulation.

Relation of Blood Sugar to Ovulation.

Dr. Oscar Riddle, with the cooperation of Dr. H. E. Honeywell, has shown that at each ovulation period there occurs a distinct suprarenal hypertrophy and concomitantly a rise in the concentration of the sugar of the blood. It has seemed probable that measurements of the blood sugar might throw light on the causes of diminished or suppressed ovulation. Studies on this subject were undertaken both because of the practical and the theoretical importance of a knowledge of the factors which regulate egg production. The practical importance is obvious, inasmuch as fecundity enters in one way or another into most genetic studies. The theoretical importance to our sex studies we have long recognized, since different sex-ratios are obtained from pigeon clutches according as they are separated from preceding clutches by long or short intervals.

A year ago Drs. Riddle and Honeywell reported (Year Book, 1922, p. 121) that one condition that inhibits ovulation, namely, inactivity or confinement in small space, reduces within 3 weeks the blood sugar of common pigeons from 0.180 to 0.125 per cent. Such pigeons produce few or no eggs. During the present year the same investigators have shown that the onset of cold weather also causes a distinct fall in the blood-sugar values of several kinds of pigeons. The duration of the initial decrease is, however, neither long nor constant. This period of decreased sugar values is one of decreased ovulation rate in our entire collection of birds.

These points established, Dr. Riddle injected insulin into the pigeons; this lowered the blood sugar and nearly all ovulations were inhibited. Precisely, the dose of insulin used lowered the blood sugar of ring-doves from 0.149 per cent to about 0.080 per cent during 1 to 4 hours twice daily. Under this treatment only about one-tenth of the normal or expected number of ovulations was realized. In the course of this study it was learned that ova which have approached to within 48 to 72 hours of ovulation are not suppressed by this dosage of insulin, and this led to a study of the effects of insulin on the size of ova. These studies seem to establish the principle that conditions which lower the concentration of sugar in the blood are incompatible with ovulation.

Death of Embryos in Eggs Abnormally Retained in the Oviduct.

Various students of poultry have observed that an occasional fresh-laid egg of the common fowl already contains a live or dead embryo of one, two, or three days of growth. In these cases it is obvious that such eggs were retained or delayed within the hen's oviduct during at least the period represented by the age of the embryo. Why some of these embryos are dead and others alive is a question which seems never to have been investigated.

In our own studies, and in all genetic work with birds, it may be of importance to know the cause of failure or death in all eggs or embryos which

fail or die. For example, in some pigeons the two eggs of the pair or clutch have unequal prospective sex values, and in any complete analysis of sex-ratios in these forms it is necessary to know whether the first or the second of the clutch is more subject to delay and consequent death. Dr. Riddle has, accordingly, carried out and published a study the full purpose of which was to determine if the retention of eggs in the oviduct is a source of embryonic death; to find out the factor immediately responsible for the death; to measure the amount of death from this cause; and to evaluate the extent to which these eliminations by death may affect sex and genetic ratios obtained in pigeon breeding. In the published results the following conclusions were reached:

"Approximately 1 egg of each 100 produced by a collection of doves and pigeons has been found to be abnormally retained in the oviduct for periods varying between 5 and 122 hours. The retention or delay of eggs within the oviduct of doves during more than 5 hours results in the death of about one-half of the embryos. About 0.5 to 0.6 per cent of all embryos die as a result of egg-retention in the oviduct. Death usually occurs at or near the 0.5-day stage of development. Increased frequency of death occurs in the eggs longest delayed and in eggs provided with thickest shells. Death probably results from an oxygen supply inadequate to the increasing requirements of the post-laying embryonic stages thus retained in utero.

"The occasional survival of retained embryos during these early developmental stages is a result of specially favorable respiratory conditions, namely, the presence of a thin shell or a delay in the secretion of the normal amount of shell.

"An egg occasionally remains in the shell gland during two or three times the normal period for shell secretion without obtaining more than the normal amount of shell. More commonly eggs thus retained receive a heavier secretion of shell material.

"The retention of a 'first' egg of the clutch or pair often or usually results in the suppression of the second egg of the pair. The total result is the death or elimination from breeding data of approximately one-half of the embryos of both eggs of such pairs.

"The retention of a 'second' egg of the pair suppresses no later ovulation. This retention of a second egg occurs, however, only once in 200 ovulations, and approximately one-half of these retained embryos survive. The selective elimination of so small a number of second eggs can have but slight effect on the sex-ratio. Even in pure species, where second eggs of the pair are predominantly female-producing eggs, the proportion of females to males is affected from this source by probably less than 0.3 per cent."

Parthenogenetic Cleavage and the Origin of the Subgerminal Cavity in the Pigeon's Egg.

The close observation of all infertile eggs encountered in Dr. Riddle's pigeon breeding indicated that such eggs give evidence of the presence of a "subgerminal" cavity—a condition normally associated with an early stage of embryonic development. It had also been learned that the yolks of such incubated infertile eggs absorb very appreciable amounts of water. The interest of Professor G. W. Bartelmez, Department of Anatomy, University of Chicago, was enlisted in the appearances connected with some such infertile eggs obtained from "virgin" females, with the result that parthenogenetic cleavage in these eggs was demonstrated. This parthenogenesis in birds is of course a fact of genetic interest, not only because it is found in our

own genetic material, but because it strongly supports Lecaillon's report of parthenogenesis in the fowl. The mode of origin of the subgerminal cavity as this could be learned from pigeon eggs is of real interest to developmental physiology. The results which have been written and sent to press were abstracted as follows:

"Incubated unfertilized pigeons' eggs acquire a fluid-filled space in the region normally occupied by the subgerminal cavity, and another—peripheral to the germ disk—beneath the vitelline membrane. This increase by osmosis of its water-content at the expense of the surrounding albumen begins in fertilized and unfertilized ova alike as soon as the ovum is liberated from the ovary. This increased fluidity is observable at all points immediately within the vitelline membrane and in the space normal to the subgerminal cavity.

"Early cleavage stages occur parthenogenetically in the pigeon's egg. Cleavage proceeds only to a stage approximately equal to that attained 10 hours after fertilization in normal development. The degenerative changes which follow are closely confined to the segmented region, and these are accompanied by a pronounced increase of fluid beneath the segmented area. The development, therefore, stops at a stage preceding that at which the subgerminal cavity normally arises, but the cavity forms nevertheless. This cavity in such eggs, therefore, arises not as a result of 'development' but as a result of a progressive increase of water within the ovum. The origin of the subgerminal cavity is thus given a purely mechanical explanation."

Relation of Age, Sex, Species, and Certain Diseases to the Blood Sugar of Pigeons.

After Dr. Riddle found the measurement of the sugar of the blood a most convenient method of studying certain metabolic aspects of sex and of reproduction in pigeons, it became necessary to learn the sources of error and variability in this material. In collaboration with Dr. Honeywell he has made about 1,400 sugar determinations. As a result of this study it has been possible to eliminate several sources of error from their own published data; to demonstrate some hitherto unrecognized sources of error in blood-sugar determinations; to evaluate the relation of age and sex to the normal blood sugar in the pigeon better than it has been done in any other species, with the possible exception of man; and to supply, for this aspect of the carbohydrate metabolism, the fullest data now available for any order of animals.

OTHER INVESTIGATIONS.

Physico-Chemical Properties of Plant-Tissue Fluids.

As has been emphasized in preceding reports, the cellular fluids represent at the same time the product and the environment of the protoplasm. It is under the influence of these fluids that all the vital processes, cytoplasmic and nuclear, must take place.

A series of investigations by Dr. Harris and a number of collaborators during the past several years has shown wide variations in the physical and chemical properties of plant-tissue fluids. Such properties as osmotic concentration, specific electrical conductivity, hydrogen-ion concentration, and the concentration of other ions have been shown to differ not merely from flora to flora, when these are growing under different environmental conditions, but to be characteristic of species so closely related that genetic studies may be made upon them when growing under the same conditions.

Brief reports on the various phases of this work have been made in preceding Year Books. During 1922 and 1923 progress has been made along the following lines:

Tropical Element in North American Flora.

Evidence is gradually accumulating that the intrusion and persistence of the West Indian element in the flora of subtropical Florida is due in part to the physico-chemical properties of their tissue fluids. During the winter of 1922, Dr. Harris, assisted by C. W. Crane, made extensive series of determinations in southern Florida, extending as far as Flamingo in the Cape Sable region. Here conditions remarkably similar to those investigated in the Jamaican coastal deserts by Harris and Lawrence a number of years ago were encountered.

Selective Elimination of Species Due to Changes in Salinity of the Soil Solution.

The opening of Jupiter Inlet on the Florida east coast, after it had remained closed for several years, has given an opportunity to consider the action of natural selection with respect to sap properties. The considerable areas of fresh-water swamp which will be occasionally touched by salt water had already been studied by Dr. Harris to some extent, and the sap properties of the species which are growing under these conditions are known. Future observations will determine to what extent the elimination of species is dependent on their sap properties, and to what extent the sap properties of other species change with the modification of conditions.

Other Studies on Natural Vegetation.

Such measurements on the sap properties of alpine and desert plants as could be made incidentally to experimental work in Utah and Arizona have been carried out by Dr. Harris. The time demanded by experimental studies is rapidly rendering this environmental work difficult, and it will soon be brought to a close as a major project, and be continued only incidentally to experiments under way in the various regions.

A report on the investigations in the highly saline and other environments of Tooele Valley, Utah, on the southern shore of the Great Salt Lake, reviewed in a preceding Year Book, has been completed for publication.

Investigations on the Cereals.

As noted in preceding reports, Dr. Harris has made investigations on the physico-chemical properties of wheat and other small grains in the arid region of the Great Basin since 1920. These studies have been made possible by the U. S. Department of Agriculture with the cordial but informal cooperation of the Utah Agricultural Experiment Station and Agricultural College.

From 1920 to 1922, inclusive, attention was devoted primarily to the change which takes place with the advance of the season when the various varieties are grown with and without irrigation. These studies have, however, involved preliminary comparisons between fall and spring wheats, and general comparisons between the varieties. This phase of the work is now in an advanced stage of preparation for publication. In 1923 the purpose of the work was changed so as to deal more intensively with the differentiation

of various varieties and selections with respect to drought resistance. During 1923 the field work was mainly in charge of Mr. W. F. Hoffman, of the University of Minnesota, assisted by Dr. G. O. Burr, Mr. W. B. Sinclair, and Mr. Charles W. Crane, Dr. Harris going to the field only toward the end of the season.

ADMINISTRATIVE RECORD.

ARCHIVES OF EUGENICS RECORD OFFICE.

The care of the archives has remained in the hands of Dr. Elizabeth B. Muncey, who has been assisted by Misses Helen Bowen and Margaret Martin as indexers. An estimate of the extent of the records and their index as of September 1, 1923, is as follows: 961,271 cards in the index, 1,618 books in the archives. The field reports (F) and (V) number 59,250 sheets, the special-traits file (A) 25,068 sheets, the records of family-traits (R) and (M) files, 5,658 parts. During the summer we had the assistance of the members of the training class in preparation of materials for the archives and in the arranging of the records. Misses Harriet Abbott, Grace Allen, Ann March, and Beatrice Meyers collated data on extinction of families, heredity of exostoses, and other topics.

The archive room is full to overflowing and, indeed, the entire Eugenics Record Office building has nearly reached the limit of its capacity. There is urgent need of an addition to it.

Collection of Data.

More records than ever before have come to the Eugenics Record Office through the generous cooperation of college and university professors. There were 852 Records of Family Traits thus supplied, besides 2,130 other schedules, nearly all filled out with great care. The number of teachers and institutional directors who thus collaborated was 42. The Record Office is deeply grateful to these collaborators for their cooperation.

Training Corps.

10 women and 3 men were trained for eugenical field work from June 27 to August 7. Of these, 4 have secured or are considering appointments in eugenical field work. To date, 246 persons have received this training.

Special Activities of and Changes in Staff.

The Director was absent on a trip to Europe September 13 to October 30, 1922. He visited the leading centers of genetical research and returned impressed by the courage with which men of science in Europe are continuing their researches against great odds, and by the great responsibility devolving on American geneticists to produce results proportional to their relatively great opportunities.

On August 8, 1923, Dr. H. H. Laughlin sailed for Europe to attend the meeting of the International Commission of Eugenics, at Lund, Sweden, and to make studies on immigration. During his absence Dr. Banker assumed some of the duties of the Assistant Director of the Eugenics Record Office.