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# CELL SIZE, NUCLEAR SIZE AND THE NUCLEO-CYTOPLASMIC RELATION DURING THE LIFE OF A PEDIGREED RACE OF OXYTRICHA FALLAX

LORANDE LOSS WOODRUFF

*Sheffield Biological Laboratory, Yale University*

ONE FIGURE

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## I. INTRODUCTION

The fundamental importance of the primary differentiation of protoplasm into cytoplasm and nucleus has naturally led to numerous studies, both morphological and physiological, on the interrelations of these parts of the cell complex. One trend of investigation has been to determine the absolute and relative size of nucleus and cytoplasm in different types of cells, and in similar cells under varying physiological conditions. Among the studies from this standpoint the following may be mentioned as pertinent to the problem in hand. Strasburger ('93) determined, by a series of careful measurements in embryonic stages of over forty species of plants, that there is quite a definite ratio between cell and nuclear size, and advanced the view that this is determined by the extent of the 'working sphere of the nucleus.' Gerassimoff ('02) concluded that cell size is determined by nuclear size, on the basis of the fact that when the daughter nuclei,

at division stages of Spirogyra, are both retained in one of the daughter cells, that cell becomes unusually large. Boveri ('02, '05) found that the size of the nuclei of the cells of sea urchin larvae is directly proportional to the number of chromosomes which enter them, and that cells which contain small nuclei are below the normal size, because such cells divide more frequently than those with large nuclei.

In a number of publications, the earliest of which appeared in 1890, Minot advanced the view that differentiation, senescence and death are concomitant with, and the result of, an increase in the proportion of cytoplasm to nucleus in cells.

Minot holds that the egg, at the earliest stage of its development, is actually in a senile state in which there is an excessive amount of cytoplasm as compared with the nucleus. During the process of cleavage, however, this relation is reversed by a rapid increase in the nuclear material. Thus rejuvenation is brought about chiefly by the segmentation of the egg, and depends upon the increase of the nucleus; and senescence gradually appears as development proceeds on account of the increase of the cytoplasm which is necessary for the differentiation of the cells. He wrote ('97, p. 370):

The animal, when it is young, has cells with a small amount of protoplasm. . . . In order that perfection of the adult structure should be attained, it is necessary that the mere undifferentiated cells, each with a small body of protoplasm, should acquire first an increased amount of protoplasm, and then from the increased protoplasm should be taken the material to result in differentiation, in specialization. . . . The increase of the protoplasm is . . . the mark both of advancing organization and advancing age.

In regard to the lower forms Minot says:

If it be true that there is among Protozoa, among unicellular animals, anything comparable to the gradual decline in the growth power which occurs in us we shall expect it to be revealed in the condition of the cells—to see in those cells which are old an increase in the proportion of protoplasm, and consequently a diminution in the relative amount of nucleus (p. 515).

The interest in the relative amount of nuclear to cytoplasmic materials in cells has been largely augmented by the contributions

of R. Hertwig and his school. As a result of his work on the conjugation of Infusoria, published in 1889, Hertwig has been led to attach great importance to what he terms the 'Kernplasmarelation.' In 1903 he discussed his theory and maintained that there exists for each type of cell a definite normal volume relation between nucleus and cytoplasm, and emphasized the fact that the normal relation may be disturbed by various environmental changes. More recently he has emphasized definite periodic changes of the Kernplasmarelation between successive cell divisions, the immediate cause of cell division being a state of cytoplasmic and nuclear tension (Kernplasma-Spannung) induced by an overgrowth of the nucleus, and the resolving of the single cell into two restores the functional Kernplasmarelation. Hertwig interprets the large nucleus of the immature egg as a sign of a state of depression, and nuclear reduction, which is accomplished by the phenomenon of maturation, and fertilization are followed by a period of rapid cell division resulting in the formation of the embryo. From the study of various Protozoa (Actinosphaerium, Paramecium, Dileptus) he finds that senescence, 'depression' and 'physiological degeneration' are concomitant with great nuclear growth. For example, certain Infusoria, which have been supplied with a superabundance of food, show physiological degeneration which is characterized chiefly by a disproportionate development of the nucleus. A return to the normal condition of the cell is brought about by a direct elimination of the superfluous nuclear material, or, for example, by conjugation. "Die Befruchtung ist kein excitatorischer, sondern ein regulatorischer Vorgang."

It is unnecessary at this time to review the many papers which have recently appeared, under Hertwig's stimulus, on the effects of various internal and external conditions upon the Kernplasmarelation, or its finer analysis during the various phases of cell life. Popoff, for example, who has made some of the most important studies on the subject, found that in the infusorian *Frontonia leucas* immediately after division there is a decrease in the nucleus, which is followed by a slow growth (functional growth) and then by a rapid growth (divisional growth) of the nucleus

which brings about again the Kernplasma-Spannung which is the inciting cause of the succeeding bipartition of the cell.

In a word, some investigations seem to show that there is for each kind of cell a characteristic size relation between nucleus and cytoplasm—variations from which lead to various phenomena, normal and abnormal. Minot holds that a decrease of nuclear as compared with cytoplasmic material leads to a slowing of the division rate, while Hertwig and his school believe that an increase of nuclear as compared with cytoplasmic material leads to a slowing of the rate of division.

Child ('10), from interesting experiments upon senescence and rejuvenescence in a planarian, puts emphasis on physiological rather than morphological factors as the fundamental cause of these phenomena.

Senescence in nature consists physiologically in a decrease in the rate of metabolism and this is determined morphologically by the accumulations in the cells of structural obstacles to metabolism, e.g., decrease in permeability, increase in density, accumulation of relatively inactive substances, etc. Rejuvenescence consists physiologically in an increase in the rate of metabolism and is brought about in nature by the removal in one way or another of the structural obstacles to metabolism.

Conklin ('12) has recently extensively reviewed the subject of cell size and nuclear size on the basis of studies on gasteropods from the standpoint of cell-lineage, and he states:

My observations do not support the view that senescence is due to a decrease (Minot) or an increase (Hertwig) of nuclear, as compared with protoplasmic material; nor that rejuvenescence is accomplished during cleavage by the great increase of nuclear material relative to the protoplasm. On the other hand senescence seems to be associated with a decrease, rejuvenescence with an increase of metabolism (Child).

In a sense there are two aspects of the nucleo-cytoplasmic relation theory. One considers the nuclear-cytoplasmic relation during the various phases of the life of the individual cell and its fluctuations during vegetative and reproductive phases. The other takes into account the general nuclear-cytoplasmic relation which obtains in the various cell generations in the life history of an organism, all the cells of which are lineal descendants of a single cell—the fertilized egg (Metazoa) or exconjugant

(Protozoa). It is the latter application of the nucleocytoplasmic relation theory which is considered in the present study.

The studies on pedigreed races of various Protozoa, under conditions in which occur, sooner or later, a gradual decline in the power of reproduction and finally death, have shown diverse results in regard to the absolute variations in size of the cells and their nuclei and also in regard to the relative changes in size of cytoplasm and nucleus during the various phases of reproductive activity.

For example, Maupas ('88) in his extensive studies on the life histories of various Infusoria found a decrease in size of the cells and an increase in the size of the nuclei as the organisms proceeded to what he termed 'senile degeneration.' Calkins ('02, '04) from studies on the life history of *Paramecium caudatum*, in which he followed with great care several races of this species through a number of depression periods which finally culminated in death, stated that "The first clearly marked period of depression . . . was characterized by a well-defined reduction in size (down to 109 microns), and by vacuolization of the endoplasm . . ." ('04, p. 443). Concerning the animals in later periods of low fission rate he wrote: "These became smaller and smaller," and again: "These unstimulated individuals died out in about 508 generations after becoming much emaciated and reduced in size and with reduced nuclei" (p. 444).

Popoff ('07) in his study of the life history of *Stylonychia mytilus* and of *Paramecium caudatum* clearly described and illustrated a marked decrease in size of the cells as degeneration proceeded and this was accompanied by a marked increase in size of the nuclei. Similarly, in his work on *Frontonia leucas* ('08) he found that slow division rate was accompanied by a decrease in cell size and increase in nuclear size. On the other hand, Woodruff ('05) found a marked increase in cell size as well as an increase in nuclear size during the life of a pedigreed race of *Oxytricha fallax*,<sup>1</sup> and Gregory ('09) in her study of the

<sup>1</sup> In the very last stage of degeneration, when the two or three remaining cells of the race were unable to divide at all, there was a marked shrinking before death occurred. This, obviously, has no bearing on the present problem.

life history of *Tillina magna* found the largest cells when the division rate was lowest. But she noted that the nucleus may or may not increase in size during periods of low activity; "if an increase does take place it is generally found that the cytoplasmic material has increased also and the ratio between the two is the same as in the periods of high activity." Moody ('12) from measurements of cells from a pedigreed culture of *Spathidium spathula* found that:

When the division energy was low and the culture had already entered on a period of depression, both the coefficient and Kernplasmarelation indicate a decrease in nuclear volume, rather than the abnormal nuclear growth claimed by Popoff in 1908. Hertwig maintained that an increase in nuclear mass led to a slowing of the division rate this is not true of *Spathidium* where a slow division rate is coincident with a decrease of nuclear material.

From the above-mentioned typical investigations, it is to be noted that those of Maupas, Calkins and Popoff showed a decrease in the size of the cell as physiological degeneration increased, while those of Woodruff and Gregory showed an increase in the size of the cell under similar conditions. The results of Calkins and Moody indicated a decrease in the size of the nucleus as physiological degeneration increased; those of Gregory showed no uniform increase or decrease under such conditions, while those of Woodruff and Popoff clearly indicated a marked increase in the size of the nucleus. From the standpoint of Hertwig's Kernplasmarelation theory: Maupas' and Popoff's results are affirmative; Gregory's and Moody's are negative; while the results of Calkins and of Woodruff give no data from which the proportion of cytoplasmic to nuclear material can be computed.

The present paper presents the results derived from a detailed investigation of cell size, nuclear size and the nucleo-cytoplasmic relation during the life of one of the pedigreed races of *Oxytricha fallax* employed in my study published in 1905.

I have determined to make this intensive study of cell and nuclear size in this race of *Oxytricha fallax* for several reasons. First, in response to several requests for detailed information in regard to the proportion of cytoplasm to nucleus during the

different phases of reproductive activity illustrated in my earlier paper. Second, because, on the basis of my microphotographs of a few specimens, some readers have determined (supposedly) that I found an increase in the proportion of nuclear to cytoplasmic material. And finally, because it appears to me that the study of the morphological changes which accompanied the protracted physiological degeneration of this culture offers a particularly favorable opportunity to determine exactly how great the variations in cell size and nuclear size are during the life of a race of *Oxytricha* and whether these variations are of such a character as to support either of the current morphological conceptions of cytoplasmic and nuclear relations with reference to physiological degeneration.

## II. MATERIALS

This race of *Oxytricha fallax* is one of several cultures of hypotrichous Infusoria which I bred some ten years ago in Professor Calkins' laboratory at Columbia University. The chief object of the work at that time was to repeat some of the work of Maupas and to determine whether hypotrichous forms show cyclical changes in their vitality similar to those which Calkins had found to occur in his race of *Paramecium* and, if such did occur, to endeavor to reinvigorate the organisms by artificial stimulation. The data at hand from this work led me to conclude that "the species studied pass through periods of greater or less general vitality as measured by the rate of division. This cyclical change is most prominent in the *Oxytricha A* culture." (The one under consideration in the present study.) "The periods of depression lead to death if the culture is subjected continuously to the same environment." (p. 626).

Subsequently I have reinvestigated the problem of the cyclical character of infusorian life history in *Paramecium* with special reference to the effect of the environment (culture medium) and have secured results which, I believe, conclusively prove that this form, at least, has unlimited power of reproduction by division, under favorable environmental conditions, without conjuga-

tion or artificial stimulation. This conclusion is based on the fact that I have been able to breed a pedigreed race of *Paramaecium aurelia* for (so far) over six years or through more than 3800 generations without periods of physiological depression—the animals of the present generation being in as normal morphological and physiological condition as the specimen isolated to initiate the culture. It is apparent, however, that closely related species, and different races of the same species, are adapted to more or less diverse environmental conditions, and it is this factor which is undoubtedly at the foundation of the diverse results which have been obtained by various students of the longevity of infusoria in culture without conjugation (cf. Woodruff '11).

Therefore, in the light of studies during the past decade, I would interpret the cycle of my *Oxytricha A* culture as being the result of the fact that the race of *Oxytricha fallax* which was employed was not adapted to live indefinitely "when continuously subjected to the same environment" of hay infusion ('05, p. 627). I believe that if an entirely suitable environment had been secured this culture would have given evidence of unlimited power of reproduction by division without conjugation as my present *P. aurelia* race has done. In other words this culture of *O. fallax* affords detailed data in regard to the cytological changes which occur during a long period of gradual physiological degeneration induced by the environment, which should be of great value in determining variations in cell and nuclear size and also the Kernplasmarelation under such conditions.

### III. METHODS

The methods employed in conducting the cultures of this race are stated in detail in the original paper and are similar to those which I have subsequently used in all my pedigreed culture work. It is only necessary to repeat here that the culture was started by the isolation of a wild specimen on a depression slide in about five drops of hay infusion and when this animal by division had produced four individuals each of these was isolated to form one of the four lines of this culture, *O. fallax A*. These four lines



were continued by the daily isolation of a specimen from each which was placed in fresh culture medium on a clean slide. The accompanying graph (fig. 1) shows the daily rate of division of all four lines averaged together and this again averaged for each ten-day period of the life of the culture.

Infusions of hay or grass were used to the exclusion of all other culture media except at certain periods of acute physiological depression. These exceptions are of no interest for the present problem because the animals preserved from such periods have been excluded from the series measured; for example during periods 21 and 22 when beef extract was employed in an endeavor to rejuvenate the race. The great rise in the rate of reproduction which occurred later was attributed to this temporary environmental change. The culture was subjected to the ordinary room temperatures throughout the work, but such variations as occurred are unimportant as the mean temperature during parts *A*, *B*, *C* and *D* is essentially the same and produces an error which is negligible, when the length of the periods and the number of specimens under consideration is taken into account.

As the present study is based entirely upon measurements of mounted specimens the following statement of the methods employed in preservation is important:

The specimen to be preserved is isolated by means of a fine-pointed pipet on a clean depression slide . . . . . with as little of the culture medium as possible. To this is added three or four drops of bichlorid of mercury in saturated solution with 5 per cent of glacial acetic acid. After about five minutes the specimen is transferred to another slide and a few drops of 75 per cent alcohol is added. A slide is now smeared with a trace of egg-albumin and the specimen is taken from the 75 per cent alcohol and gently spurted onto the albumin. After a short time, when the alcohol has coagulated the albumin, the slide with the specimen adhering to it is transferred to a jar of 75 per cent alcohol and is thereafter treated by the ordinary slide method. For staining, Ranvier's picrocarmin was used . . . . . Clearing was done with xylol, and damar was used in mounting.

All the specimens were fixed, stained and mounted in exactly the same way so that alterations due to shrinkage should be approximately the same. Some two hundred slides were made

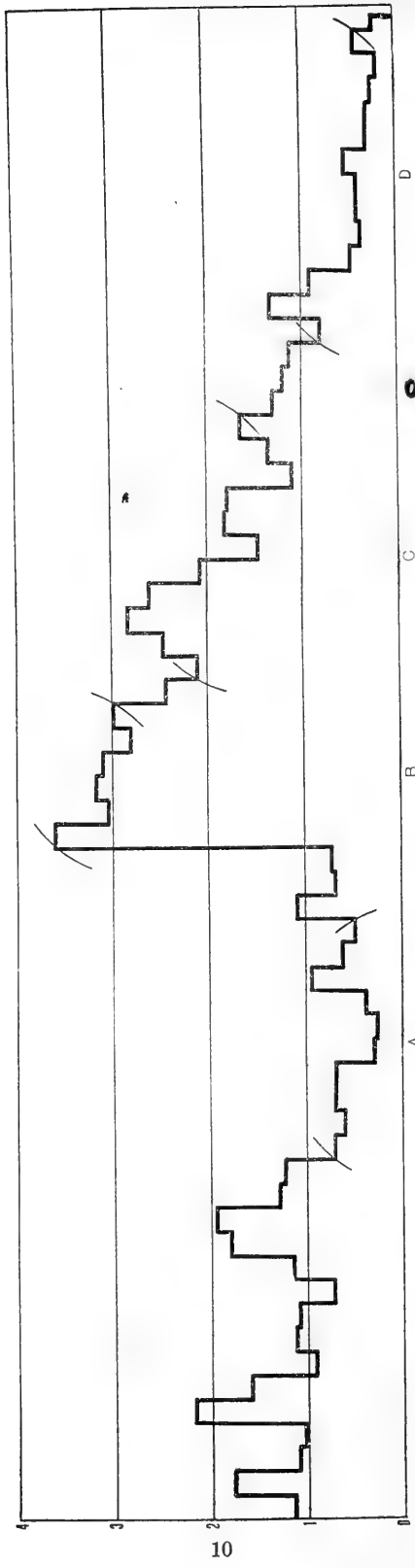


Fig. 1 Life history of *Oxytricha fallax*, culture A (October, 1901, to July, 1903)

which comprise about seven hundred specimens. It is important to emphasize the fact that in the present study are considered specimens only from periods in which the regular culture medium was employed, and also those only from the direct lines of the culture. The specimens preserved from the stock cultures (i.e., mass cultures seeded from the main lines) have been omitted. This left over 450 specimens of the pedigreed race for measurement. These specimens cover nearly the entire range of the life of the race, but of necessity a majority are representatives of periods of comparatively high division rate (fig 1, *B* and *C*). The methods necessary for the breeding of pedigreed cultures make it impossible to kill and preserve a large number of specimens when the rate is low, because at such periods it is essential to keep alive all animals available to ensure the continuance of the strain. It is believed, however, that whatever may be lost in accuracy, by not having larger numbers to measure at certain depression periods, is far more than counterbalanced by the fact that the ancestry and the environmental conditions of those studied is accurately known.

It was apparent when the material was surveyed that it was impossible to secure measurements which would give accurately the volume of either cytoplasm or nucleus during the periods of marked physiological depression owing to the irregular contours of the cells and particularly of the nuclei. Accordingly, it was determined first to make accurate measurements of the areas of the cells and their nuclei throughout the entire life of the race—as only by this method could the size of the cell and nucleus during marked depression periods (*A*, *D*) be compared with those during periods of comparatively high reproductive activity (*B*, *C*).

Since from these data it was impossible to determine the relative volume of cytoplasm and nucleus at the different periods of the life history, it was decided to investigate this point with respect to the specimens from periods *B* and *C*, during which the rates of division were characteristically different, while the form of the cells and their nuclei was sufficiently regular to permit the computation of their volumes from linear measurements.

The first series of measurements, those of areas, were made with an ocular micrometer cross-ruled into squares. The areas of the cells and nuclei were each plotted twice to obviate errors as far as possible. The unit in which the areas are expressed in the tabulations equals 225 square microns. The second series of measurements were made with an ordinary ocular micrometer. The length and breadth of each cell was determined and also the length and breadth of each of the two macronuclei. It was impossible to measure the thickness of either the cell or nucleus, but from a series of observations it was apparent that the average thickness of the cell is very nearly one half that of the width and that the thickness of the macronucleus is the same as its width.<sup>2</sup> Therefore these dimensions, which are without doubt substantially accurate and produce a negligible error, were employed for the third dimension. The results are expressed in cubic microns.

Throughout this study the term 'nucleus' has taken into account the macronuclei only and the micronuclei have been disregarded as in the investigations of former workers in this field. This is justifiable from several standpoints. In the first place the area and the volume of the micronuclei is insignificant in comparison with that of the macronuclei, and actual measurements fail to reveal variations which would appreciably affect the results. Second, variations in the number of micronuclei which were noted occurred characteristically during periods of marked physiological degeneration when it was impossible to compute the volumes of the parts of the cell (Periods *A* and *D*), and when the macronuclear increase was so great that the micronuclear variations would not appreciably affect the area determinations. Third, it is well established that the macronucleus comprises the chromatin specialized for the general vegetative cell activities—and so is of paramount interest in the present connection.

<sup>2</sup> It is apparent, of course, that since these hypotrichous forms are considerably flattened dorso-ventrally, the mounted specimens present to view either the dorsal or ventral side of the animal.

IV. CELL SIZE AND NUCLEAR SIZE

In order to analyze the data accurately four parts of the life of the race were selected, designated respectively *A*, *B*, *C*, and *D*, each of which comprises, as it were, a different phase of the reproductive activity. Part *A* includes cells in which the rate of reproduction and general vitality is very low (less than one division in twenty-four hours); part *B* includes cells in which the reproductive activity is actually and relatively very high (average about three divisions in twenty-four hours); part *C* includes cells in which the reproductive activity is about 'normal', though considerably less than *B* (average about two divisions in twenty-four hours); and part *D* includes cells which have again reached a stage of marked morphological and physiological degeneration.

The data may be considered best in connection with the tables. Table 1 gives, for each of the twenty-one ten-day periods for

TABLE 1

PART	NUMBER OF TEN-DAY PERIOD	NUMBER OF SPECIMENS	SMALLEST CELL		SMALLEST NUCLEUS		LARGEST CELL		LARGEST NUCLEUS	
			Cytoplasm	Nucleus	Cytoplasm	Nucleus	Cytoplasm	Nucleus	Cytoplasm	Nucleus
A.....	16	3	16.25	2.75	24.65	1.50	24.65	1.50	16.25	2.75
	18	3	10.72	1.52	15.65	1.44	17.28	1.80	17.28	1.80
	24	4	10.75	1.72	10.75	1.72	16.00	2.75	13.75	2.80
	25	8	9.73	1.04	9.73	1.04	22.50	2.30	22.50	2.30
	29	62	2.75	0.65	6.55	0.52	11.38	0.92	7.80	1.16
B.....	30	11	4.97	0.71	6.35	0.63	8.65	0.69	6.75	0.79
	32	43	3.69	0.60	4.16	0.50	11.96	1.04	11.96	1.04
	33	91	4.00	0.54	4.20	0.46	11.20	0.98	11.20	0.98
	34	37	4.00	0.70	5.73	0.48	12.18	1.04	12.18	1.04
	36	63	5.76	0.80	11.50	0.72	13.50	1.44	13.50	1.44
C.....	37	53	8.44	0.84	8.81	0.60	18.40	1.44	18.40	1.44
	38	10	7.24	0.91	8.26	0.69	10.90	0.90	7.24	0.91
	39	33	3.36	0.64	4.08	0.44	8.12	1.04	8.12	1.04
	40	10	4.01	0.60	5.00	0.56	7.89	0.77	6.72	0.79
	41	8	8.44	0.77	10.11	0.76	12.10	0.89	10.74	1.07
D.....	42	9	7.47	0.70	7.47	0.70	14.30	1.08	14.30	1.08
	50	2	8.95	0.70	8.95	0.70	11.00	1.20	11.00	1.20
	51	7	8.36	0.72	10.42	0.68	11.21	0.84	11.21	0.84
	52	1	11.70	0.76	11.70	0.76	11.70	0.76	11.70	0.76
	56	1	11.16	1.20	11.16	1.20	11.16	1.20	11.16	1.20
	62	2	12.53	1.62	12.53	1.62	14.07	1.68	14.07	1.68

which comparable material was available, the area of the smallest cell and its nucleus, and of the largest cell and its nucleus and also the area of the smallest nucleus and its cell and of the largest nucleus and its cell. Table 2 gives in parallel columns for each part (*A*, *B*, *C* and *D*) of the life of the race: (1) the number of the ten-day period from which the specimens were taken, (2) the number of specimens measured in each ten-day period, (3) the range of cytoplasmic area during each ten-day period, (4) the mean of this range, (5) the range of nuclear area for each ten-day period, (6) the mean of this range. A further analysis of these data, in which the cells of each part (*A*, *B*, *C* and *D*) of the life of the race is taken as a unit, is given in table 3.

From these data it is clear that, on the basis of measurements of area, and this is a sufficient criterion for the particular aspect of the question under immediate consideration, there is great variation in cell size during each period but that the mean size of the cell is greatest in part *A* when the reproductive activity is low and least in part *B* when the reproductivity activity is highest, and then becomes larger in parts *C* and *D* as the rate of reproduction gradually wanes. The data for the nuclear area also indicate that there is great variation in nuclear size during each period but that the mean size of the nucleus is greatest in part *A* and smallest in part *B*, and is again larger in parts *C* and *D*. The difference between the means of the cytoplasm and also of the nucleus in *C* and *D* is near the limits of the probable error of the determination so that it is not positively established that there is a real increase in size of the nucleus and cytoplasm during part *D* as compared with part *C*. It is clear, however, that the mean size of the nucleus is smallest when the reproductive activity is greatest. It should be emphasized that the greatest difference in size of both cytoplasm and nucleus is seen in comparing *A* and *B*—the periods which are characterized by the most marked change in divisional activity. It is apparent then, from measurements of the area of the cytoplasm and nucleus of cells of this race of *Oxytricha* that *the mean size of both the cells and their nuclei is smallest at the period of greatest reproductive activity.*

TABLE 2

PART	NUMBER OF TEN-DAY PERIOD	NUMBER OF SPECIMENS	CYTOPLASM		NUCLEUS	
			Range	Mean	Range	Mean
A.....	16	3	16.25-24.65	19.86	1.50-2.75	2.22
	18	3	10.72-17.28	11.21	1.44-1.80	1.58
	24	4	10.75-16.00	13.87	1.72-2.80	2.44
	25	8	9.73-22.50	15.28	1.04-2.30	1.63
B.....	29	62	2.75-11.38	6.13	0.52-1.16	0.72
	30	11	4.97- 8.65	6.19	0.63-0.79	0.70
	32	43	3.69-11.96	6.28	0.50-1.04	0.71
	33	91	4.00-11.20	6.39	0.46-0.98	0.65
	34	37	4.00-12.18	6.81	0.48-1.04	0.78
	36	63	5.76-13.50	10.15	0.72-1.44	1.03
C.....	37	53	8.44-18.40	11.73	0.60-1.44	0.95
	38	10	7.24-10.90	8.83	0.69-0.91	0.80
	39	33	3.36- 8.12	4.91	0.44-1.04	0.70
	40	10	4.01- 7.98	6.06	0.56-0.79	0.69
	41	8	8.44-12.10	10.61	0.76-1.07	0.88
D.....	42	9	7.47-14.30	10.92	0.70-1.08	0.90
	50	2	8.95-11.00	9.97	0.70-1.20	0.95
	51	7	8.36-11.21	9.50	0.68-0.84	0.73
	52	1	11.70-11.70	11.70	0.76-0.76	0.76
	56	1	11.16-11.16	11.16	1.20-1.20	1.20
	62	2	12.53-14.07	13.30	1.62-1.68	1.65

TABLE 3

*Cytoplasm*

PART	NUMBER OF SPECIMENS	RANGE	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
A.....	18	9.73-24.65	15.05±0.89	3.80±0.42	25.28±2.84
B.....	244	2.75-12.18	6.36±0.07	1.70±0.05	26.72±0.81
C.....	186	3.36-18.40	9.44±0.15	3.18±0.11	33.68±1.17
D.....	13	8.36-14.07	10.45±0.35	1.89±0.25	18.08±2.40

*Nucleus*

A.....	18	1.04-2.80	1.90±0.08	0.53±0.059	27.89±3.13
B.....	244	0.46-1.16	0.70±0.006	0.13±0.003	18.57±0.57
C.....	186	0.44-1.44	0.90±0.009	0.20±0.006	22.22±0.78
D.....	13	0.68-1.68	0.94±0.06	0.35±0.045	37.23±4.93

## V. NUCLEO-CYTOPLASMIC RELATION

In order to determine whether there is any characteristic change in the nucleo-cytoplasmic relation as the power of reproduction wanes, it was important to measure the volume of cytoplasm and nucleus. Here, for reasons already noted, it was necessary to confine the study to specimens from parts *B* and *C*, but results from these cells should adequately answer the question. For this purpose 100 cells from *B* and 100 cells from *C* were measured. In *B* the 244 available cells were distributed through ten-day periods, nos. 29, 30, 32, 33 and 34; and in *C* the 186 available cells were from ten-day periods no. 36 through no. 42 (fig. 1). The 100 cells in *B* and the 100 cells in *C* which were measured were taken proportionately from each of the ten-day periods on the basis of the total number available in each of these periods. The actual cells measured were taken at random and the data secured therefore should be entirely representative of the groups measured for areas and already described.

The data secured in regard to the volume of cytoplasm and nucleus are presented in tables 4 and 5, from which it is apparent that, although there is great variation in cell and nuclear size during each period, the mean volume of the cytoplasm and also the mean volume of the nucleus is smaller in period *B*, during which the division rate was higher, than in period *C* in which

TABLE 4

PART	TEN-DAY PERIOD	NUMBER OF SPECIMENS	VOLUME OF CYTOPLASM		VOLUME OF NUCLEUS		C N
			Range	Mean	Range	Mean	
B	29	25	2948.91-41563.36	17776.99	666.01-1884.96	1009.26	16.61
	30	5	11904.57-30159.36	21328.85	913.15-1135.16	983.03	20.63
	32	18	7095.82-47048.60	20572.81	660.01-1507.96	1059.07	18.42
	33	37	6588.98-33510.40	16760.64	452.38-1478.64	922.17	17.17
	34	15	7602.67-21447.65	14160.16	565.48-1545.66	1004.81	13.09
C	36	34	12990.24-42101.62	26709.61	995.88-2314.28	1404.80	18.01
	37	28	21790.13-62832.00	39658.80	678.58-2408.56	1525.94	24.98
	38	5	15551.02-26464.83	21269.26	522.64-1206.36	737.86	27.82
	39	18	3901.86-18765.82	9311.07	343.47-1234.64	811.97	10.46
	40	5	9047.90-24950.58	13238.30	527.78-1005.30	788.32	15.79
	41	5	21184.85-39885.75	32080.75	867.07-1373.92	1118.39	27.68
	42	5	20357.66-44635.86	34346.28	800.05-2094.40	1451.83	22.65



TABLE 5

PART	VOLUME OF CYTOPLASM		STANDARD DEVIATION	COEFFICIENT OF VARIATION	$\frac{C}{N}$
	Range	Mean			
B	6588.98-47048.60	17539.26±600.46	8902.34±424.59	50.75±2.41	
C	3901.86-62832.00	26908.45±945.53	14018.35±668.59	52.09±2.48	
	VOLUME OF NUCLEUS				
B	452.38-1884.96	984.17±17.00	252.12±12.02	25.61±1.22	16.82
C	343.47-2408.56	1255.87±31.18	462.40±22.05	36.81±1.85	20.42

the rate was lower. Thus measurements of volume substantiate those already presented for areas.

The data now in hand (table 5) show\* that the increment of mean volume of cytoplasm in period *C* (as compared with period *B*) is greater than that of the nucleus, as the nucleo-cytoplasmic relation in period *B* is 1:16.82, while in period *C* it is 1:20.42. Therefore the measurement of the cells of this race indicate that *as the power of reproduction wanes the mean proportion of nuclear to cytoplasmic material becomes lower, owing to greater growth of the cytoplasm than of the nucleus*, although individual cells show a wide variation at all periods of the life of the race.

In the above discussion the terms 'cytoplasm' and 'nucleus' have been employed to include these regions of the cell as usually defined. It is important, however, to consider the more obvious changes in the morphological condition of these elements during the life of the race. During part *B* both cytoplasm and nucleus showed the normal, comparatively homogenous appearance. But beginning with ten-day period 36, part *C*, about the 542d generation, "the cytoplasm showed signs of vacuolization, and this increases steadily and at approximately the 600th generation (ten-day period 38) the nuclear apparatus begins to depart from the normal." These changes are very slight, though progressive, as we pass from the early to the latter part of *C*, but during part *D* "the cytoplasm is greatly vacuolated, the ventral cirri reduced, the macronuclei distorted and fragmented and the micronuclei increased beyond the typical number; a condition closely similar to that which obtained at the 230th generation"

(ten-day period 24, part *A*). This vacuolization of cytoplasm and nucleus is not peculiar to the race of *Oxytricha* under observation, as similar phenomena are mentioned by other students of the life history of *Infusoria*.

Conklin ('12) first called attention to one aspect of this question:

Neither Minot nor Hertwig took account of the fact that a large part of the nuclear content belongs to both nucleus and protoplasm. The Kernplasmarelation depends very largely upon the quantity of protoplasmic material temporarily in the nucleus. . . . Neither of the authors named, in describing the enormous growth of the nuclear material during cleavage, took account of the growth of the protoplasm during cleavage at the expense of the yolk.

This, it seems to me, emphasizes a fundamental difficulty in many studies on the nucleo-cytoplasmic relation, and one which, in somewhat different aspect, has to be considered in the present work. The vacuolization of cytoplasm and nucleus in all probability does not represent strictly a reversible interchange of material between these two elements of the cell, but it certainly indicates a dilution of the fundamental constituents of the cytoplasm and nucleus (employing these terms in their usual sense) and, insofar as this is true may, and undoubtedly does, alter the proportion.

The question of importance, then, is whether the increase in size of cytoplasm and nucleus, and the decrease in the relative amount of nuclear to cytoplasmic material, which has been shown to occur in this race with declining reproductive activity (periods *C* and *D*), indicates an actual change in these elements or whether the vacuolization is sufficient substantially to alter the results. A careful study of the cells with this in mind shows unmistakably that the cytoplasm and the nucleus, in the strictest interpretations of these terms, actually increase in volume with decreased fission rate. The mean difference in nuclear size and cell size between periods *A* and *B*, and *B* and *C* are of such a magnitude as to be clearly beyond any error due to vacuolization. Careful study also shows that the vacuolization which is present in part *C* is not sufficiently extensive appreciably to affect the nucleo-cytoplasmic relation.

## VI. CONCLUSIONS

The results presented are suggestive from several points of view. The recent extensive and thorough work of Jennings on *Paramecium* shows clearly that a wild population of either of the common species, *aurelia* or *caudatum*, can be resolved into several diverse races or genotypes which are distinguished by differences in size, and that these differences are independent of environmental factors. In other words, the various races maintain their relative sizes when bred under diverse conditions, provided only that the conditions are identical for each race. The present data bear directly on this problem in that they indicate the wide range of variation in size which may occur at the same and at different periods of the life of a pure line of *Oxytricha*, and emphasize the fact that even extreme variations in size are of little or no specific value, and that very large numbers of cells of a pure line under identical culture conditions and at the same division rate must be measured in order to establish morphological criteria of infusorian genotypes. In diverse races, the division rate of which is different under identical environmental conditions, the investigation is obviously still more difficult.

Although most studies on pedigreed races of Protozoa indicate that cell size decreases as the rate of division decreases, it is usually held, from studies in embryology, that cells which divide infrequently are larger, all things being equal, than those which divide more often. For example, Conklin has shown that the 'turret' cells of *Crepidula* are the smallest cells in the entire embryo when they are formed but since they divide only twice during the whole cleavage process they grow very large, whereas the cells from which they are derived, the apical cells, give rise during the same period to twelve cells whose combined volume is not much greater than the volume of one full-grown 'turret' cell. This is in accord with the present observations on *Oxytricha* and those of Gregory on *Tillina magna*.

It seems clear from my data that neither the size of the cell nor the size of the nucleus is the inciting cause of division, but rather that the rate of cell division is probably indirectly responsible for the size of the cell and the nucleus. The wide varia-

tion in size during each period clearly indicates that the cell may function normally in diverse size relations (parts *B* and *C*), and that the inciting cause of cell division must be sought in other factors. The fact that cells of a protozoan genotype show great size differences which bear no direct relation to division is of great significance as it indicates clearly that the conditions which induce growth may be distinguished from those which incite fission.

The data presented also indicate that the mean proportion of nuclear to cytoplasmic material is smaller as the rate of reproduction wanes (*B* and *C*) and thus are in agreement with those of Moody's study of *Spathidium*. This result is contrary to the observations of Hertwig and his school though it is, at first glance, in agreement with Minot's nucleo-cytoplasmic conception of cytomorphic changes leading to senility. But Minot's theory, if I interpret it correctly, does not include cases of physiological degeneration other than those brought about by an inherent tendency to 'grow old,' and consequently, since the present data concerns cells which are degenerating as a result of their environment, the results only indirectly bear on his theory.

When we survey the results of the already numerous investigations on cell size and nuclear size and their relation to each other in the cell complex, one cannot fail to be impressed with the fact that the data are remarkably diverse and contradictory—much more so than would, a priori, be anticipated if the Kern-plasmarelation idea were substantially sound. To cite but a single instance, the results of Popoff on *Stylonychia mytilus* and my own on *Oxytricha fallax* may be considered. These two races of closely related hypotrichous ciliates were bred under conditions which should render them directly comparable, and each ended with the extinction of the lines after a considerable number of generations. Yet the cytoplasmic change in *Stylonychia* consisted in an unmistakable decrease as the division rate decreased, while that of *Oxytricha* showed an equally marked increase as the rate of division waned. In each there was nuclear enlargement with falling divisional activity—but obviously the decreased cytoplasm resulted in an increase in the proportion

of nucleus to cytoplasm in *Stylonychia*, while the increase of both cytoplasm and nucleus in *Oxytricha* resulted (as we have seen) in a decrease in the proportion of nucleus to cytoplasm, owing to the slightly smaller increment of the nucleus as compared with that of the cytoplasm.

It cannot be doubted that the relative volume of cytoplasm and nucleus plays an important part in the life of every cell and that inconceivably small variations may, and probably do, produce far reaching disturbances many of which are not detectable. Likewise, I believe, it is a priori beyond the limits of probability that significant morphological changes would be as a rule of such a magnitude as to be measurable. Undoubtedly a morphological basis should be sought for the various physiological manifestations in degenerating cells, but, I believe, that the results so far at hand indicate that it is prejudging the problem and attempting to reduce it to unduly simple terms, when mere measurable mass relations are estimated as fundamental criteria.

My results with various cultures of Protozoa lead me to agree with the view that senescence seems to be associated with decreased metabolism and rejuvenescence with increased metabolism, but I am unable to correlate with these physiological factors any constant morphological changes such as the current nucleo-cytoplasmic relation theories demand.

#### VII. SUMMARY

1. A wide variation in the size of the cells and of the nuclei occurs at all periods of the life of the race.

2. The mean size of the cell is smallest at periods of high reproductive activity and becomes progressively larger as the division rate falls.

3. The mean size of the nucleus is smallest at periods of high reproductive activity and becomes progressively larger as the division rate falls.

4. The nucleo-cytoplasmic relation of individual cells shows a wide variation at all periods of the life of the race.

5. The mean proportion of nuclear to cytoplasmic material is highest during the period of greatest reproductive activity.

6. The size of the cell and the size of the nucleus as well as the nucleo-cytoplasmic relation are interpreted as an incidental result rather than as a cause of the rate of cell division.

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# THE PHYSIOLOGY OF CELL-DIVISION

## V. SUBSTITUTION OF ANESTHETICS FOR HYPERTONIC SEA-WATER AND CYANIDE IN ARTIFICIAL PARTHENOGENESIS IN STARFISH EGGS

RALPH S. LILLIE

*From the Marine Biological Laboratory, Woods Hole, and the Physiological Laboratory, Zoological Department, University of Pennsylvania*

### I. INTRODUCTION

The problem of fertilization presents various analogies with the problem of stimulation—some superficial, others indicating that the two processes possess certain fundamental physiological features in common. In both cases the cell gives a qualitatively constant or specific response to a change of condition which itself need not be specific. Thus, a muscle contracts when artificially excited by chemical, electrical, or mechanical stimuli which never act upon it in the intact organism, in the same manner as in response to the normal physiological stimulus or nerve-impulse. Similarly, the egg-cell begins its characteristic cycle of cell divisions after subjection to various artificial forms of treatment, as well as after the normal contact and entrance of the spermatozoon. It is a special peculiarity of the egg-cell that these cell-divisions, once started, continue automatically in a regular and predetermined rhythm and are associated with the definitely directed and progressive processes of growth and differentiation which constitute development. But this process, however complex, is none the less the constant and distinctive mode of response of the egg-cell, just as contraction is of the muscle-cell. A relatively slight and non-specific disturbance initiates in either type of cell its own characteristic and complex type of physiological activity.

There are indications that something more is involved here than a mere formal resemblance or analogy. In stimulation a temporary change in the electrical polarization of the limiting membrane of the irritable element is the essential or critical event.<sup>1</sup> This change—so far as the present evidence extends—appears to be conditioned by a temporary variation in the properties of the limiting membrane. Apparently the latter undergoes a rapid and automatically reversible increase of permeability; hence the electrical polarization of the membrane, which in the resting cell is a function of the normal semi-permeability,<sup>2</sup> is temporarily diminished; the characteristic electrical variation of stimulation is an expression of this change. In other words, the membrane temporarily loses its semi-permeability during stimulation. The evidence of this is largely indirect, in the nature of the case, but fortunately one irritable tissue is known in which there is clear and unequivocal indication that the membranes lose their semi-permeability during excitation; this is the osmotic motor mechanism of sensitive plants, where movement results from a sudden loss of turgor. Since turgor is dependent on semi-permeability, its sudden loss can only mean loss of semi-permeability. Evidence that similar conditions exist in irritable animal tissues is seen in the increase of electrical conductivity of muscle during excitation,<sup>3</sup> and in the temporary loss of irritability (the so-called refractory period) which accompanies the rising phase of the electrical variation: it is clear that if the membranes of the irritable tissue lose their semi-permeability during stimulation, any repetition of stimulation will be impossible until the semi-permeability necessary to this process is regained, since relative impermeability to ions is a necessary condition of the polarizing action of the electrical current, and it is this action—as Nernst has shown—which is the essential in electrical stimulation. I have also adduced evidence in favor

<sup>1</sup> This is the necessary inference from the studies of Nernst and his successors on electrical stimulation.

<sup>2</sup> Increase of permeability, however induced, decreases or abolishes the demarcation-current potential.

<sup>3</sup> Cf. McClendon, *American Journal of Physiology*, 1912, vol. 29, p. 302; Galeotti, *Zentralblatt für Physiologie*, 1912, vol. 26, p. 536.



of this general theory from observations on the pigmented larvae of *Arenicola*, where stimulating solutions, for example, 0.55m NaCl, cause visible increase of permeability; stimulation and permeability-increase show an unmistakable parallelism in this organism, and both processes are simultaneously decreased or prevented by the same agencies, such as calcium or magnesium salts and anesthetics.<sup>4</sup>

In fertilization also the primary change is a surface-change, which almost certainly involves an increase in the permeability of the plasma membrane. Loeb's researches have demonstrated the general effectiveness of cytolytic substance in initiating cleavage in sea-urchin eggs.<sup>5</sup> Pure solutions of neutral sodium and potassium salts have the same effect, which is the more marked the more energetic the permeability-increasing action, and is inhibited by salts like calcium or magnesium chloride which check or prevent this action.<sup>6</sup> The egg-cell immediately after normal fertilization shows increased electrical conductivity<sup>7</sup> and increased permeability to substances like sugar and alkali,<sup>8</sup> as well as to its own pigment.<sup>9</sup> All of these facts indicate that the fertilization-process involves an initial increase in permeability; such a change ought theoretically to be accompanied by a change in the electrical polarization of the membrane, similar to that which, on the membrane theory, conditions the action-current in irritable tissues; and Miss Hyde's observations on *Fundulus* eggs<sup>10</sup> indicate that this is in fact the case. A depolarization-process thus probably accompanies the initial stage of fertilization, as well as of stimulation, and it is highly probably that it forms the critical or initiatory event in this process also.

<sup>4</sup> I have discussed this subject in fuller detail in a recent paper in the *American Journal of Physiology*, 1911, vol. 28, p. 197.

<sup>5</sup> J. Loeb, *Chemische Entwicklungserregung des tierischen Eies*, and his numerous earlier papers there cited.

<sup>6</sup> R. S. Lillie, *American Journal of Physiology*, 1911, vol. 27, p. 289. *Journal of Morphology*, 1911, vol. 22, p. 695.

<sup>7</sup> McClendon, *American Journal of Physiology*, 1910, vol. 27, p. 240

<sup>8</sup> E. N. Harvey, *Science*, N. S. 1910, vol. 32, p. 565. *Journal of Experimental Zoology*, 1911, vol. 10, p. 547.

<sup>9</sup> Lyon and Shackell, *Science*, N. S., 1910, vol. 32, p. 249.

<sup>10</sup> I. H. Hyde *American Journal of Physiology*, 1904, vol. 12, p. 241.

If this is true, an externally induced depolarization ought to initiate cell-division in unfertilized eggs; the facts of electrical parthenogenesis thus favor this view. It is well known that cytolytic substances cause in muscle an electrical variation or 'injury-current,' similar in direction to the action-current, and indicating—according to the membrane theory—increased permeability of the membranes; the same change presumably occurs in egg-cells treated with cytolytic substances. There seems thus to be good reason for assuming that the initial event in fertilization, as in stimulation, is a depolarization-process, which is conditioned by a temporary increase in the permeability of the plasma membrane. Since this initial depolarization is in both cases reversible, we may conclude that the primary phases of both processes—those in which the membranes are concerned—are essentially similar and differ chiefly in their time-relations, the latent period being relatively long and the rate of the electrical variation relatively slow in egg-cells.<sup>11</sup> There is also reason to believe not only that a depolarization-change is associated with the fertilization-process, but that a rhythm of alternating polarization and depolarization is similarly associated with the rhythm of cleavage.<sup>12</sup> The appearance and reappearance of the characteristic system of cytoplasmic relations, coincidently with the rhythm of cleavage, seems satisfactorily accounted for on the assumption of an alternating polarization and depolarization of the plasma membrane; and the existence in sea-urchin eggs of a parallel rhythm of carbon dioxide production and of susceptibility to poisons<sup>13</sup> constitutes a further and independent indication of a rhythmical variation of permeability.

In stimulation the initial depolarization, corresponding to the rising phase of the action-current curve, is promptly and completely reversible, and the same—with characteristic differences in time-relations—is probably true of the fertilized egg-cell.

<sup>11</sup> The rate of electrical variation varies widely in different tissues, being most rapid for nerve and least rapid for slowly responding tissues like smooth muscle and heart muscle.

<sup>12</sup> R. S. Lillie, *Biological Bulletin*, 1909, vol. 17, p. 207. *American Journal of Physiology*, 1910, vol. 26, p. 126.

<sup>13</sup> Lyon, *American Journal of Physiology*, 1902, vol. 7, p. 56 and 1904, vol. 11, p. 52.

A corresponding reversibility in the associated permeability-change is thus implied. Now, the initial cytolytic process caused in sea-urchin eggs by fatty acids or other cytolytic substances appears to be imperfectly reversible unless the egg is afterwards subjected to a second treatment, which consists in exposure for a certain time to oxygen-containing hypertonic sea-water (for twenty to thirty minutes at 20°), or to cold (for several hours) or to cyanide-containing sea-water (for several hours) Sea-urchin eggs subjected to a simple membrane-forming treatment and then returned to sea-water typically undergo cytolysis, usually after some irregular form-change with perhaps an occasional cleavage. But after appropriate treatment with hypertonic sea-water, cold, or cyanide, many of these eggs are restored to an essentially normal state and proceed normally with their development.<sup>14</sup> The necessity of such after-treatment, following the initial membranolytic or permeability-increasing process appears, however, to vary widely in different eggs. Sea-urchin eggs are characterized by a certain inertia or inability to recover a normal condition without such supplementary treatment. Starfish eggs, on the contrary, may develop normally in a large proportion of cases after simple membrane-formation by heat or fatty acid.<sup>15</sup> In other eggs a local mechanical irritation may also be followed by normal development, as in Bataillon's experiments with amphibian eggs.<sup>16</sup> In some of the experiments described in the present paper the starfish eggs were found to develop more favorably *without* the after-treatment with hypertonic sea-water, cyanide, or anesthetics, than with it. In these cases it is to be assumed that the egg spontaneously reverts to the normal condition of semi-permeability, and hence is enabled to proceed normally with its development. Such spontaneous recovery of the normal properties after artificial membrane-formation rarely occurs with sea-urchin eggs, where the after-treatment

<sup>14</sup> Cf. J. Loeb, loc. cit.

<sup>15</sup> See below. Cf. also my earlier paper on parthenogenesis by temporary warming, *Journal of Experimental Zoölogy*, 1908, vol. 5, p. 375.

<sup>16</sup> Bataillon, *Archives de Zoölogie expérimentale et générale*, 1910, Ser. 5, vol. 6, p. 101.

is indispensable to the production of a large proportion of larvae; if this treatment is omitted the vast majority of eggs invariably undergo cytolysis.

These conditions suggest that in the sea-urchin egg the cytolysis following simple membrane-formation is an expression of the undue persistence of a condition of increased permeability; and that the after-treatment serves essentially to bring the permeability again to the norm.<sup>17</sup> In the normal egg a curve of polarization-change (dependent on permeability-change) of the following general form probably follows the entrance of the spermatozoon; there is an initial depolarization resulting from the increase of permeability, and lasting for perhaps fifteen minutes; following this the permeability and the correlative polarization return to or toward the original condition, where they remain unchanged until the period of the first cleavage, when a second temporary depolarization takes place; a similar change recurs with each succeeding cleavage. In parthenogenetic fertilization with a cytolytic agent it is to be assumed that the initial treatment also causes an increase of permeability with accompanying depolarization, from which however the egg recovers only imperfectly unless subjected to a second treatment whose general effect on permeability is of the reverse kind, that is, of a kind tending to restore the original semi-permeability of the membrane. On this hypothesis the beneficial effect of the after-treatment depends on what may be broadly characterized as an *anti-cytolytic* action. The total process thus consists in a preliminary permeability-increasing treatment—equivalent to cytolytic if the condition is not soon reversed—followed after the proper interval by one whose general effect is permeability-decreasing or anti-cytolytic. If this is true it ought to be possible to substitute for the after exposure to hypertonic sea water or other favorable condition a treatment with other substances or agencies whose general effect is to *decrease* the permeability of the plasma membrane or to oppose any further increase of permeability. Such treatment ought to check or prevent the progressive breakdown or cytolysis otherwise following the

<sup>17</sup> Cf. R. S. Lillie, *American Journal of Physiology*, 1911, vol. 28, p. 285; Godlewski, *Archiv für Entwicklungsmechanik*, 1911, vol. 33, p. 225.

simple membrane-formation, and thus enable a larger proportion of eggs to recover the normal state and proceed with their development.

During the summer of 1912 at Woods Hole I have experimented from this point of view with solutions of various anesthetics. Substances of this class have been found to check the permeability-increasing action of pure isotonic solutions of sodium and potassium salts on sea-urchin and starfish eggs. These anti-cytolytic effects are seen in certain definite concentrations, which correspond closely with those which anesthetize the neuromuscular system of marine organisms like *Arenicola* larvae; in these concentrations anesthetics have the general effect of rendering the plasma membranes of cells—egg-cells, muscle cells, pigment cells, as well as structures like cilia—more resistant than normally to agencies which tend to increase permeability (or cause breakdown in the case of cilia).<sup>18</sup> Anesthetics thus counteract the action of permeability-increasing agencies, and it is probably for this reason that they prevent stimulation (which involves temporary increase of permeability) as well as retard cytolysis. In some cases they have been definitely shown to decrease the normal permeability of the plasma-membranes.<sup>19</sup> The alterations which they induce in the properties of the membranes are thus, generally speaking, opposite in kind to those caused by permeability-increasing or cytolytic agencies. We should thus expect that if, as above assumed, the plasma membrane of the egg is deprived of its normal semi-permeability by the membrane-forming process—and is thus brought into a state which eventually leads to cytolysis—an after-treatment with anesthetics will tend to restore the original semi-permeability and will thus render the condition of the egg more favorable to the continuance of development. Solutions of anesthetics, in other words, ought to produce effects similar to those of hypertonic sea-water or cyanide.

<sup>18</sup> Cf. my recent papers in the *American Journal of Physiology* on antagonisms between salts and anesthetics: 1912, vol. 29, p. 372; vol. 30, p. 1; 1913, vol. 31, p. 255.

<sup>19</sup> Cf. the recent experiments of Osterhout, *Science*, 1913, N. S., vol. 37, p. 111; also my observations on *Arenicola* larvae; *American Journal of Physiology*, 1909, vol. 24, pp. 25 seq.

In the experiments about to be described I have found that anesthetics produce very definite effects of this kind with starfish eggs. Unfertilized mature eggs of *Asterias forbesii* treated briefly with isotonic NaCl solution, dilute fatty acid solution, or warm sea-water (35°), returned to sea-water for ten minutes, and then exposed for half an hour to sea-water containing a favorable anesthetic in appropriate concentration, yield a large proportion—in some cases 80 to 90 per cent—of active larvae. Such after-treatment is often more favorable than that with hypertonic sea-water. With *Arbacia* eggs, on the other hand, the results of similar experiments have been essentially negative. So far I have been unable to produce in these eggs any marked increase in the proportion developing to a larval stage by any method other than the use of hypertonic sea-water. Cyanide, in my experience, has proved only slightly effective. After-treatment with sea-water containing an increased proportion of magnesium and calcium salts (without altering the osmotic pressure), or with sea-water containing anesthetics (as in the solutions used below), is ineffective with *Arbacia* eggs. Just why the two species should thus differ cannot definitely be said at present; in general the starfish egg is the more responsive of the two, and may be made to form membranes and develop by various methods which have no effect on the *Arbacia* egg;<sup>20</sup> it is also more variable in its behavior and less resistant to injurious influences. It is significant that the plasma membranes of starfish eggs are much less resistant to the permeability-increasing action of salt-solutions than are those of *Arbacia* eggs,<sup>21</sup> and the greater effectiveness of after-treatment with anesthetics may possibly indicate that the membranes undergo the reverse kind of modification (*permeability-decrease*) also with greater readiness. The difference in the degree of responsiveness would on this view be an expression of differences in the properties of the plasma membranes; these differences indicate differences of protoplasmic composition, since the membrane is presumably a haptogen film

<sup>20</sup> Brief warming, shaking, isotonic NaCl solutions, weak solutions of mineral acids, exposure to cold.

<sup>21</sup> Cf. my papers in the *American Journal of Physiology*, 1910, vol. 26, p. 125; 1911, vol. 27, p. 289.

formed by a surface-condensation of certain protoplasmic constituents, particularly those which lower surface tension. The starfish egg is deficient in cholesterol according to Mathews,<sup>22</sup> and this circumstance may be important, since the presence of cholesterol seems in general to render plasma membranes relatively resistant to chemical alteration.<sup>23</sup> The plasma membrane of the mature starfish egg seems unusually sensitive to changes in the surrounding medium, and the responsiveness of these eggs to the above form of after-treatment is probably an expression of this peculiarity. The experiments now about to be described relate entirely to starfish eggs.

## II. EXPERIMENTAL

In all the experiments described below the unfertilized eggs of starfish (*Asterias forbesii* and *vulgaris*) were used.<sup>24</sup> The eggs were first subjected to a membrane-forming treatment; then, after an interval of ten minutes in normal sea-water, they were exposed for thirty minutes to sea-water containing anesthetics in the concentrations given below. For comparison, part of the eggs remained in sea-water without after-treatment, and part were exposed for thirty minutes to hypertonic sea-water and to sea-water containing potassium cyanide in m/1000 concentration. The eggs were then returned to normal sea-water and on the following day the proportion developing to a free-swimming larval stage was approximately determined.

<sup>22</sup> A. P. Mathews, unpublished observations made at Woods Hole.

<sup>23</sup> Cf. Höber, *Physikalische Chemie der Zelle und der Gewebe*. 3rd Ed., 1911, p. 232.

<sup>24</sup> In 1912 good starfish eggs were abundant at Woods Hole throughout the summer. A difference was observable between the eggs obtained toward the middle and end of June, and those obtained later (from July on). The latter showed the more regular behavior and were in general more responsive, while eggs obtained towards the end of June were frequently resistant to fertilization and showed irregularities of behavior similar to those described in my recent paper in the *Biological Bulletin* (1912, vol. 22, p. 328). Two maxima of egg-production are thus indicated, which probably correspond to the two specific types, *A. forbesii* and *A. vulgaris*, recognized as occurring in this region. In the experiments described below the majority of the eggs showed normal behavior in regard to post-maturational cytolysis and response to fertilization.

Three types of artificial membrane-forming treatment were employed: (1) exposure for five minutes to pure isotonic sodium chloride solution (0.55m. NaCl); (2) exposure for one to two minutes to a weak solution of a fatty acid (acetic or butyric) in sea-water; and (3) exposure to warm sea-water (at 35°) for seventy seconds.

In the majority of experiments the eggs were thus treated at about the time of separation of the first polar body—that is, from one to one-and-a-quarter hours after removal from the animals—this being the time at which the eggs respond most favorably to either normal or parthenogenetic fertilization. In a number of instances the same treatment was applied also after maturation was complete, at three to four hours after removal. Of eggs treated at this time the proportion reaching larval stages is typically much smaller than in the first class of cases; this decrease in responsiveness to artificial treatment is probably correlated with the decreased susceptibility to sperm-fertilization which appears at this time;<sup>25</sup> the unfertilized eggs seem then normally to enter on a refractory or relatively irresponsive phase in the life-cycle. In several series of experiments, however, development proved more favorable in eggs which were treated after maturation was complete; this occurred chiefly in abnormal lots of eggs which exhibited delay in the post-maturational cytolysis and imperfect development after sperm-fertilization. In all those experiments where eggs treated two or three hours after maturation formed a considerable proportion of larvae (10–20 per cent), sodium chloride solution was the membrane-forming agent; this solution appears to act more energetically than the other two agents and is possibly for this reason better able to overcome the increased resistance which the eggs show at this stage.

The experiments with each of the above types of membrane-forming treatment will now be described separately in some detail.

<sup>25</sup> R. S. Lillie, *Journal of Experimental Zoölogy*, 1908, vol. 5, p. 411.



*Series I. Treatment with anesthetics after membrane-formation by isotonic sodium chloride solution*

Brief exposure (five to ten minutes) to 0.55 m. NaCl is typically followed by membrane-formation in a considerable proportion of eggs, of which a minority develop to a larval stage.<sup>26</sup> This mode of treatment is less favorable than exposure to acid-containing sea-water or temporary warming, and even when a favorable after-treatment is used the proportion of eggs reaching larval stages rarely exceeds 20 or 30 per cent. Presumably the initial cytolytic action of this solution is too energetic to be readily counteracted or reversed by the subsequent anti-cytolytic treatment.

If eggs thus treated with salt solution are afterwards exposed for half an hour to hypertonic sea-water or cyanide solution, or sea-water containing anesthetics in appropriate concentrations, the proportion of eggs forming blastulae is typically and often markedly increased. The results of a series of such experiments are summarized in table 1.

In this series each form of after-treatment caused well-marked increase in the proportion of eggs developing to a blastula stage. It is to be noted that hypertonic sea-water was less effective than the other solutions; this is very generally the case with starfish eggs. After-treatment with hypertonic sea-water following membrane-formation by salt-solution appears often to be more effective with eggs treated *after* the completion of maturation than before. In three series where eggs from the same lots were thus treated both (a) during maturation and (b) three hours afterward, the proportions forming larvae were: (a) < 1 per cent, (b) 20-25 per cent; (a) *ca.* 1 per cent; (b) *ca.* 20 per cent; (a) 1 per cent; (b) *ca.* 10 per cent. No such differences were ever observed with hypertonic sea-water used after the other two membrane-forming agents. Cyanide and the anesthetics show well-marked action in the above experiments. In the series shown in table 2 the effects of after-treatment with alcohols (ethyl, propyl, butyl, and amyl) are illustrated.

<sup>26</sup> American Journal of Physiology, 1910, vol. 26, p. 119.

TABLE 1

July 4, 1912: 10.30 a.m. The eggs from several starfish were placed, about one-and-a-quarter hours after removal from the animals, in 0.55 m. NaCl solution. After five minutes in this solution they were returned to seawater. Part of the eggs remained permanently in sea-water (for control). The rest, ten minutes later were placed for thirty minutes in the several solutions of the series; they were then returned to sea-water. Next morning the proportion of mature eggs that had formed blastulae was approximately estimated. The results were as follows:

AFTER-TREATMENT	CONDITION NEXT MORNING
1. None (Control treated with 0.55m. NaCl alone)	Only one living blastula found; almost all eggs dead
2. Hypertonic sea-water (250 cc. sea-water + 30 cc. 2.5m NaCl)	Blastulae few though increased in number,—ca. 1 % of mature eggs
3. Sea-water + m/1000 KCN	Marked increase in blastulae: 25-30 % of mature eggs
4. Sea-water + 0.6 vol. % ether	Well-marked increase: ca. 10-15 %
5. Sea-water + 0.1 % chloral hydrate	Well-marked increase: ca. 10-15 %
6. Sea-water + 0.2 % ethyl urethane	Less favorable: ca. 2-4 %
7. Sea-water one-tenth saturated with chloretone	Considerable increase: ca. 5 %

*Fertilized control.* Eggs fertilized one-and-a-quarter hours after removal yielded a large proportion of normal larvae.

*Unfertilized control.* Eggs left in sea-water without treatment: next morning all mature eggs are dead and coagulated, without membranes or cleavage.

TABLE 2

July 6: 3.38 p.m. Eggs were placed about one hour after removal from the animals in 0.55m NaCl solution for five minutes, then returned to sea-water and after ten minutes placed in the following solutions for thirty minutes, then again returned to sea-water. The results were as follows:

AFTER-TREATMENT	PROPORTION OF MATURE EGGS FORMING LARVAE
1. None (control treated with 0.55 m NaCl alone)	Proportion small (ca 1 %)
2. Hypertonic sea-water	Well-marked increase: 10-15 %
3. Sea-water + m/1000 KCN	Well-marked increase: 10-15 %
4. Sea-water + 5 v. % ethyl alcohol	Like 2 and 3: 10-15 %
5. Sea-water + 2.5 v. % n-propyl alcohol	Little if any increase <1 %
6. Sea-water + 1 v. % n-butyl alcohol	Fair increase: ca. 5 %
7. Sea-water + 0.25 v. % n-amyl alcohol	Well-marked increase: 10-15 %

*Controls.* Eggs fertilized one hour after removal developed normally. Unfertilized eggs were all dead and coagulated next morning.

In this series the alcohols, with the exception of propyl alcohol, showed well-marked though not pronounced action. The concentrations in which the alcohols act most favorably correspond closely with the minimal concentrations for producing muscular anesthesia in *Arenicola* larvae.<sup>27</sup> The above solutions of propyl and butyl alcohols appear to be somewhat above the optimal strength; in most of the following experiments weaker solutions, 2 v. per cent and 0.8 v. per cent respectively, were used. The following series (table 3) shows results similar to the above.

TABLE 3

July 27: The treatment was the same as in the preceding series

AFTER-TREATMENT	PROPORTION OF EGGS FORMING LARVAE
1. None (treated with 0.55m NaCl alone)	Very few form larvae: < 1 %
2. Hypertonic sea-water	Well-marked increase: 10-15 %
3. Sea-water + m/1000 KCN	Marked increase: 40-50 %
4. Sea-water + 5 v. % ethyl alcohol	Marked increase: 20-25 %
5. Sea-water + 2 v. % n-propyl alcohol	Marked increase: 30-40 %
6. Sea-water + 0.8 v. % n-butyl alcohol	Less favorable: 15-20 %
7. Sea-water + 0.25 v. % n-amyl alcohol	Still less favorable: 5-10 %

*Controls.* Nearly all eggs fertilized one hour after removal develop normally. Unfertilized eggs undergo normal coagulation without membrane-formation or cleavage.

In the experiments with sodium chloride solution, followed by after-treatment as above, the proportion of eggs developing to a larval stage was in some cases considerable—at times reaching 30 or 40 per cent—but never very high. In several other similar experiments less favorable results were obtained; usually the after-treatment caused a definite increase in larvae, but exceptions to this rule sometimes occurred, especially when the eggs were in any way abnormal. As already mentioned, sodium chloride solution gives in general less favorable results than the other two membrane-forming agents, probably because the action of the salt is too energetic and tends to produce effects not readily reversible by the subsequent action of the anesthetic, hypertonic sea-water, or cyanide. The experiments with fatty acid about

<sup>27</sup> American Journal of Physiology, 1913, vol. 31, p. 264.

to be described are more favorable and in some cases exhibit a close approach to the results of normal fertilization.

*Series II. Treatment with anesthetics after membrane-formation by fatty acids*

In this series the results of after-treatment with anesthetics were more decidedly and uniformly favorable than in the series with sodium chloride solution. The proportion of eggs forming larvae after such treatment is almost invariably much higher than after the simple membrane-formation with acid, although to this rule also there are certain exceptions, the more significant of which are considered below. Experiments with acetic acid solution followed by anesthetics were performed last summer with eighteen different lots of eggs at different times; in four of these series butyric acid was also used, making a total of twenty-two series of experiments with fatty acid. As a rule, starfish eggs treated with acid alone, without after-treatment of any kind, yield only a small proportion of larvae, rarely exceeding 10 per cent; this was the case in twenty out of the twenty-two series; in all of those series where the behavior of the control eggs was normal (seventeen in all) the after-treatment led to a marked increase in the proportion of favorably developing eggs, in some cases eighty per cent or more of the eggs forming active blastulae. In three series out of the twenty the eggs were unfavorable and yielded only a few larvae after normal fertilization, and correspondingly the proportion of parthenogenetically developing eggs was small; in two out of these three series the after-treatment with alcohol caused a definite though slight increase in the proportion of larvae; in the third the increase was doubtful. The remaining two series showed unusual behavior of a quite different kind; the eggs treated with fatty acid without any after-treatment yielded a high proportion of larvae, and the after-treatment appeared rather injurious than beneficial; these series will be discussed more fully below.

Last summer six series of experiments were performed with the solutions of table 4. Five of these series were carried out within a single week toward the end of June, and the sixth a few

days later. The table gives a summarized account of the results of all six series.

The results of these experiments show considerable uniformity. The number of larvae resulting from acid treatment alone was small, and each form of after-treatment caused a decided increase in the proportion of eggs forming larvae. It is noteworthy that hypertonic sea-water is on the whole less favorable than the other solutions. Cyanide and chloral hydrate show somewhat better results than the other solutions; this, however, is not always the case, as the following experiments with alcohols will illustrate.

TABLE 4

*In each series the eggs were exposed, about one hour after removal from the animals, to a solution containing 6 cc. n/10 acetic acid, plus 100 cc. sea-water, for one to two minutes. Ten minutes later they were brought into the several solutions of the series, where they remained for thirty minutes; they were then returned to sea-water. Part of the acid-treated eggs received no after-treatment (for control). The hypertonic sea-water consisted in all cases of a mixture of 250 cc. sea-water plus 30 cc. 2.5m NaCl. The solutions (in sea-water) of cyanide and anaesthetics were kept in corked flasks. The results are given in approximate percentages of mature eggs forming swimming larvae (blastulae or gastrulae), under the date of the experiment. The condition of the control eggs, sperm-fertilized and unfertilized, is recorded in each case.*

AFTER-TREATMENT	RESULTS					
	June 25	June 26	June 27	June 28	June 30	July 4
1. None (acid s.w. alone).....	ca. 1-2%	1-2%	1% or less	<1%	<1%	ca. 1%
2. Hypertonic sea-water.....	ca. 5%	ca. 5%	10-15%	ca. 10%	ca. 5%	5-10%
3. m/1000 KCN.....	ca. 20%	10-15%	20-25%	20-30%	<5%	30-40%
4. 0.6 v. % ether.....	10-15%	10-15%	<5%	ca. 10%	ca. 10%	25-35%
5. 0.1 % chloral.....	25-35%	>50%	10-15%	ca. 10%	<5%	25-35%
6. 0.2 % urethane....	10-15%	5-10%	ca. 10%	ca. 10%	< 5%	ca. 25%
7. 1/10 saturated chloretone.....	15-20%	15-20%	ca. 10%	ca. 10%	<5%	ca. 25-30%
Condition of control eggs.....	Normal	Partly resistant; good proportion normal	Normal	Partly resistant; majority normal	Considerable proportion resistant	Majority normal

In the series of June 30, and to a lesser degree of June 28, the increase produced by anesthetics was relatively slight. These eggs showed certain other abnormalities of behavior; they were partly resistant to fertilization, and in many the post-maturational cytolysis was delayed.<sup>28</sup> They also showed exceptional behavior in another respect; eggs treated in the above manner two hours after maturation was complete gave a larger proportion of larvae than those treated during the maturation period. Some instances of similarly abnormal behavior were cited above in the case of eggs treated with sodium chloride solution; in these cases also the eggs responded less favorably to a parthenogenetic treatment applied during the maturation-period than to one applied later. An abnormally lowered responsiveness during the maturation period seems thus often to be associated with an abnormally increased responsiveness later. The presence of a considerable proportion of abnormal eggs in the experiments of June 28 and 30 probably accounts for the relatively unfavorable character of the result on those days.

*Effects of treatment with solutions of cyanide and anesthetics without previous membrane-formation.* Experiments were performed to determine what effect the above forms of after-treatment, *acting alone*, have upon unfertilized starfish eggs. The results of these experiments show clearly that the treatment with these solutions must be *superposed* on the preliminary membrane-forming treatment in order to produce their characteristic effects. When acting alone they are almost or quite ineffective in inducing development, or what effect they have is confined to a small proportion of eggs, presumably the abnormal minority almost invariably present in lots of starfish eggs. The following experiments will illustrate: in the series of June 25 part of the eggs were exposed to hypertonic sea-water, cyanide and anesthetics at the same time as the others, but without having been treated previously with fatty acid. The results are summarized in table 5.

Several other similar series of experiments (June 20, 21, 23, 24) gave essentially the same results. Exposure to hypertonic

<sup>28</sup> For an account of this condition, cf. Biological Bulletin, 1912, vol. 22, p. 328.

sea-water for thirty minutes usually caused considerable membrane-formation and cleavage, and in a few cases gave a small proportion of blastulae. Ether showed a similar though less marked action; the proportion of eggs affected was in all cases small. With cyanide, chloral hydrate, urethane, and chloretone there was never any indication of membrane-formation or cleavage in eggs treated during the maturation period. Some slight effect however was seen in eggs exposed in the later period, and in three series a few eggs treated with chloretone, urethane, and chloral hydrate formed larvae, though the great majority remained unchanged. There was no action with cyanide. Apparently the conditions of response to the membrane-forming treatment undergo a certain change after maturation is complete: the increased resistance to normal fertilization then appearing is associated with a change in the effects produced by the above solutions of anesthetics. The existence of a certain proportion of eggs showing a behavior more or less divergent from the norm

TABLE 5

*June 25, 1912: Unfertilized starfish eggs of the same lot were placed in the following solutions at two different intervals after removal from the animals; part A about one-and-a-quarter hours after removal, part B about two hours later. After remaining in the solutions for thirty minutes they were returned to sea-water. The condition of the eggs next day was as follows:*

SOLUTION	A (EXPOSED 1 $\frac{1}{4}$ H. AFTER REMOVAL)	B (EXPOSED 3 $\frac{1}{4}$ H. AFTER REMOVAL)
1. Hypertonic sea-water.	About half the eggs show membrane-formation; the rest have coagulated unchanged. <sup>1</sup> No blastulae	Majority coagulated unchanged; no blastulae
2. m/1000 KCN.....	No effect. All coagulated unchanged	All coagulated unchanged
3. 0.6 v. % ether.....	Almost all coagulated unchanged. A few membranes	Great majority coagulated unchanged; a few blastulae present
4. 0.1 % chloral hydrate.	All coagulated unchanged	All coagulated unchanged
5. 0.2 % urethane.....	All coagulated unchanged	All coagulated unchanged
6. 1/10 saturated chloretone.....	All coagulated unchanged	All coagulated unchanged

<sup>1</sup> That is, show the same condition as the untreated control eggs—dead and coagulated without membrane-formation or cleavage.

must also always be allowed for in lots of starfish eggs, which show marked contrast to the regularity and uniformity of behavior characteristic of *Arbacia* eggs.

Since treatment with anesthetics and cyanide alone, without preliminary membrane-formation, has typically no effect in initiating the development of starfish eggs, while after the membranes have been formed the same treatment greatly increases the proportion of eggs that develop favorably, it is to be inferred that the essential physiological effect of this treatment is of a kind quite different from that of the initial membrane-forming treatment. Expressed in general terms, the effect seems to be essentially *compensatory* in nature, and consists in restoring a physiologically balanced condition which has been disturbed by the first treatment. Thus if the initial action is cytolytic, the second is probably anti-cytolytic. In these concentrations the above anesthetics and cyanide have in fact a protective or anti-cytolytic action on eggs exposed to pure sodium chloride solution; and it seems thus probably that the favorable effects of the after-treatment depend on an action of essentially this nature—that is, consist in a modification of the plasma-membrane of the opposite kind to that produced by the primary or cytolytic treatment, in other words, in the direction of a *decrease* of permeability, or of an increase in the resistance to any permeability-increasing conditions that may be present.

*Alcohols.* After-treatment with alcohols gave results similar to the above, but on the whole more favorable. This increased favorability may however be due to the fact that most of the experiments with alcohols were performed during late July and August, at a time when the starfish eggs showed in general a greater responsiveness to parthenogenetic treatment than in June, in which month most of the experiments with the first group of anesthetics were made. During late July and early August eggs exposed to acid sea-water alone, without any after treatment, often yielded a considerable and in a few cases a high proportion of larvae. The effects of the after-treatment with alcohols are shown in table 6, in which are summarized the results of four series of experiments with both acetic and butyric acids, made on four successive days toward the end of July.



TABLE 6

In these experiments both acetic and butyric acids were used: The eggs were exposed, about one hour after removal, to sea-water containing 6 cc. n/10 fatty acid to 100 cc. sea-water, for one minute. After ten minutes they were brought into the solutions of the series where they remained thirty minutes; they were then returned to sea-water. Hypertonic sea-water and cyanide were also used for comparison. The figures give the approximate proportion of mature eggs forming free-swimming blastulae and gastrulae.

## A. Acetic acid series

AFTER-TREATMENT	RESULTS			
	July 23	July 24	July 25	July 26
1. None (acetic acid alone)...	ca. 5 %	ca. 1 %	10-15 %	10-15 %
2. Hypertonic sea-water.....	no exp't.	ca. 3-4 %	ca. 10-15%	ca. 50 %
3. n/1000 KCN.....	30-40 %	ca. 50 %	ca. 90 %	ca. 70 %
4. 5 v. % ethyl alcohol.....	30-40 %	ca. 10 %	ca. 60 %	ca. 10 %
5. 2 v. % n-propyl alcohol...	ca. 20-25%	ca. 3-4 %	ca. 60 %	25-35 %
6. 0.8 v. % n-butyl alcohol...	ca. 35-40%	ca. 3-4 %	ca. 70 %	ca. 50 %
7. 0.25 v. % n-amyl alcohol...	ca. 25-30%	ca. 3-4 %	ca. 65-75%	ca. 40 %

## B. Butyric acid series

1. None (butyric acid alone).	10-15 %	ca. 10 %	20-25 %	ca. 30 %
2. Hypertonic sea-water.....	no exp't	ca. 5 % (decrease)	30-40 %	ca. 50 %
3. m/1000 KCN.....	ca. 20 %	ca. 50 %	ca. 50 %	ca. 90 %
4. 5 v. ethyl alcohol.....	85-90 %	ca. 35-40%	5-10 % (decrease)	decrease (ca. 10 %)
5. 2 v. % n-propyl alcohol...	80-90 %	increase slight (<10 %)	40-50 %	decrease (10-15 %)
6. 0.8 v. % n-butyl alcohol...	ca. 60 %	ca. 10 %	ca. 50 %	ca. 50 %
7. 0.25 v. % n-amyl alcohol...	ca. 40 %	ca. 20 %	ca. 60 %	ca. 60 %

*Controls.* In all four series the great majority of sperm-fertilized eggs yielded normal larvae. The unfertilized eggs showed no development.

In three additional similar series with acetic acid, methyl alcohol (10 v. per cent and 8 v. per cent) and capryl alcohol,  $C_8H_{17}OH$ , (one-fifth saturated solution in sea-water) were used in addition to the above. Methyl alcohol proved unfavorable; capryl alcohol gave a well-marked increase in two of the experiments; in the third the acid treatment alone produced an unusually high proportion of larvae (70 to 80 per cent of the mature eggs) and all forms of after-treatment were unfavorable (cf. table 7).

This last phenomenon was observed in several series; those of July 25 and July 26, in table 6 (eggs treated with butyric acid), show it in part; two other series where a still higher proportion of eggs formed larvae after simple membrane-formation, illustrate this behavior more definitely. These series are summarized in table 7.

TABLE 7

*The unfertilized eggs were treated for one minute with a solution of 6 cc. n/10 acetic acid plus 100 cc. sea-water, at about one-and-a-quarter hours after removal from the starfish. After ten minutes in normal sea-water they were exposed for thirty minutes to the solution, then returned to sea-water.*

AFTER-TREATMENT	RESULT	
	July 6	July 30
1. None (acid treatment alone)	30-40 % of eggs form larvae	70-80 % form larvae
2. Hypertonic sea-water.....	No apparent increase	Slight decrease; 65-75 %.
3. m/1000 KCN.....	Decrease; ca. 10 % larvae	Slight decrease; ca. 60 %
4. 5 and 6 v. % ethyl alcohol..	All dead next day	All dead next day
5. 2.5 v. % n-propyl alcohol...	All dead next day	All dead next day
6. 1 v. % n-butyl alcohol.....	All dead next day	All dead next day
7. 0.25 v. % n-amyl alcohol....	ca. 30 % larvae	A few larvae; ca. 1 %
8. 1/5 saturated capryl alcohol	No experiment	ca. 30-40 % larvae

*Controls.* Normal: almost all fertilized eggs from larvae. Unfertilized eggs undergo coagulation without membrane-formation or cleavage.

In these experiments the after-treatment not only effected no improvement, but on the contrary was definitely injurious. It would thus appear that the after-treatment is favorable only with those eggs which are unable otherwise to recover a normal condition. Eggs which are able spontaneously to recover from the effects of the initial cytolytic action are unfavorably affected by the subsequent anti-cytolytic treatment. As already suggested, this treatment appears to be essentially compensatory in its action; and merely aids in restoring what might be called a condition of physiological equilibrium. Hence, with eggs that spontaneously revert to the normal state after membrane-formation, the addition of the after-treatment is likely to result in over-com-

compensation and hence in injury. Whether the after-treatment is favorable or not thus depends on the physiological condition of the egg. Its action is at best supplementary, and is superfluous or injurious whenever the egg is capable of regaining the normal condition unaided.

As with the first series of anesthetics, it was found that treatment with alcohols alone, without preliminary membrane-formation, leaves the great majority of eggs apparently unchanged. A small and variable proportion of eggs so treated may, however, form membranes and develop, as in the above experiments with ether, urethane and chloretone. The essential physiological action of the alcohols, like that of the other anesthetics, is thus different from that of the membrane-forming agency, and probably consists in a modification of the membrane in the direction of decreased permeability.

*Series III. Treatment with anesthetics after membrane-formation by temporary warming*

Brief exposure of starfish eggs during the maturation-period to temperatures of 34° to 38° induces typical membrane-formation followed by cleavage, which in favorable instances leads to the production of a considerable proportion of larvae. As with the other membrane-forming agents, this proportion may be increased by after-treatment with hypertonic sea-water, cyanide, or anesthetics. Table 8 gives a summary of four typical series of experiments, in which urethane, chloretone, and ether show well-marked favorable action, while chloral hydrate and cyanide are less effective; hypertonic sea-water again proves relatively ineffective as compared with the other solutions. Two series of experiments with alcohols gave similar results, summarized in table 9.

After-treatment with alcohols is thus highly effective with eggs subjected to this type of membrane forming treatment. In these experiments (table 9) propyl, butyl and amyl alcohols appear more favorable than ethyl alcohol, and the same was very generally observed in last summer's experiments with starfish eggs. In my experiments with *Arenicola* larvae, propyl and butyl alcohols

TABLE 8

The eggs were placed at about the time of separation of the first polar body in sea-water at 35° for seventy seconds, and then returned to sea-water at ca. 20°. Ten minutes later they were placed in the following solutions where they remained thirty minutes; thence again to sea-water. The proportions of mature eggs forming larvae were approximately as follows:

AFTER-TREATMENT	PROPORTION OF EGGS FORMING LARVAE			
	June 19	June 20	June 21	June 24
1. None (warming alone).....	< 5 %	5-10 %	ca. 5 %	ca. 5 %
2. Hypertonic sea-water.....	{ slight in-crease; ca. 5 %	{ no in-crease; 5-10 %	ca. 10 %	> 5 %
3. n/1000 KCN.....	5-10 %	ca. 5 %	ca. 10 %	few: < 5 %
4. 0.6 v. % ether.....	10-15 %	20-25 %	10-15 %	ca. 20 %
5. 0.1 % chloral hydrate.....	ca. 10 %	{ few; < ex-per. 1	{ no in-crease; ca. 5 %	few; ca. 5 %
6. 0.2 % urethane.....	no exp't	ca. 20 %	ca. 20 %	40-50 %
7. 1/10 saturated chloretone..	{ few; < ex-per. 1	ca. 25-30 %	ca. 25-30 %	30-40 %

Controls showed a good proportion of normal eggs; on June 21 many eggs failed to mature; on June 24 the proportion of fertilized eggs yielding larvae was smaller than usual.

Eggs from each lot were subjected to the same treatment after maturation was complete (3½ to 4 hours after removal); the results of these experiments were almost entirely negative. After maturation the eggs become relatively irresponsive to this form of treatment.<sup>29</sup>

TABLE 9

The eggs were warmed briefly to 35° as already described, and exposed for thirty minutes to the following solutions

AFTER-TREATMENT	PROPORTION OF LARVAE	
	July 27	July 29
1. None (warming only).....	ca. 10 %	2-3 %
2. Hypertonic sea-water.....	No increase; ca. 10 %	ca. 5 %
3. m/1000 KCN.....	ca. 60 %	decrease; 1-2 %
4. 5 v. % ethyl alcohol.....	Slight increase: 10-15 %	15-20 %
5. 2 v. % n-propyl alcohol.....	50-60 %	20-25 %
6. 0.8 v. % n-butyl alcohol.....	75-85 %	15-20 %
7. 0.25 v. % n-amyl alcohol.....	50 %	15-20 %
8. 1/5 saturated capryl alcohol.....	No exp't	ca. 10 %

Both sets of controls were normal.

<sup>29</sup> Journal of Experimental Zoölogy, 1908, vol. 5, pp. 400 seq.

also showed a distinctly greater protective or anti-cytolytic action than ethyl alcohol.<sup>30</sup>

It is remarkable that hypertonic sea-water, in the numerous experiments performed last summer, never proved more than moderately effective with starfish eggs, and in no case effected so great an improvement as occurred in favorable experiments with cyanide or anesthetics. With sea-urchin eggs, on the other hand, hypertonic sea-water of the above concentration gives highly constant and favorable results. It would seem that the action of hypertonic sea-water in artificial parthenogenesis is in no sense exceptional or distinctive, but that it is for mainly incidental reasons that this treatment proves so highly favorable with certain species of eggs, while with others a quite different form of treatment is more effective. Why such different agencies should produce the same physiological effects cannot at present be said in any detail. The common factor in the production of these effects is probably the presence of a membrane whose physiologically important properties, such as electrical polarization, are influenced by agencies of widely different kind in essentially the same manner. The case of stimulation again affords perhaps the best analogy; here polarization-changes at membranes are demonstrably concerned, and we find that a large variety of stimuli may cause the same effect. The possibilities of variation in polarization, *qua* polarization, are limited; a given agency may either decrease the polarization of a membrane or increase it, and the degree, rate, and periodicity of these changes may vary. What is significant is that the number of variables is constant and limited. It is thus not surprising that a variety of external changes should produce the same physiological effect, if this effect depends on a change of polarization. The phenomena under consideration in the present paper appear to illustrate this principle.

<sup>30</sup> Cf. American Journal of Physiology, 1913, pp. 264 seq.

## III. SUMMARY

The chief experimental results and theoretical conclusions of the present paper may be briefly summarized as follows.

1. Temporary exposure of unfertilized starfish eggs to pure isotonic NaCl solution, to sea-water containing fatty acid, or to high temperature (35°), is followed by the formation of fertilization-membranes and cleavage, and a certain proportion of eggs so treated (usually from 1 to 5 per cent) may develop to a larval stage. If, after membranes are formed, the eggs are treated for thirty minutes with hypertonic sea-water or weak cyanide solution, the proportion thus developing is usually increased, often to a marked degree.

2. The same effect is produced in *Asterias* eggs by after-treatment with solutions of various anesthetics in sea-water, namely, ethyl ether, ethyl urethane, chloral hydrate, chloretone, various alcohols (ethyl, propyl, butyl, amyl, capryl). *Arbacia* eggs do not respond to this treatment.

3. The anesthetics exert this favorable action in concentrations corresponding closely to those causing typical anesthesia in *Arenicola* larvae. The essential effect of this treatment upon the egg is thus probably of the same nature as that which conditions anesthesia in irritable tissues. This effect appears to consist in imparting to the plasma membranes either a degree of permeability which is *less* than the normal, or an increased resistance to agencies whose general action is permeability-increasing (that is, cytolytic, if the action is not compensated or reversed within a certain time).

4. The above anesthetics have been shown to exert a protective or anticytolytic action on starfish eggs in the presence of certain cytolytic agents (pure salt-solutions). Their action in preventing the disintegration of eggs after artificial membrane-formation, and in thus rendering possible the continuance of development, is probably an effect of the same essential kind.

5. The view is put forward that the essential physiological effects produced by the two successive treatments in artificial parthenogenesis are opposite in character and correspond respectively to the depolarizing and the repolarizing phases of the stimulation-process in irritable tissues in general. The primary or membrane-forming treatment has a permeability-increasing and hence depolarizing effect upon the plasma membrane. This initial depolarization is probably the critical or determinative event in fertilization, as well as in stimulation. A return of the plasma membrane to or toward the original semi-permeable and electrically polarized condition within a certain time (*ca.* fifteen minutes at 20°), is, however, essential if normal development is to follow, otherwise cytolysis results. This recovery of the normal semi-permeability of the membrane, with the correlative electrical polarization, is favored by after-treatment with agencies (cold, cyanide, anesthetics, hypertonic sea-water) whose general action is permeability-decreasing or anti-cytolytic. Hence, such after-treatment increases the proportion of eggs that regain their normal properties and continue development.





# INHERITANCE IN HYDATINA SENTA

## I. VIABILITY OF THE RESTING EGGS AND THE SEX RATIO<sup>1</sup>

A. FRANKLIN SHULL

*The Department of Zoölogy, University of Michigan*

TWO FIGURES

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### INTRODUCTION

Several years ago in an attempt to effect crosses between parthenogenetic lines of the rotifer *Hydatina senta*, it was found that the proportion of fertilized eggs that would hatch varied greatly in different lines. In one cross, only one egg in several hundred hatched, although the batch of eggs was kept for weeks; in another, about one-eighth of the whole lot hatched. Since then crosses have been made which resulted in a much higher proportion of viable eggs, up to more than 50 per cent of viability.

<sup>1</sup> Contribution from the Zoölogical Laboratory of the University of Michigan.

The same condition was found among inbred eggs. Females fertilized by males of their own parthenogenetic line laid eggs which, in some lines, did not hatch at all, though the eggs were kept for many weeks, and though some of them were dried, others frozen—treatment generally supposed to favor the hatching of such eggs. Of other lots of inbred eggs, 50 to 70 per cent were viable.

In the autumn of 1911 I obtained particularly favorable material with which to test the possible inheritance of this viability of the fertilized eggs. Two lines were found, one of which produced highly viable fertilized eggs by inbreeding, the other only slightly viable. The experiments about to be described show this viability to be a rather definitely inherited character. Its behavior in inbreeding, in crosses, and in selection have been tested, and some results of theoretical interest beyond the limits of this particular problem obtained.

I take pleasure in acknowledging the invaluable assistance of Miss Frances J. Dunbar in carrying the experiments over critical periods during my enforced absence.

#### FACTORS DETERMINING VIABILITY OF EGGS

The following are some of the factors that seem to me to govern the proportion of eggs that hatch:

1. A solvent which reduces the thickness of the chitinous shell of the egg is apparently produced. The effect of this solvent is perceptible in parthenogenetic eggs shortly before hatching, when the shell, thinner at some points than at others, yields to pressure from within, and is raised in visible humps. When the embryo has emerged, the shell is plainly very much thinner than the shell of a freshly laid egg from which the egg itself has been crushed by pressure. In fertilized eggs this solvent does not usually produce any visible effect, for the young rotifer breaks out while the shell is still quite thick. It is ordinarily quite difficult to distinguish an empty shell from an unhatched fertilized egg, unless the break in the chitin can be seen. Sometimes, however, the embryo does not break from the shell until the latter is quite thin and transparent. These occasional cases show that such a solvent exists. Variations in the production

of this solvent may account for differences in viability in different lots of eggs.

2. Muscular power of the embryo probably determines the hatching in part. Shells that have been made quite thin by the solvent sometimes remain unbroken, owing to weakness of the young rotifers.

3. Some of the eggs classed as fertilized may not really be fertilized, and so incapable of development, or else incapable of producing an embryo that can escape from the shell. Several years ago I obtained, by mating a female with an old male, a family consisting in part of fertilized eggs, in part of male eggs. This is the only case of the kind ever recorded. The fertilized eggs were laid first, and one of them hatched, producing a female. They were followed by a number of smaller eggs, all of which had shells somewhat thicker than parthenogenetic eggs usually have, but not so thick as fertilized eggs. Some of these small eggs hatched, though the embryo remained in the egg stage longer than the usual twelve to fourteen hours common to parthenogenetic eggs. A number of the small eggs did not hatch, which is uncommon in parthenogenetic eggs. I believe this failure to hatch in some, and delay in hatching in others, to be due to the thicker shells. In some way impregnation of the female seemed to stimulate the production of thicker shells, even on the eggs that were not fertilized. May this not be the case frequently? Small eggs, sometimes not any larger than male eggs, were not uncommonly found in these experiments; but they had thick shells bearing the usual pilose projections, and were classed as fertilized eggs. If these were unfertilized, anything that caused a variation in the number of them produced might affect the proportion of eggs that hatched.

4. Environmental conditions no doubt play an important rôle in the hatching of the fertilized eggs. There are plain indications that this is the case, though in what way or to what extent has been only partially worked out.

Which, if any, of the first three of these factors is responsible for the variations in the viability of the eggs that were the subject of these experiments is not known.

## GENERAL ACCOUNT OF THE EXPERIMENTS

The diagram in figure 1, with the following description, will make clear the nature of the whole series of experiments. The parthenogenetic lines used were derived originally from rotifers collected in Grantwood, New Jersey, one in March, the other in May, 1911. These lines were designated *A* and *B*, respectively, in a former paper. Females of line *A* were inbred with males of the same line, and from one of the fertilized eggs a new line was started; this line is designated *C*. Females of line *A* were mated with males of line *B*, and from one of the fertilized eggs a second parthenogenetic line was started; this line is designated *D* in the diagram.

Lines *C* and *D* were each inbred ( $C \times C$  and  $D \times D$ ), and reciprocal crosses between them were obtained ( $C \times D$  and  $D \times C$ ). In these crosses the female parent is named first. The inbred eggs were discarded after determining how many of them would hatch, but from each of the reciprocal crosses a new parthenogenetic line was derived. Throughout the diagram, a batch of eggs and the parthenogenetic line or lines derived from one or more of them are regarded as a unit, since all individuals between one fertilization and the next are believed to have the same genotypic constitution, and are included in one rectangle of the diagram.

The two reciprocal crosses ( $C \times D$  and  $D \times C$ ) were each inbred [ $(C \times D) \times (C \times D)$  and  $(D \times C) \times (D \times C)$ ], and reciprocal crosses [ $(C \times D) \times (D \times C)$  and  $(D \times C) \times (C \times D)$ ] also obtained. Three of these were discarded after determining the viability of the fertilized eggs. The fourth, the inbred  $(C \times D) \times (C \times D)$  was continued as two parthenogenetic lines, one started from a family of eggs showing low viability, the other from a family showing high viability of the eggs.

Each of these two lines was inbred, and from the fertilized eggs two parthenogenetic lines started. The line from the low viability line was started from an egg in a family that hatched few of its eggs ( $L_1$ ); that from the high viability line was derived from an egg in a highly viable family ( $H_1$ ). These two lines were again inbred. Fertilized eggs were again selected from

families showing low and high viability, respectively, and from them the lines designated  $L_2$  and  $H_2$  were reared.

Each of these lines was inbred, and the fertilized eggs designated  $L_3$  and  $H_3$  were secured.  $L_2$  and  $H_2$  were also crossed reciprocally ( $L_2 \times H_2$  and  $H_2 \times L_2$ ), but no parthenogenetic lines were reared in these cases; viability was determined and the eggs discarded.

From this point the low viability and the high viability series may be followed separately. The line bred from the eggs named  $L_3$  was again inbred, the eggs being marked  $L_4$ . Two families of these eggs were selected, one for low, the other for relatively high viability. From each was reared a new line, each of which was inbred to produce a batch of fertilized eggs ( $L$  from  $L$ , and  $H$  from  $L$ ). With these eggs the experiments with the low viability series terminated.

Of the lot of eggs in  $H_3$ , two families were selected, one showing relatively low, the other high viability; from each a line was started. These parthenogenetic lines were inbred to produce the lots of eggs called  $L_1$  from  $H$ , and  $H_1$  from  $H$ . Selection of families for low and high viability, respectively, was made in these lots of eggs and a parthenogenetic line bred from each. These lines were in turn inbred, the fertilized eggs being designated  $L_2$  from  $H$ , and  $H_2$  from  $H$ . With these eggs the experiments in the high viability series terminated.

#### DETAILS OF THE EXPERIMENTS

The following account of the individual experiments is divided and labeled in accordance with the diagram in figure 1, in connection with which the description may be easily followed.

A. The data for this line were published in part in a former paper. It comprised 55.2 per cent of male-producers.

B. This line was likewise given in an earlier publication. It included 16.5 per cent of male-producers.

C. The resting eggs of this lot were obtained by mating females with males of line A on June 28 and 29, 1911. These females laid on the average 8 eggs apiece. The eggs were dried soon after laying, and remained in this condition until August 31, 1911,

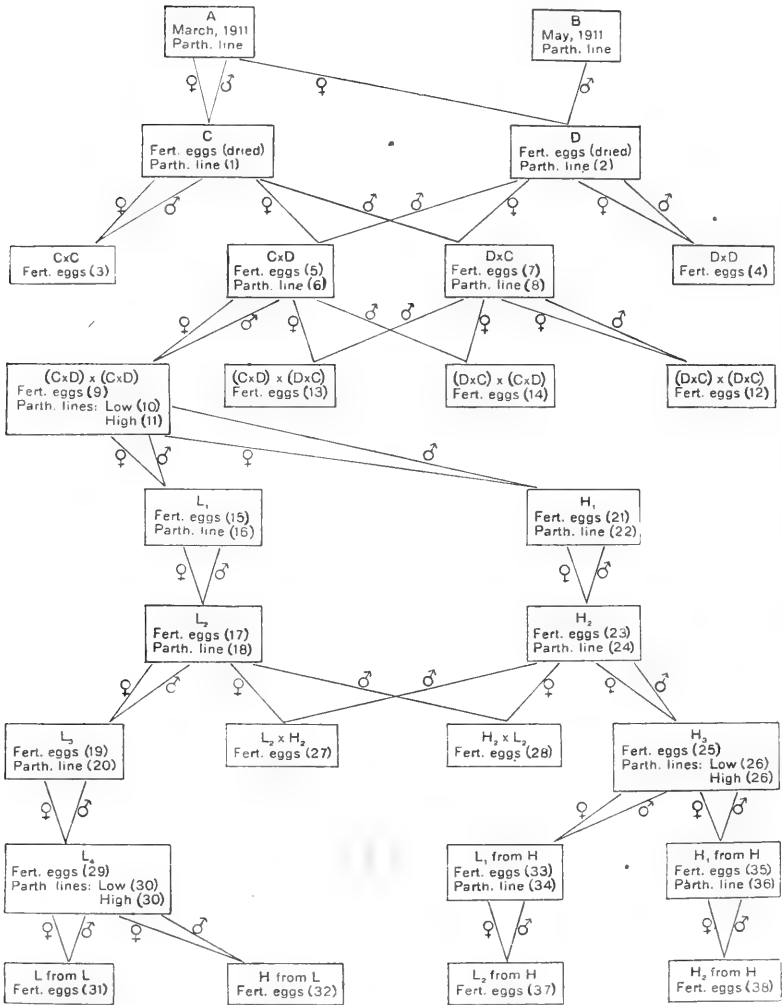


Fig. 1 Diagram showing the relation of the experiments to each other. The parthenogenetic line in each rectangle was derived from one of the fertilized eggs in the same rectangle. Letters at the top of the rectangles refer to experiments; numbers in parentheses refer to tables.

TABLE 1

Showing details of the parthenogenetic line *C* in figure 1. Male-producers are designated ♂ ♀, female-producers ♀ ♀

NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂ ♀	NUMBER OF ♀ ♀	NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂ ♀	NUMBER OF ♀ ♀
1	Oct. 12	35	16	5	Oct. 20	1	11
2	14	23	25		21	30	22
3	17	0	21	6	22	43	8
4	18	33	16	7	27	0	16
				8	29	23	14
Total.....						188	149
Percentage of ♂ ♀.....						55.7	

when water was put on them. On September 9 one female hatched, from which was bred the parthenogenetic line designated *C* in figure 1. The details of this line are given in table 1.

*D.* Females of line *A* were mated with males of line *B* on June 24 and 25, 1911, and laid on the average 10.9 eggs apiece. These eggs were dried and remained in this condition until August 30, when they were again covered with water. One female hatched on September 2 and gave rise to line *D* of figure 1, the details of which are shown in table 2.

*Viability in crosses and in inbreeding*

*C* × *C.* Females of line *C* were mated with males of the same line October 22 to 26, 1911. From the 50 matings thus made, 805 fertilized eggs, or 16.1 per female, were obtained. These eggs were kept under observation until December 20 to determine what proportion of them would hatch. Table 3 gives the details of the hatching. It appears that 368 eggs, or 45.7 per cent of the whole lot, were viable. It is also worthy of note that the number hatching daily gradually increased up to the fifth or sixth day, and thereafter, with certain fluctuations, decreased. Furthermore, practically the whole lot of viable eggs hatched within a period of twelve or thirteen days, though the eggs were kept much longer. The brief period in which the hatching occurred is in such contrast with the case next to be described that I have sought some method of measuring it. As

TABLE 2

*Showing details of parthenogenetic line D in figure 1*

NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂	NUMBER OF ♀	NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂	NUMBER OF ♀
1	Sep. 3	0	5	18	Oct. 1	6	45
2	5	0	22	19	3	30	19
3	7	0	23	20	5	8	41
4	9	0	46	21	7	10	39
5	10	0	26	22	9	22	22
6	12	0	46	23	11	0	50
7	14	4	19	24	13	32	16
8	16	6	23	25	15	20	18
9	17	0	26	26	17	23	22
10	19	0	28	27	19	6	36
11	20	7	45	28	21	18	27
12	22	11	44	29	23	5	44
13	23	7	44	30	25	1	41
14	25	3	44	31	28	16	28
15	26	9	40	32	30	3	40
16	28	2	48	33	Nov. 2	19	21
17	29	14	30				
Total.....						282	1068
Percentage of ♂ ♀ .....						20.8	

TABLE 3

*Showing number of eggs that hatched, out of 805 in lot C × C, figure 1*

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
Nov. 1	7	Nov. 7	28	Nov. 13	2
2	19	8	28	14-15	0
3	14	9	65	16	1
4	33	10	15	17-Dec. 3	0
5	83	11	8	4	1
6	63	12	1	5-20	0
Total.....					368
Percentage of viable eggs.....					45.7

Standard deviation:  $\sigma_H = 2.83$      $\sigma_L = 1.74$



the curve of hatching is similar to a probability curve, the variability of the hatching time may be measured by the standard deviation. This is obtained by the formula  $\sigma = \sqrt{\frac{\sum pa^2}{n}}$ , in which  $p$  is the number hatching each day,  $a$  the deviation from the mean duration of the egg stage, and  $n$  the total number hatching. The standard deviation in this case, as shown at the bottom of table 3, is 2.83 (days). A comparatively low standard deviation indicates that the hatching occurred within a brief period. A certain correction may be necessary, but this will be mentioned later.

$D \times D$ . Females of line  $D$  were mated with males of the same line October 22 to 31, 1911. From 32 matings thus made, 543 eggs, or 16.9 per female, were secured. These eggs were kept under observation until December 20, during which time 29, or 5.3 per cent hatched. There was great variability in the hatching time of these 29 eggs, that is, the hatching was spread nearly evenly over four weeks, with scattering ones later, as shown in table 4, instead of over two weeks as in  $C \times C$ . This is shown by the high standard deviation,  $\sigma = 10.78$ .

TABLE 4

*Showing number of eggs that hatched, out of 543 in lot  $D \times D$ , figure 1*

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
Nov. 4	1	Nov. 16	1	Dec. 1	2
5	1	17-18	0	2	0
6-8	0	19	1	3	1
9	1	20	3	4	1
10	2	21	0	5-8	0
11	0	22	2	9	1
12	1	23	1	10	0
13	3	24-25	0	11	1
14	1	26	1	12-17	0
15	3	27-30	0	18	1
				19-20	0
Total.....					29
Percentage of viable eggs.....					5.3
Standard deviation: $\sigma_H = 10.78$ $\sigma_L = 3.64$					

These inbred eggs are so different from those of  $C \times C$ , both in their percentage of viability and in the variability of the duration of the egg stage, that reciprocal crosses between the lines producing them ( $C$  and  $D$ ) are of considerable interest. These crosses are next described.

$C \times D$ . Females of line  $C$  were mated with males of line  $D$  October 14 to 16 and 20 to 21, 1911. From 55 such matings were obtained 827 eggs. These were observed daily until December 4, at which time 426, or 51.5 per cent had hatched. More than half the eggs were observed eight days longer, but none of them hatched, so the 426 recorded up to December 4 are probably about the total. Table 5 gives the details of the hatching. The eggs were obtained in two distinct lots, separated by an interval of several days, and these lots were observed separately. In the table, they are combined, and instead of the actual date the day of hatching, counting from the day the first egg was laid, is given in the table. From this table it appears that the variability of the duration of the egg stage is larger than in  $C \times C$ , but much smaller than in  $D \times D$  ( $\sigma = 3.44$ ).

A parthenogenetic line, started from a female selected at random from the third day's hatch is recorded in table 6. The proportion of male-producers in this line was 44.6 per cent.

TABLE 5

*Showing number of eggs that hatched, out of 827 in lot  $C \times D$ , figure 1*

DAY OF HATCHING	NUMBER HATCHED	DAY OF HATCHING	NUMBER HATCHED	DAY OF HATCHING	NUMBER HATCHED
7	8	14	31	24	2
8	34	15	16	25-31	0
9	47	16	8	32	1
10	120	17	5	33-45	0
11	66	18	12	46	1
12	35	19	5	47-49	0
13	35	20-23	0		
Total.....					426
Percentage of viable eggs.....					51.5
Standard deviation: $\sigma_H = 3.44$ $\sigma_L = 1.57$					

*D* × *C*. Females of line *D* were mated with males of line *C* October 16 to 21, 1911. From 47 such matings were obtained 660 eggs which were kept under observation until December 8, at which time 214 eggs, or 32.4 per cent, had hatched. Half the eggs were kept until December 20, and two more were hatched; but these two are not included in table 7 as they would not greatly

TABLE 6  
*Showing details of parthenogenetic line C × D, figure 1*

NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂ ♀	NUMBER OF ♀ ♀	NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂ ♀	NUMBER OF ♀ ♀
1	Nov. 3	23	32	5	Nov. 22	18	29
2	5	16	24	6	24	26	25
3	7	21 <sup>1</sup>	24 <sup>1</sup>	7	26	32	17
4	10	0 <sup>1</sup>	15 <sup>1</sup>	8	28	9 <sup>1</sup>	14 <sup>1</sup>
Total.....						145	180
Percentage of ♂ ♀.....						44.6	

<sup>1</sup> Remainder of family not recorded.

TABLE 7  
*Showing number of eggs that hatched, out of 660 in lot D × C, figure 1*

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
Oct. 26	7	Nov. 7	0	Nov. 19	1
27	31	8	2	20	4
28	21	9	20	21	0
29	9	10	19	22	1
30	9	11	13	23	3
31	1	12	2	24	1
Nov. 1	3	13	9	25-26	0
2	11	14	1	27	1
3	8	15	1	28-Dec. 5	0
4	6	16	3	6	1
5	4	17	0	7	1
6	20	18	1	8	0
Total.....					214
Percentage of viable eggs.....					32.4
Standard deviation: $\sigma_H = 7.79$ $\sigma_L = 2.15$					

affect the result. The standard deviation of the duration of the egg stage is 7.79, less than in  $D \times D$ , but considerably greater than in  $C \times C$  or  $C \times D$ . The hatching has been spread over a considerable time, but not so great a time, relative to the number hatching, as in  $D \times D$ .

Some correction in the standard deviation of the duration of the egg stage seems necessary. If the eggs be collected over a long period, and all be put into one lot for records of hatching, it is obvious that the hatching will be spread over a correspondingly longer period, and the standard deviation thereby increased. Since the four lots of eggs to be compared ( $C \times C$ ,  $C \times D$ ,  $D \times C$  and  $D \times D$ ) were not obtained in the same length of time, the apparent variability of the duration of the egg stage is not the true variability. To correct this error in some measure, I have computed the standard deviation of the laying of the eggs, and deducted it from the standard deviation of the hatching. Some computation was necessary to determine when the eggs were laid; but as the number of females mated in one day, and the number of eggs laid by each, is known, and as the fertilized eggs of one female are laid over a period of about five days, a fair approximation of the time of laying may be had. After this adjustment, the standard deviation was computed, and is given at the bottom of each of the four tables of hatching (indicated by  $\sigma_L$ ;  $\sigma_H$  indicates the standard deviation of hatching). It is believed that deducting  $\sigma_L$  from  $\sigma_H$  is a sufficiently accurate correction within the limits in which it is used.

If, now, the four lots to be compared are arranged in a series, as in figure 1, with the inbred eggs ( $C \times C$  and  $D \times D$ ) at the ends, and the reciprocal crosses between them, each one nearest the line that furnished the female parent, some interesting facts appear. The standard deviations of hatching of the four lots are 2.83, 3.44, 7.79 and 10.78, respectively; or, if we deduct from each the standard deviation of laying, they are 1.09, 1.87, 5.64 and 7.14. From left to right, these four lots of eggs form a series of increasing variability of the duration of the egg stage. The most important feature is that the reciprocal crosses are

apparently unequal. While both lie between the extremes of their parents, each is more like the line that furnished its female parent.

This inequality of the reciprocal crosses also appears in the percentage of viable eggs. If the series be again arranged in the same order as in figure 1, these percentages are 45.7, 51.5, 32.4 and 5.3, respectively. Of the two reciprocal crosses, the one whose mother came from the high viability line had the more viable eggs, and conversely. The apparent inequality of the reciprocals is further tested in the experiments next to be described.

It should be mentioned here in passing that from one of the eggs of lot  $D \times C$ , that hatched on the third day of hatching, a parthenogenetic line was reared as in table 8. The percentage of male-producers was 31.2.

$(C \times D) \times (C \times D)$ . Females of the parthenogenetic line  $C \times D$  were paired with males of the same line November 24 to 30, 1911. From 54 matings thus made, 904 eggs were secured. These eggs were observed daily until December 20, by which time 425, or 47.0 per cent, had hatched. Table 9 records the complete data. The standard deviation of the duration of the egg stage is found to be 2.30. It does not seem necessary to

TABLE 8  
*Showing details of parthenogenetic line  $D \times C$ , figure 1*

NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀	NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀
1	Nov. 4	2	23	5	Nov. 21	1 <sup>1</sup>	21 <sup>1</sup>
2	5	12	38	6	23	9	22
3	8	12 <sup>1</sup>	18 <sup>1</sup>	7	25	35	13
	9	1 <sup>1</sup>	21 <sup>1</sup>	8	28	12	22
4	10	1 <sup>1</sup>	9 <sup>1</sup>				
Total.....						85	187
Percentage of ♂♀.....						31.2	

<sup>1</sup> Remainder of family not recorded.

compute the standard deviation of laying, since in the four experiments to be compared the number of days on which the eggs were obtained does not vary so greatly.

To test whether the viability of the eggs could be altered by selection, two parthenogenetic lines were started from fertilized eggs of the lot  $(C \times D) \times (C \times D)$ . Of the 54 females laying these eggs, twelve were kept isolated and the hatching of their eggs observed separately. There was considerable variation in the proportion of the various families that hatched. The least viable family hatched 4 out of 18 eggs, or 22.2 per cent of the family. From the first of the 4 that hatched a parthenogenetic line was reared, as in table 10. The most viable family hatched 11 out of 15, or 73.3 per cent. From the first of the 11 that hatched a parthenogenetic line was reared, as in table 11. The

TABLE 9

*Showing number of eggs that hatched, out of 904 in lot  $(C \times D) \times (C \times D)$ , figure 1*

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
Dec. 7	4	Dec. 12	107	Dec. 17	0
8	8	13	87	18	12
9	13	14	10	19	0
10	58	15	6	20	4
11	114	16	2		
Total.....					425
Percentage of viable eggs.....					47.0
Standard deviation: $\sigma_H = 2.30$					

TABLE 10

*Showing details of parthenogenetic line derived from low viability family in  $(C \times D) \times (C \times D)$ , figure 1*

NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀	NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀
1	Jan. 11	6	35	3	Jan. 15	17	30
2	13	9	28	4	16	31	14
Total.....						63	107
Percentage of ♂♀.....						37.0	

effect of this selection on viability is described later under the headings  $L_1$  and  $H_1$ . I merely call attention here to the difference in the proportion of male-producers, 37.0 per cent in one line, 20.5 per cent in the other.

$(D \times C) \times (D \times C)$ . Females of the parthenogenetic line  $D \times C$  were mated with males of the same line November 26 to 30, 1911. From 38 such matings were secured 576 eggs. By December 20, when the hatching must have been nearly complete, 319 of these eggs, or 55.3 per cent had hatched (table 12). The greater number of these eggs were hatched on a few successive days, hence the standard deviation is low ( $\sigma = 1.65$ ).

TABLE 11

Showing details of parthenogenetic line derived from high viability family in  $(C \times D) \times (C \times D)$ , figure 1

NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀	NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀
1	Jan. 11	0	14	6	Jan. 21	8	30
2	13	0	17		21	6	23
3	15	0	9	7	21	8	11
4	17	6	42	8	23	10	34
5	18	21	29	9	25	8	28
6	20	4	29		25	5	28
Total.....						76	294
Percentage of ♂♀ .....						20.5	

TABLE 12

Showing number of eggs that hatched, out of 576 in  $(D \times C) \times (D \times C)$ , figure 1

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
Dec. 7	1	Dec. 12	57	Dec. 17	1
8	3	13	100	18	1
9	5	14	11	19	1
10	52	15	10	20	1
11	72	16	4		
Total.....					319
Percentage of viable eggs.....					55.3
Standard deviation: $\sigma_H = 1.65$					

Attention may now be called to the fact that although the eggs of  $D \times C$  showed lower viability and greater variability in the duration of the egg stage than did those of  $C \times D$ , yet when the parthenogenetic lines from each were inbred, the eggs in lot  $(D \times C) \times (D \times C)$  were a little more viable and a little less variable in their hatching time than were those of  $(C \times D) \times (C \times D)$ . The differences are small, however, as compared with the differences between  $D \times C$  and  $C \times D$ , and are probably insignificant. The inequality of the reciprocal crosses disappears when the parthenogenetic lines derived from them are compared. Further evidence that the reciprocal crosses  $C \times D$  and  $D \times C$  are not after all unequal is found in the reciprocal crosses between the reciprocal crosses, now to be described.

$(C \times D) \times (D \times C)$ . Females from the parthenogenetic line  $C \times D$  were paired with males from line  $D \times C$  November 9 and 10, 1911. From 20 matings thus produced, 278 eggs were obtained. Up to December 20, 174 of these eggs, or 62.5 per cent, had hatched (table 13). The duration of the egg stage is not greatly variable, hence the standard deviation is low ( $\sigma = 2.42$ ).

$(D \times C) \times (C \times D)$ . Females of the line  $D \times C$  were mated with males of the line  $C \times D$  November 7 to 10, 1911. From 23 such matings were secured 357 eggs. Up to December 20,

TABLE 13

*Showing number of eggs that hatched, out of 278 in  $(C \times D) \times (D \times C)$ , figure 1*

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE*	NUMBER HATCHED
Nov. 19	5	Nov. 25	10	Dec. 1-5	0
20	9	26	2	6	1
21	20	27	1	7-14	0
22	59	28	0	15	1
23	44	29	1	16-20	0
24	19	20	2		
Total.....					174
Percentage of viable eggs.....					62.5
Standard deviation: $\sigma_H = 2.42$					



244 of these eggs, or 68.3 per cent, had hatched (table 14). The variability in hatching time is not great, hence the standard deviation is low ( $\sigma = 2.19$ ).

The reciprocal crosses described in the last two paragraphs are approximately equal both in percentage of viability, and in variability of hatching time, though their parents  $C \times D$  and  $D \times C$ , were widely different in both characters. This supports similar evidence from the inbreeding of  $C \times D$  and the inbreeding of  $D \times C$ . The reciprocal crosses  $C \times D$  and  $D \times C$  were,

TABLE 14

Showing number of eggs that hatched, out of 357 in  $(D \times C) \times (C \times D)$ , figure 1

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
Nov. 18	3	Nov. 23	53	Nov. 28	2
19	14	24	30	29	0
20	16	25	26	30	1
21	19	26	17	Dec. 1-6	0
22	59	27	3	7	1
				8-20	0
Total.....					244
Percentage of viable eggs.....					68.3
Standard deviation: $\sigma_H = 2.19$					

after all, nearly equal. The inequality existed only in the egg stage. When these eggs grew up, so to speak, to produce parthenogenetic lines, these lines were essentially equal in the viability and variability of the fertilized eggs which they produced.

*Effect of selection for low viability in a hybrid line*

$L_1$ . Females of the parthenogenetic line derived from a low viability parent in  $(C \times D) \times (C \times D)$  were paired with males of the same line January 16 to 18, 1912. From 40 such matings, 515 eggs were obtained. These were kept under observation until February 22, at which time 149 eggs, or 28.9 per cent, had hatched (table 15). Standard deviation of hatching time has not been computed for any of the selection experiments, as it was obvious that no marked difference would be found.

Of the 40 females laying these eggs, 12 were kept isolated and the hatching of their eggs recorded separately. The least viable of these twelve families of eggs was one that hatched only 1 out of 21 (viability 4.7 per cent). From the one that hatched, a parthenogenetic line was reared (table 16) to produce a further generation of eggs. This line included 39.3 per cent of male-producers.

*L*<sub>2</sub>. Females of the parthenogenetic line *L*<sub>1</sub> were paired with males of the same line February 14 to 24, 1912. From 60 matings thus made were obtained 770 eggs. By March 17, when observations ceased, 272 eggs, or 35.3 per cent, had hatched (table 17).

From the 60 females that laid these eggs, one was selected that laid 18 eggs, of which but one hatched (viability 5.5 per cent). From this one was bred a parthenogenetic line (table

TABLE 15

*Showing number of eggs that hatched, out of 515 in lot L<sub>1</sub>, figure 1*

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
Jan. 26	5	Jan. 31	23	Feb. 8	11
27	14	Feb. 1	6	9	12
28	2	2	0	10	1
29	10	3	1	11-22	0
30	64	4-7	0		
Total.....					149
Percentage of viable eggs .....					28.9

TABLE 16

*Showing details of parthenogenetic line L<sub>1</sub>, figure 1*

NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀	NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀
1	Feb. 8	10	31	4	Feb. 15	24	19
2	10	24	22	5	17	10	39
3	12	24	19				
Total.....						92	142
Percentage of ♂♀ .....						39.3	

18) to be used for further selection for least viable eggs. This line included 40.7 per cent of male-producers.

*L*<sub>3</sub>. Females of the parthenogenetic line *L*<sub>2</sub> were paired with males of the same line April 3 to 8, 1912. From 42 such matings, 487 eggs were obtained. To and including April 30, there had hatched 191 eggs, or 39.2 per cent (table 19).

From the 191 eggs that hatched, one was selected at random, without reference to the viability of the family to which it belonged. From this egg a parthenogenetic line was reared (table 20) which included 36.1 per cent of male-producers.

TABLE 17

*Showing number of eggs that hatched, out of 770 in lot L<sub>2</sub>, figure 1*

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
Feb. 24	20	Mar. 1	26	Mar. 7	2
25	30	2	31	8-10	0
26	16	3	9	11	2
27	29	4	8	12	0
28	52	5	12	13	5
29	29	6	0	14	1
				15-17	0
Total.....					272
Percentage of viable eggs.....					35.3

TABLE 18

*Showing details of parthenogenetic line in L<sub>2</sub>, figure 1*

NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀	NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀
1	Mar. 28	13	43	10	Apr. 15	26	22
2	30	2	42	11	17	12	37
3	Apr. 2	14	31	12	19	26	19
4	4	15	28	13	21	21	28
5	6	21	19	14	23	24	19
6	8	36	13	15	24	22	12
7	10	15	28	16	26	16	16
8	11	14	27	17	28	2	22
9	13	21	31				
Total.....						300	437
Percentage of ♂♀.....						40.7	

TABLE 19

*Showing number of eggs that hatched, out of 487 in lot L<sub>3</sub>, figure 1*

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
Apr. 13	1	Apr. 18	32	Apr. 22	3
14	22	19	10	23-24	0
15	33	20	6	25	1
16	53	21	1	26-30	0
17	29				
Total.....					191
Percentage of viable eggs.....					39.2

TABLE 20

*Showing details of parthenogenetic line L<sub>3</sub>, in figure 1*

NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀	NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀
1	May 12	5	22	4	May 18	11	27
2	14	6	7	5	21	19	19
3	16	15	24				
Total.....						56	99
Percentage of ♂♀.....						36.1	

TABLE 21

*Showing number of eggs that hatched, out of 728 in lot H<sub>1</sub>, figure 1*

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
Jan. 30	2	Feb. 7	42	Feb. 15	0
31	7	8	32	16	3
Feb. 1	28	9	79	17	1
2	19	10	38	18-21	0
3	2	11	29	22	1
4	11	12	57	23-25	0
5	4	13	11	26	1
6	12	14	2	Feb. 27-Mar. 1	0
Total.....					381
Percentage of viable eggs.....					52.3

*Effect of selection for high viability in a hybrid line*

Let us now turn to the high viability series, beginning with the parthenogenetic line in  $(C^* \times D) \times (C \times D)$ , whose parent hatched 11 out of 15 eggs (viability 73.3 per cent).

$H_1$ . Females of the parthenogenetic line bred from a highly viable family in  $(C \times D) \times (C \times D)$  were paired with males of the same line January 20 to 28, 1912. From 53 such matings, 728 fertilized eggs were obtained. By March 1, 381 of these, or 52.3 per cent, had hatched (table 21). Of the low viability lot of eggs,  $L_2$ , there hatched at the same time but 28.9 per cent.

One of the above 53 females laid 16 eggs, of which 12, or 75 per cent, hatched. From the first of her twelve daughters the parthenogenetic line designated  $H_1$  was reared. It included but 18.9 per cent of male-producers (table 22).

$H_2$ . Females of the parthenogenetic line  $H_1$  were paired with males of the same line, February 17 to March 8, 1912. From 43 such females were obtained 583 eggs, of which 288, or 49.3 per cent, hatched before April 10 (table 23). The low viability lot of eggs,  $L_2$ , corresponding to the high viability lot  $H_2$ , hatched but 35.3 per cent. It should be noted that these two lots of eggs were not on the whole hatching simultaneously. Whereas the bulk of the eggs in  $L_2$  hatched at the end of February, the lot  $H_2$  reached its maximum about March 20. This was unavoidable owing to the fact that the scarcity of male-producers in

TABLE 22

*Showing details of parthenogenetic line  $H_1$ , in figure 1*

NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀	NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀
1	Feb. 18	7	44	4	Feb. 27	16	36
2	21	3	37	5	29	10	34
3	24	3	36	6	Mar. 3	20	25
	25	3	26	7	5	6 <sup>1</sup>	29 <sup>1</sup>
	26	4	24	8	7	0 <sup>1</sup>	16 <sup>1</sup>
Total.....						72	307
Percentage of ♂♀.....						18.9	

<sup>1</sup> Remainder of family not recorded.

the high viability parthenogenetic line prevented the fertilization of a sufficient number of eggs. The fluctuation of the viability, which will be apparent later, may be due to the fact that the two lots did not hatch simultaneously.

Of the 43 females that laid the eggs in lot  $H_2$ , one laid 13 eggs, of which 10, or 76.9 per cent, hatched. From the first of her

TABLE 23

*Showing number of fertilized eggs that hatched, out of 583 in lot  $H_2$ , figure 1*

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
Feb. 28	3	Mar. 13	12	Mar. 25	9
	29	14	4	26	2
Mar. 1	3	15	3	27	3
	2	16	5	28	1
	3	17	6	29	0
	4	18	7	30	2
	5	19	21	31	0
6-8	0	20	34	Apr. 1	2
9	2	21	38	2-3	0
10	0	22	13	4	1
11	1	23	39	5-10	0
12	0	24	69		
Total.....					288
Percentage of viable eggs.....					49.3

TABLE 24

*Showing details of parthenogenetic line  $H_2$ , in figure 1*

NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀	NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀
1	Mar. 22	3	19	6	Apr. 3	12	32
2	25	19	26	7	5	7	39
3	28	11	25	8	7	15	34
	29	0	43	9	9	7	33
4	30	7	48	10	11	0	9
5	Apr. 1	4	27	11	13	5	19
Total.....						90	354
Percentage of ♂♀.....						20.2	

ten daughters was bred the parthenogenetic line  $H_2$  (table 24). It included 20.2 per cent of male-producers.

$H_3$ . Females of the parthenogenetic line  $H_2$  were paired with males of the same line March 29 to April 7, 1912. From 42 such females, 640 eggs were obtained, of which 407, or 63.5 per cent, hatched before April 30 (table 25). The low viability lot  $L_3$  hatched, at about the same time, only 39.2 per cent.

TABLE 25

*Showing number of fertilized eggs that hatched, out of 640 in lot  $H_3$ , figure 1*

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
Apr. 8	2	Apr. 14	86	Apr. 20	6
9	17	15	53	21	2
10	34	16	35	22	3
11	33	17	39	23	1
12	24	18	20	24-30	0
13	47	19	5		
Total.....					407
Percentage of viable eggs.....					63.5

From the 42 females that laid the eggs in lot  $H_3$ , two were selected for further breeding. One laid 16 eggs, of which but 7, or 43.7 per cent, hatched; the other laid 21 eggs, of which 17, or 80.9 per cent, hatched. From the first daughter of each was bred a parthenogenetic line, as given in table 26. The purpose was to test the effect of selection within the high viability series, the results of which are described later. The first of these lines included 10.0 per cent, the second 11.1 per cent of male-producers.

The percentage viability of the eggs in lots  $L_1$ ,  $L_2$ , and  $L_3$  were 28.9, 35.3, and 39.2, respectively; in lots  $H_1$ ,  $H_2$ , and  $H_3$ , it was 52.3, 49.3, and 63.5, respectively. The high viability lots were consistently more viable than the lots selected for low viability. With the exception of the second lots, the difference between the two lots is nearly constant; and this exception is probably due to the fact that  $L_2$  and  $H_2$  were not hatched at the

same time, and hence not under the same conditions. Selection affected viability, but this effect was all in the first selection, and further selection did not increase the difference in viability.

*Effect of crossing on viability in two lines made different by selection*

Whether selection produced a permanent difference, or whether the difference was maintained only by continuous selection, was determined by the remaining experiments. First among these were reciprocal crosses between the parthenogenetic lines in  $L_2$  and  $H_2$ .

$L_2 \times H_2$ . Females of the parthenogenetic line  $L_2$  (see fig. 1) were mated with males of the line  $H_2$  April 11 to 19, 1912. From 36 such females were obtained 427 eggs, of which 245, or 57.3 per cent, hatched before May 6, as in table 27.

TABLE 26

*Showing details of two parthenogenetic lines in  $H_3$ , figure 1, one derived from a family in which only 43.7 per cent of the fertilized eggs hatched, the other from a family in which 80.9 per cent of the eggs hatched.*

LOW VIABILITY				HIGH VIABILITY			
NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀	NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀
1	May 2	0	28	1	May 2	2	17
2	4	0	50	2	4	1	53
3	6	0	42	3	6	0	25
4	7	1	33	4	7	9	34
5	9	3	36	5	9	7	18
6	11	4	39	6	11	3	18
	12	15	50	7	13	3	25
7	12	8	15	8	15	0	27
8	15	1	31	9	17	13	26
9	17	7	28	10	19	1	35
10	19	6	39	11	21	0	33
11	21	1	22				
Total.....		46	413	Total.....		39	311
Percentage of ♂♀....			10.0	Percentage of ♂♀....			11.1



$H_2 \times L_2$ . Females of the parthenogenetic line  $H_2$  were mated with males of the line  $L_2$  April 7 to 15, 1912. From 30 such females, 294 eggs were obtained. Of these, 203, or 69.0 per cent, hatched before May 6 (table 28).

These reciprocal crosses bear precisely the same relation to each other and to their parents as do the reciprocal crosses  $C \times D$  and  $D \times C$ . The reciprocals are unequal, that one having the higher viability whose mother belonged to the high viability line. In both cases, the cross whose mother came from the high viability line ( $C \times D$  and  $H_2 \times L_2$ ) possessed greater viability than either parent line.

TABLE 27

Showing number of fertilized eggs that hatched, out of 427 in lot  $L_2 \times H_2$ , in figure 1

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
Apr. 18	9	Apr. 23	10	Apr. 29-30	0
19	56	24	5	May 1	1
20	80	25-27	0	2	1
21	48	28	1	3-6	0
22	34				
Total.....					245
Percentage of viable eggs.....					57.3

TABLE 28

Showing number of fertilized eggs that hatched, out of 294 in lot  $H_2 \times L_2$ , in figure 1

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
Apr. 16	1	Apr. 22	35	Apr. 27	4
17	0	23	11	28	3
18	1	24	4	29-30	0
19	10	25	10	May 1	3
20	57	26	3	2-6	0
21	61				
Total.....					203
Percentage of viable eggs.....					69.0

*Effect of selection for high and low viability in the low viability series (repeatedly inbred)*

*L*<sub>4</sub>. Females from the parthenogenetic line *L*<sub>3</sub>, whose parent was selected at random without reference to viability, were mated with males of the same line May 19 to 23, 1912. From 38 such females were obtained 241 eggs, of which 53, or 21.9 per cent, hatched before June 7 (table 29).

Twelve of the above 38 females were kept isolated and the hatching of their eggs recorded separately. In the least viable family, 1 egg in 12, or 8.3 per cent, hatched; in the most viable, 4 out of 11, or 36.3 per cent, hatched. From the first daughter in each family a parthenogenetic line was reared (table 30). The low viability line included 37.5 per cent of male-producers, the high viability line 13.6 per cent.

*L from L*. Females of the parthenogenetic line in *L*<sub>4</sub> bred from a low viability family were mated with males of the same line June 11 to 13, 1912. From 26 such females 223 eggs were obtained, of which 74, or 33.1 per cent, hatched prior to July 14 (table 31).

*H from L*. Females of the high viability line (selected for high viability from low viability eggs) in *L*<sub>4</sub> were mated with males of the same line June 15 and 16, 1912. From 12 such females 74 eggs were obtained, of which 24, or 32.4 per cent, hatched prior to July 14 (table 32).

TABLE 29

*Showing number of fertilized eggs that hatched, out of 241 in lot L<sub>4</sub>, figure 1*

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
May 27	5	May 31	3	June 4	0
28	14	June 1	9	5	0
29	10	2	4	6	0
30	6	3	2	7	0
Total.....					53
Percentage of viable eggs.....					21.9

TABLE 30

Showing details of two parthenogenetic lines in  $L_4$ , figure 1, one line being reared from a family in which few eggs hatched, the other from a family in which relatively many eggs hatched

LOW VIABILITY				HIGH VIABILITY			
NUMBER OF GENERATION	DATE OF FIRST YOUNG	NO. OF ♂♀	NUMBER OF ♀♀	NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀
1	June 3	5	31	1	June 3	0	7
2	4	0	6	2	4	0	6
	4	0	4		6	0	16
3	6	12	28	3	7	2	33
	6	0	8	4	10	3	24
4	10	18	15	5	12	1	30
5	12	18	10	6	13	9	19
6	14	15	11	7	15	4	24
				8	17	7	19
				9	20	0	7
					21	7	24
Total.....		68	113	Total.....		33	209
Percentage of ♂♀....		37.5		Percentage of ♂♀....		13.6	

TABLE 31

Showing number of eggs that hatched, out of 223 in lot ( $L$  from  $L$ ), figure 1

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
June 20	1	June 25	9	June 29	2
21	11	26	4	30	1
22	15	27	0	July 1	2
23	18	28	1	2-14	0
24	10				
Total.....					74
Percentage of viable eggs.....					33.1

Since *L* from *L*, and *H* from *L* show almost precisely the same viability, selection for high and low viability within the low viability lot was entirely ineffective.

*Effect of selection for high and low viability in the high viability series (repeatedly inbred)*

*L*<sub>1</sub> from *H*. Females of the parthenogenetic line bred from a low viability family in *H*<sub>3</sub> were mated with males of the same line May 13 to 23, 1912. From 18 such females were obtained 161 eggs, of which 94, or 58.3 per cent, hatched prior to June 5 (table 33).

One of these 18 females laid nine eggs, of which only one hatched (11.1 per cent of viable eggs). From that one a parthenogenetic line was reared, as in table 34, including 16.1 per cent of male-producers.

TABLE 32

*Showing number of fertilized eggs that hatched, out of 74 in lot (H from L), figure 1*

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
June 24	2	June 27	3	June 30	1
25	8	28	2	July 1	4
26	2	29	2	2-14	0
Total.....					24
Percentage of viable eggs.....					32.4

TABLE 33

*Showing number of eggs that hatched, out of 161 in lot (L<sub>1</sub> from H), figure 1*

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
May 22	1	May 27	30	June 1	3
23	7	28	14	2	1
24	13	29	2	3	0
25	7	30	5	4	0
26	10	31	1	5	0
Total.....					94
Percentage of viable eggs.....					58.3

*H*<sub>1</sub> from *H*. Females of the parthenogenetic line bred from a high viability family in *H*<sub>3</sub> were mated with males of the same line May 11 to 20, 1912. From 23 such females were obtained 259 eggs, of which 125, or 48.2 per cent, hatched prior to June 19 (table 35).

Comparison of *L*<sub>1</sub> from *H* with *H*<sub>1</sub> from *H* shows that a single selection for low viability within the high viability series was entirely ineffective.

TABLE 34

*Showing details of parthenogenetic line in (L<sub>1</sub> from H), figure 1*

NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀	NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂♀	NUMBER OF ♀♀
1	May 30	1	26	7	June 11	1	26
2	31	4	18	8	13	6	26
3	June 3	9	30	9	15	6	14
4	4	8	36	10	16	0	30
5	7	5	34	11	18	10	18
6	9	1	36		18	9	18
Total.....						60	312
Percentage of ♂♀.....							16.1

TABLE 35

*Showing number of fertilized eggs that hatched, out of 259 in lot (H<sub>1</sub> from H), figure 1*

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	
May 20	2	May 28	21	June 5	1	
21	0	29	2	6	1	
22	9	30	3	7-9	0	
23	5	31	15	10	1	
24	15	June 1	3	11	0	
25	6	2-3	0	12	4	
26	23	4	1	13-19	0	
27	13					
Total.....						125
Percentage of viable eggs.....						48.2

One of the 23 families in lot  $H_1$  from  $H$  laid 14 eggs, of which 11, or 78.5 per cent, hatched. From the first of her 11 daughters a parthenogenetic line was reared, as in table 36, including 35.8 per cent of male-producers.

$L_2$  from  $H$ . Females of the parthenogenetic line  $L_1$  from  $H$  were mated with males of the same line June 9 to 17, 1912. From eight such females were obtained 65 eggs, of which 36, or 55.3 per cent, hatched prior to July 1 (table 37).

$H_2$  from  $H$ . Females of the parthenogenetic line  $H_1$  from  $H$  were mated with males of the same line June 8 to 13, 1912. From 23 such females were obtained 164 eggs, of which 92, or 56.0 per cent, hatched prior to July 14 (table 38).

Comparison of  $L_2$  from  $H$  with  $H_2$  from  $H$  indicates that a second selection for high and low viability within the high viability series had no effect.

TABLE 36

*Showing details of parthenogenetic line in ( $H_1$  from  $H$ ), figure 1*

NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂ ♀	NUMBER OF ♀ ♀	NUMBER OF GENERATION	DATE OF FIRST YOUNG	NUMBER OF ♂ ♀	NUMBER OF ♀ ♀
1	May 29	0	13	5	June 7	11	16
2	31	0	9	6	9	0	3
3	June 3	1	4		11	0	2
4	4	14	28	7	11	27	20
Total.....						53	95
Percentage of ♂ ♀.....						35.8	

TABLE 37

*Showing number of fertilized eggs that hatched, out of 65 in lot ( $L_2$  from  $H$ ), figure 1*

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
June 18	4	June 21	5	June 24	1
19	11	22	4	25	1
20	7	23	3	June 26-July 1	0
Total.....					36
Percentage of viable eggs.....					55.3

TABLE 38

Showing number of fertilized eggs that hatched, out of 164 in lot ( $H_2$  from  $H$ ), figure 1

DATE	NUMBER HATCHED	DATE	NUMBER HATCHED	DATE	NUMBER HATCHED
June 17	1	June 23	7	June 28	3
18	26	24	5	29	2
19	12	25	6	30	2
20	8	26	2	July 1	6
21	4	27	2	2-14	0
22	6				
Total.....					92
Percentage of viable eggs.....					56.0

RESULTS OF THE EXPERIMENTS

The results of the experiments may be stated under three heads: (1) The effect of crossing on the viability of eggs and on the variability of the duration of the egg stage; (2) effect of selection on viability of eggs in a hybrid line and in relatively 'pure' lines; and (3) inheritance of the sex-ratio. Figure 2 will aid in presenting these results.

1. *The effect of crossing. Viability of eggs.* This was tested twice in the experiments: once in reciprocal crosses between lines  $C$  and  $D$ , which were found, already distinctly different in viability, in previous experiments, and again in reciprocals between  $L_2$  and  $H_2$ , which had been made to differ in viability by means of selection. In both cases the average viability of the two lots of  $F_1$  fertilized eggs (for instance,  $C \times D$  and  $D \times C$ ) was considerably greater than the average viability of the inbred eggs of the two lines crossed. In both cases, the two reciprocal crosses were unequal, that one being most viable which had the high viability mother, and conversely. These results indicate that  $L_2$  and  $H_2$ , made different by selection, differed from one another in precisely the same way as did  $C$  and  $D$ , which were found different in previous experiments.

The inequality of the reciprocal crosses lasted, however, only through the egg stage. For when parthenogenetic lines were

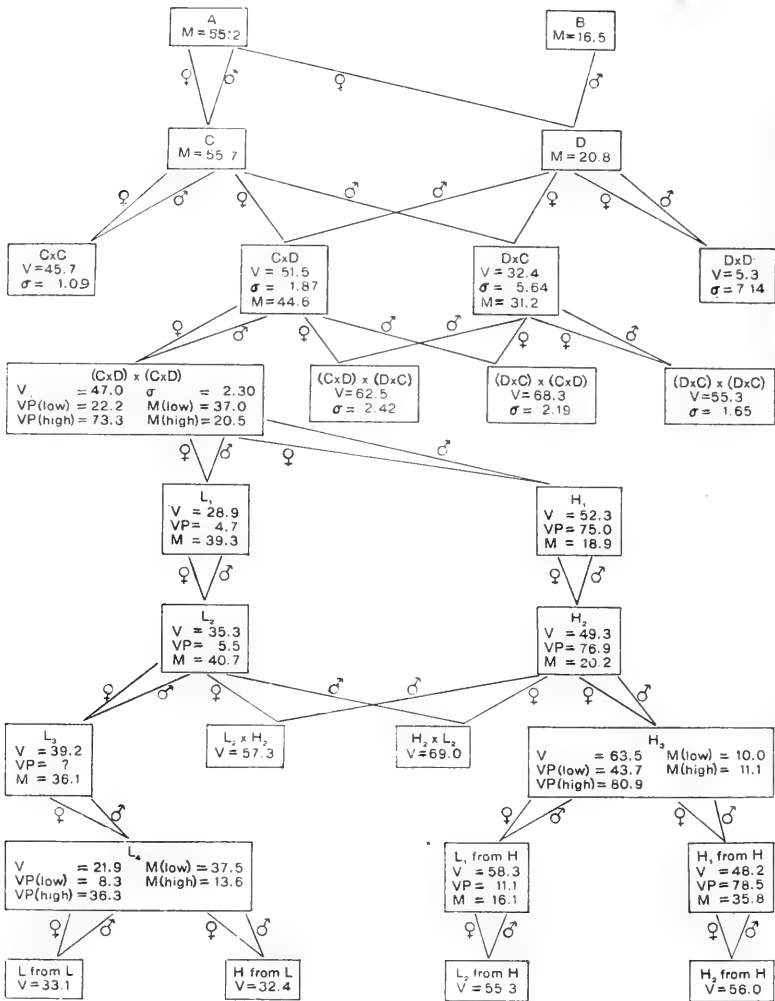


Fig. 2 Diagram showing results of all experiments described in text (see also fig. 1).  $M$  = percentage of male-producers in parthenogenetic line.  $V$  = percentage of viable fertilized eggs.  $VP$  = percentage viability in family of fertilized eggs from which parthenogenetic line was derived.  $\sigma$  = standard deviation of duration of egg stage.

reared from the fertilized eggs in  $C \times D$  and  $D \times C$ , and these lines were in turn inbred and reciprocally crossed, the following result appeared: the two lots of inbred eggs were approximately



equal in viability, and the two reciprocal crosses were approximately equal.

*Variability of the duration of the egg stage.* The effect of crossing on the variability of the duration of the egg stage was tested in two successive generations, beginning with a cross between lines *C* and *D*. The standard deviation of the hatching time for inbred eggs of these two lines were widely different (1.09 and 7.14 respectively). The standard deviations of the two reciprocal crosses were intermediate between those of the inbred eggs, but were very unequal (1.87 and 5.64); each cross was nearer its female parent, in point of variability of hatching time.

This inequality existed only in the egg stage, however, for when the two reciprocal crosses ( $C \times D$  and  $D \times C$ ) were in turn inbred and reciprocally crossed, the variability of hatching time did not materially differ in the four lots of eggs (standard deviation being 2.30, 2.42, 2.19 and 1.65, respectively).

2. *The effect of selection on viability of eggs. In hybrid lines.* In the lot of eggs designated  $(C \times D) \times (C \times D)$ , which were only once inbred from an  $F_1$  (and hence hybrid) parthenogenetic line, selection was made for families of high and low viability, respectively. From the selected eggs, parthenogenetic lines were reared, and among their inbred eggs, selection for families of high and low viability was again made. Three successive selections resulted in an average difference of about 21 per cent in the viability of the eggs; but *practically all this difference was produced by the first selection, and was not thereafter appreciably increased.*

*Selection in relatively 'pure' lines.* Three successive inbreedings, as described above, must have changed many heterozygous characters to the homozygous condition, if random segregation and recombination occur; hence the parthenogenetic lines  $L_3$  and  $H_3$  must have been relatively 'pure,' that is, homozygous. Was the difference in viability between  $L_3$  and  $H_3$  fertilized eggs a permanent product of selection, or had it been maintained only by continued selection?

Random selection from  $L_3$ , without reference to the viability of the parents, resulted in a lowered viability in  $L_4$ . This may

have been due to external conditions; at any rate, *viability did not increase when selection for low viability was discontinued*. Selection was then made for high and low viability in this "random" lot of eggs, but the results, as shown in *L from L* and *H from L*, figure 2, show that *selection was wholly ineffective*. The low viability eggs were permanently of low viability, not kept low by continued selection; selection within the low viability series was of no avail.

In like manner, selection for high and low viability among the high viability families of  $H_3$  (cf. *L<sub>1</sub> from H*, and *H<sub>1</sub> from H*; also, *L<sub>2</sub> from H*, and *H<sub>2</sub> from H*) *did not alter the viability in the direction of selection*. The high viability eggs were inherently of high viability, not maintained so by selection.

3. *Inheritance of the sex-ratio*. In every case here recorded in which two lines were crossed (for example, lines *A* and *B* to produce *D*, also reciprocal crosses between *C* and *D*), the *cross was intermediate between the parent lines in the proportion of male-producers*. In the one case of reciprocal crosses recorded, *the reciprocals were unequal; each was nearer the line that furnished the female parent*. Unfortunately, only one of these reciprocals was tested further, so it is not known whether this inequality of the reciprocal crosses disappeared, as was the case with viability, in the subsequent parthenogenetic lines.

When a parthenogenetic line was inbred, the parthenogenetic line derived from its fertilized eggs usually yielded about the same proportion of male-producers as did the parent line. That this was not necessarily the case, however, was shown when two lines were reared from the same lot of inbred eggs. Thus, in  $(C \times D) \times (C \times D)$ , the line bred from a low viability family of eggs included 37 per cent of male-producers, while the line from a high viability parent yielded only 20.5 per cent. This was not due to the conceivable fact that one line was bred only during a period of many male-producers, the other only during a period of few male-producers; for when these two lines were inbred, and their descendants inbred several times in succession, the 37 per cent series maintained, with one exception, a proportion of male-producers about 35 per cent ( $L_1$  to  $L_4$ ), while the

20 per cent series, with one exception, yielded less than 21 per cent ( $H_1$  to  $L_1$  from  $H$  and  $H_1$  from  $H$ ). Thus, in  $(C \times D) \times (C \times D)$ , selection for viability of fertilized eggs was accompanied by a differentiation with respect to the proportion of male-producers such that the least viable eggs yielded the parthenogenetic line with the most male-producers. Precisely the same result appeared in  $L_4$ , where selection for low viability produced a parthenogenetic line with 37.5 per cent of male-producers, while the high viability eggs gave rise to a line with only 13.6 per cent of male producers.

One can not safely generalize, however, and say that a high percentage of male-producers is associated with low viability of fertilized eggs; for in  $H_3$  a similar selection for viability of eggs was made, without, as figure 2 shows, any appreciable effect on the percentage of male-producers in the descendent parthenogenetic lines.

#### INTERPRETATION OF RESULTS

*Viability of fertilized eggs* appears to be dependent on segregating factors or genes. Otherwise, two eggs from the same source could not give rise to parthenogenetic lines whose fertilized eggs differ markedly in viability. Selection for viability was effective in a hybrid (heterozygous) lot of eggs because of this segregation.

Since a single selection produced the maximum effect on viability, since this effect was not augmented by subsequent selection, it appears probable that but few genes are concerned with viability. The eggs of the first selected families must have been practically homozygous, or subsequent selection would have augmented the effect. Had the genes affecting viability been numerous, the chance of selecting a completely homozygous egg the first time would have been quite small. I hesitate to suggest that a single pair of genes is involved; but it is quite possible that only one of the elements of viability discussed in a previous section has been studied here, and that the irregularities noted are but the effect of external conditions.

That inbreeding coupled with selection had produced practically homozygous eggs is further indicated by the negative results of selection in  $L_4$  and  $H_3$ . Selection for high and low viability within each of these lots of eggs was without effect, as would be expected if the parthenogenetic lines producing the eggs were homozygous with respect to viability.

The greater viability in eggs produced by crossing two lines, as compared with the inbred eggs produced by the same lines, may be the result of increased vigor due to heterozygosis. I have already shown (Shull '12 b) that inbreeding results in decrease of vigor in *Hydatina*, and Whitney ('12), following a single experiment of mine, has proven that crossing increases vigor. It seems probable that the fact that the reciprocal crosses between  $C$  and  $D$  and between  $L_2$  and  $H_2$  show an average viability plainly in excess of the average viability of the inbred eggs of the same lines, is merely another example of the increase of vigor due to heterozygosis. Which of the several possible elements contributing to viability is affected by heterozygosis can not be determined.

The inequality of the reciprocal crosses with respect to viability of eggs is discussed in a separate section.

*Variability in the duration of the fertilized egg stage* is not so certainly explainable because the experiments in which marked differences in this variability appeared were few. All of the results of the experiments here described accord fairly well with the assumption that this variability is dependent on a single pair of genes, and that low variability of hatching time is dominant over high variability. Because of the small number of tests obtained, this interpretation may well be incorrect.

The inequality of the reciprocal crosses  $C \times D$  and  $D \times C$ , together with the equality of the inbred and of the crossed eggs obtained from them, is discussed in another section, in connection with the inequality of reciprocal crosses with respect to viability of eggs.

*The sex-ratio*, or its equivalent, the percentage of male-producing females, behaves as if dependent on a number of genes; but it is difficult to formulate a general statement. In the experi-

ments described in this paper, the only two parthenogenetic lines ( $C \times D$  and  $D \times C$ ) that were derived from crosses between other lines, were intermediate between their parents in respect to the sex-ratio. This is what would be expected if the sex-ratio were dependent on a number of genes. But in a previous paper (Shull '11) I described a case in which a cross gave a much higher proportion of male-producers than either of its parent lines. There is a bare possibility that the two lines crossed in this earlier experiment were old; that their percentage of male-producers had been reduced by long continued parthenogenesis, as I have shown (Shull '12 a) to be a not infrequent occurrence; and that when these lines were young their sex-ratios were so high as to have made the  $F_1$ , which was later obtained by crossing, and which was itself a young line, actually intermediate between them. This possibility was discussed in the earlier paper, but the view taken that it was probably not the correct explanation. If my earlier supposition was well grounded, there is still no simple explanation for the inheritance of the proportion of male-producers.

The assumption of numerous genes for the sex-ratio would harmonize with the fact that inbreeding usually does not change the sex-ratio in the resulting parthenogenetic line. Repeated inbreeding showed little alteration in the sex-ratio. Since but one or two eggs were selected from each lot to breed parthenogenetic lines, the chances were many to one that these would be similar to their parent line. The few exceptions, such as the low percentage of male-producers in the high viability line in  $L_4$  (13.6), and the high percentage in  $H_1$  from  $H$  (35.8), may be regarded as due to selecting the extremes for parents, or, which seems to me more probable, to the fact that the lines were reared only a few generations during a period of few male-producers in the former line, and through a period of many male-producers in the latter line.

## NUCLEUS AND CYTOPLASM IN HEREDITY

Unequal reciprocal hybrids are not uncommon both in animals and in plants. Some of them are due to sex-limited inheritance, and have received an explanation that is at least plausible, involving the accessory chromosome. In *Oenothera* the reciprocals may resemble the mother most in some crosses, and resemble the father<sup>2</sup> most in other crosses; or may be more like the father in some characters, more like the mother in others; these cases must have a special explanation. In some cases, as echinoderms (Baltzer '10), the inequality seems to be due to the elimination of chromosomes in one of the reciprocals. But there remain a number of cases which apparently belong to none of these categories. A few of these have been collected and discussed by Jones ('12), who himself reports unequal reciprocals in *Digitalis*. Pearl ('10) obtained a case in fowls very similar to that which I here report in *Hydatina*; the 'hatching quality' of the eggs was unequal in the two reciprocals.<sup>3</sup> He suggested that the greater infertility of the one reciprocal might be due to unfavorable action of the secretions of the oviduct on the sperm in that particular cross, but obviously unfavorable action by the egg itself would bring about the same result. Toyama ('12) has obtained unequal reciprocals in the silkworm.

All cases in which the reciprocals are nearer the female parent, unless having some other obvious explanation, might be used as evidence that the cytoplasm is a partial bearer of the hereditary qualities, and some of the cases found have been so interpreted. Certain echinoderm crosses, not necessarily reciprocals, in which the zygote possessed purely maternal larval characters, have received the same explanation. Unfortunately, in some of the most striking cases, the hybrids could not be bred further, owing to infertility, or to the fact that they never reached maturity. *Hydatina* is particularly valuable in this regard, because

<sup>2</sup>Since this paper went to press Professor Goldschmidt has explained certain patrocline *Oenothera* hybrids as due to the degeneration of the maternal chromosomes in the fertilized ovum.

<sup>3</sup>Dr. Pearl states that the inequality of the reciprocal hybrids was diminished in the next generation.

the reciprocals were quite easily reared. The results obtained from *Hydatina* are stated in a preceding section of this paper. When the unequal reciprocals were inbred, and reciprocally crossed, it was found that all traces of the supposed influence of the cytoplasm of the egg had disappeared. The inequality lasted only during the egg stage, and ceased when the eggs gave rise to parthenogenetic lines that could be compared.

In this case it seems necessary to regard the cytoplasm as part of the environment of the zygote. When new cytoplasm had been manufactured under the influence of the combined nuclei of the two lines, this cytoplasm was approximately equal in the two reciprocal lines.

#### SUMMARY

The fertilized eggs of *Hydatina senta*, unlike the parthenogenetic eggs, do not all hatch. The proportion hatching in various lots of eggs has varied from zero to seventy per cent. The capacity for hatching I have called the 'viability' of the eggs. Some of the factors apparently governing the viability of the eggs are discussed in an early part of this paper.

The viability of eggs produced by inbreeding males and females of the same parthenogenetic line is characteristic of the line producing them. One line produced eggs, none of which hatched; another line produced eggs of which 5 per cent were viable; another showed 40 per cent of viability, and so on.

The viability of the eggs is inherited. When a line whose inbred eggs possessed a viability of 5 per cent was reciprocally crossed with another line with 45 per cent of viable eggs, *the reciprocal hybrid lots of eggs were unequal in viability*. Each reciprocal was *more nearly like the inbred eggs of the maternal line*. This result was obtained in two distinct pairs of reciprocal crosses.

The age of fertilized eggs at hatching varies greatly. One egg I have observed hatched in four days after laying; two weeks is a more common age; while many eggs doubtless never hatch. The degree of uniformity of the duration of the egg stage is a characteristic of the line producing the eggs. The inbred eggs of one line practically finished hatching (45 per cent were viable)

within a period of two weeks; in another line, the hatching was spread nearly uniformly over a period of four weeks or more. This variability of the duration of the egg stage may be measured by the standard deviation. In the first of the two lines just mentioned, the standard deviation of the hatching time was low; in the second, the standard deviation was high.

The variability of the duration of the egg stage is inherited. When a line whose inbred eggs showed great variability (high standard deviation) of the hatching time was reciprocally crossed with a line showing small variability (low standard deviation) of the hatching time, *the reciprocal hybrid lots of eggs were intermediate between the parents in variability, but were unequal. Each lot was more nearly like the inbred eggs of its maternal line.*

The same pair of reciprocal hybrids were unequal both in viability and in variability of hatching time. This inequality was not permanent, however; that is, it lasted only during the egg stage. For when a parthenogenetic line was derived from each lot of eggs, and these two 'reciprocal' lines were inbred and reciprocally crossed, *the inequality observed in the former lots of eggs did not appear in the eggs of the reciprocal lines. In the latter lots of eggs, the inbred lots were approximately equal, and the reciprocal hybrids were approximately equal.*

The inequality of the reciprocal hybrids in the egg stage can not be interpreted as due to the influence of the cytoplasm in heredity. The cytoplasm must rather be regarded as part of the environment.

In the inheritance of viability of the eggs of *Hydatina*, segregation occurs in the production of the gametes. For this reason, selection for high and low viability within a hybrid (heterozygous) lot of eggs was plainly effective. But the whole effect was obtained by the first selection, this effect not being increased by subsequent selections. I conclude from the rapidity with which the maximum effect of selection was reached, that the number of segregating units concerned with viability of eggs is small.

After several successive inbreedings from a line that was plainly heterozygous with respect to viability, selection for high and

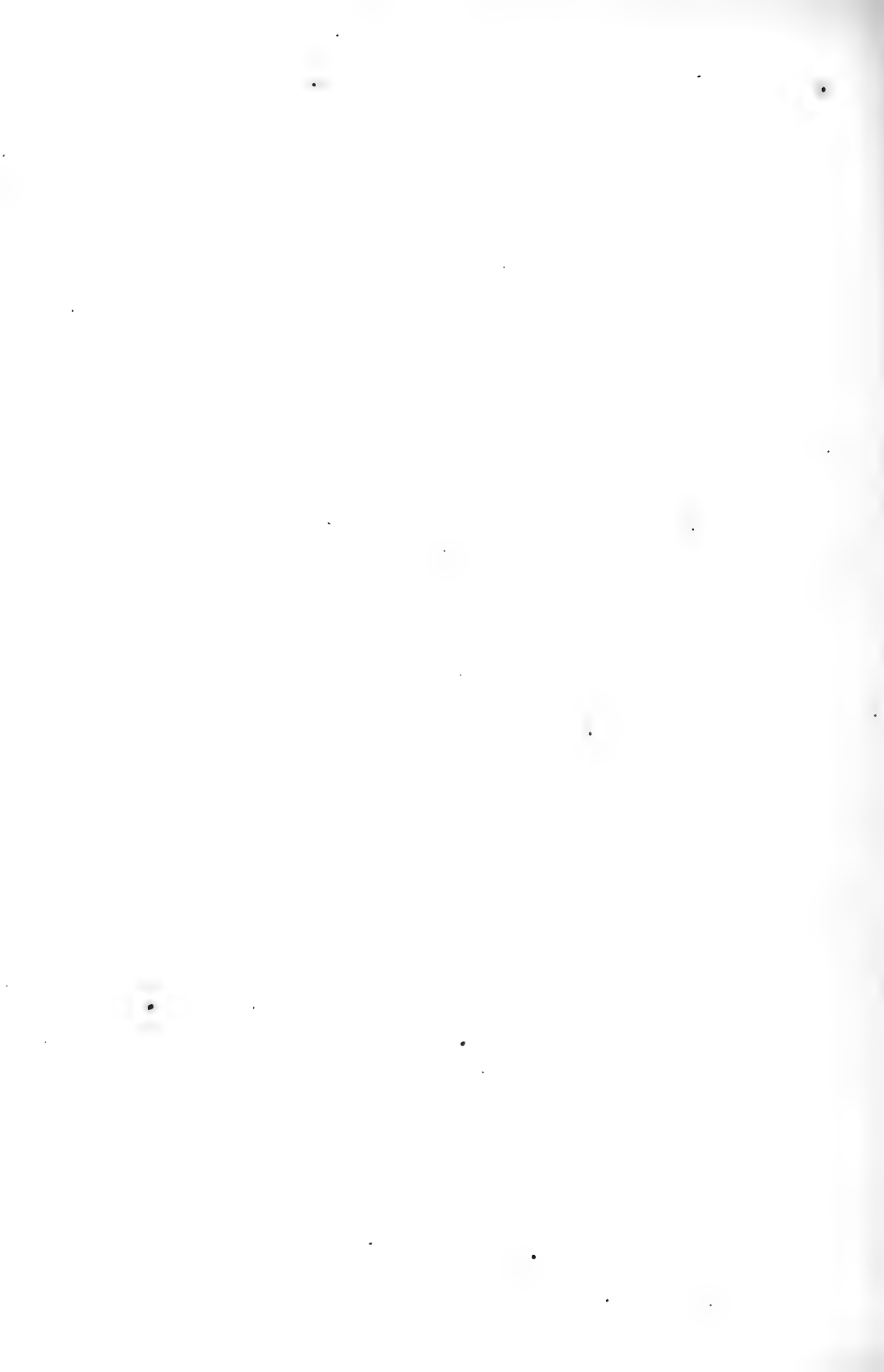


low viability was wholly ineffective. From this I judge that inbreeding (and selection?) had produced a line homozygous with respect to viability.

The sex ratio (proportion of male-producing females) is inherited. In two series of parthenogenetic lines, each line of which was produced by inbreeding from the preceding line, the sex ratio remained nearly constant. In one series the ratio was about 35 per cent of male-producers, in the other series about 20 per cent. The effect of crossing on the sex ratio is not clear, as the labor of rearing several parthenogenetic lines prohibits the acquiring of sufficient data. The experiments described in this paper are in fair accord with the assumption that numerous factors, or genes, unite in producing the sex ratio. A possible method of harmonizing this assumption with contradictory results previously published is discussed above.

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EXPERIMENTALLY INDUCED TRANSITIONS IN THE  
MORPHOLOGICAL CHARACTERS OF ASPLANCHNA  
AMPHORA HUDSON, TOGETHER WITH REMARKS  
ON SEXUAL REPRODUCTION<sup>1</sup>

CLAUDE W. MITCHELL

THREE CHARTS

The species of rotifer, *Asplanchna amphora*,<sup>2</sup> may show, according to the recent observations of Dr. J. H. Powers, the following types or developmental stages: first, from the fertilized or resting egg, there arises a small saccate female, which usually reproduces its own type through several generations; second, from these early saccate females there usually arises a larger humped type, which is the only form generally known to exist in nature as belonging to the species *A. amphora*; third, there arises or may arise from the saccate, or more frequently from the humped form, a still larger type, which has been appropriately described as campanulate.

The question as to the number of generations normally involved in these various stages and of the cause or causes for the transition from one type to another are important, both for the understanding of the species in question and for the bearing they have on the general questions of polymorphism, mutation, and regulation.

Dr. Powers suggested the definite problems of determining the constancy of the saccate type, the number of generations through

<sup>1</sup>Studies from the Zoological Laboratory, The University of Nebraska, No. 108.

<sup>2</sup>In thus naming this rotifer we do not wish for the present to enter into the vexed question of priority or synonymy. We are simply following as closely as possible English usage. We wish, however, at this point, to call attention to, and credit the brief experimental work of Arno von Lange—*Zur Kenntnis von Asplanchna sieboldii* Leydig.—which appeared in the *Zool. Anz.*, Bd. 38, November, 1911. This rotifer is at least very closely allied to *A. amphora*. Von Lange has demonstrated a saccate and a humped type, but not the third or campanulate.

which it passes, and the cause or causes of the transition from this type to the better known, more predominating, humped form. The following experiments have been conducted at the Zoölogical Laboratories of the University of Nebraska, under the supervision of Dr. Powers, to whom I wish to acknowledge my indebtedness and extend my most sincere thanks, for his interest, valuable suggestions and aid.

#### EXPERIMENTAL CONDITIONS

In practically all the earlier experiments *Paramecia* were used as food. In the later experiments, various Protozoa, especially *Oxytricha* and *Euglena*, as well as the rotifers *Brachionus bakeri*, *B. urceolaris*, and *Hydatina senta*, were employed. The *Paramecia* used were taken from a tub in which a general culture of this organism had been maintained for nearly two years. The water in this tub was stale and the odor of decomposition strong. But as it seemed to be a favorable medium for *Asplanchna*, and as the quantity present sufficed for numerous experiments, it was used as the culture medium for the rotifers. Fresh tap water replaced from time to time the amount thus removed from the general *Paramecium* culture, but the quantity added was not large enough to affect appreciably the character of the culture medium.

Isolation cultures were conducted in all pedigree series. But mass cultures were also employed for certain experiments. As containers watch glasses were used of two sizes. At the close of each experiment these glasses were thoroughly cleansed and dried. Different pipettes were used for different purposes, those for food never being employed for transferring individuals. All transfers were made under a hand lens of  $8 \times$ . This gave sufficient magnification to enable the observer to see each individual clearly. As only one individual was transferred at a time it was considered unnecessary to heat either pipettes or dishes before a second use. Tests were nevertheless made at different times to discover whether or not a rotifer could possibly have been carried by accident in the dry dishes; but no such possibility was detected and it is safe to assume that no error in number or pedigree was made by this means during the course of the experiments.

## EXPERIMENTS

*Observations on a mass culture*

In order first to observe the development of the species under normal conditions a mass culture was reared from resting eggs. These eggs were transferred from the pond water in which they had been produced to fresh tap water. Many had hatched by September 28, or in about six days. These were all small saccates and rapidly gave rise to large numbers of the same type. By October 10 the entire culture had changed to the humped form, males had been produced, and resting eggs were present. This culture was conducted for a considerable period and displayed the typical development of the species.

*Observations on the development of the saccate type in isolation culture*

On October 4 resting eggs were again taken from the same supply as the above and placed singly in a series of twenty watch glasses each containing the culture medium. By October 7 five of these had hatched. One was chosen as the parent of a pedigree series termed *A*. Later, still more of the eggs hatched and on the thirteenth, a newly born saccate individual was isolated to become the parent of a pedigree series *D*. In series *A* were bred twenty-six generations, and in *D* one hundred and eight, the purpose being to determine the effect of isolation upon individuals in a genetic series. The results obtained from all of *A* and from fifty generations of *D* are recorded in table 1. Briefly stated they are: first, a marked persistence and uniformity of the saccate type, and, second, an almost total absence of males. The saccate type did not give rise to the humped form, a fact contrary to all observations in general cultures. This was wholly unlooked for. The water in both general and isolation cultures was taken from the same source; the food introduced was identical, and the temperature conditions very similar; yet the two results are markedly at variance.

*Mass cultures of A and D*

The question now arose—were the stocks, thus isolated in *A* and *D*, unable to produce the humped type, or had the conditions in regard to some essential, but as yet unrecognized, factor been unfavorable to its production? To determine this, thirteen individuals were isolated from the pedigree cultures and allowed to reproduce in as many mass cultures. The results are given in table 2.

TABLE 1

*History of pedigree Series A*

Gen. = generation; ind. = individual; sac. = saccate; fol. = following

NO. OF GEN.	DATE OF BIRTH OF FIRST YOUNG 1910	NO. OF YOUNG IN FAMILY	DATE OF PARENT OF FOL. GEN. 1910	NO. OF IND. IN FAMILY SERIES	PER CENT		TYPE
					♂ ♀	♀ ♀	
1	Oct. 7	1					
2	9	5	Oct. 11	2	0	100	sac.
3	14	7	14	1	0	100	sac.
4	15	5	15	1	0	100	sac.
5	17	9	17	2	0	100	sac.
6	23	6	24	4	0	100	sac.
7	26	7	27	4	14	86	sac.
8	30	3	30	2	0	100	sac.
9	Nov. 3	7	Nov. 3	1	0	100	sac.
10	4	4 <sup>1</sup>	7	3	67	33	sac.
11	8	5	10	5	0	100	sac.
12	11	1	11	1	0	100	sac.
13	12	5	12	1	0	100	sac.
14	15	2	15	2	0	100	sac.
15	18	1	18	1	0	100	sac.
16	20	4	20	1	0	100	sac.
17	22	10	22	2	0	100	sac.
18	24	8	25	4	0	100	sac.
19	26	3	26	2	0	100	sac.
20	30	2	Dec. 1	2	0	100	sac.
21	Dec. 4	3	4	1	0	100	sac.
22	6	5	8	2	0	100	sac.
23	10	2	10	1	0	100	sac.
24	12	5	12	1	0	100	sac.
25	14	3	15	3	0	100	sac.
26	18	1	dead				

<sup>1</sup> One individual dead.

TABLE 1—(Continued)  
*History of pedigree Series D*

NO. OF GEN.	DATE OF BIRTH OF FIRST YOUNG 1910-1911	NO. OF YOUNG IN FAMILY	DATE OF PARENT OF FOOL. GEN. 1910-1911	NO. OF IND. IN FAMILY SERIES	PER CENT		TYPE
					♂	♀	
1	Oct. 13, 1910	1					
2	15	11	Oct. 15, 1910	1	0	100	sac.
3	16	14	16	2	0	100	sac.
4	17	10	18	2	0	100	sac.
5	20	10	23	3	0	100	sac.
6	25	6	25	1	0	100	sac.
7	28	8	31	8	0	100	sac.
8	Nov. 1	3	Nov. 2	2	0	100	sac.
9	4	3	4	1	0	100	sac.
10	5	3	5	1	0	100	sac.
11	6	10	6	1	0	100	sac.
12	9	7	9	1	0	100	sac.
13	11	6	11	1	0	100	sac.
14	13	3	13	1	0	100	sac.
15	15	9	15	1	0	100	sac.
16	17	6	17	1	0	100	sac.
17	19	7	19	1	0	100	sac.
18	20	8	20	1	0	100	sac.
19	22	12	22	1	0	100	sac.
20	23	6	23	2	0	100	sac.
21	25	7	25	1	0	100	sac.
22	28	6	28	1	0	100	sac.
23	Dec. 1	5	Dec. 1	1	0	100	sac.
24	4	5	4	1	0	100	sac.
25	6	2	6	1	0	100	sac.
26	8	7	8	1	0	100	sac.
27	10	7	11	4	0	100	sac.
28	14	9	15	4	0	100	sac.
29	17	8	17	1	0	100	sac.
30	21	5	21	1	0	100	sac.
31	23	4	23	1	0	100	sac.
32	25	7	26	4	0	100	sac.
33	28	8	29	2	0	100	sac.
34	31	8	31	2	0	100	sac.
35	Jan. 2, 1911	3	Jan. 3, 1911	2	0	100	sac.
36	5	8	5	1	0	100	sac.
37	9	8	10	6	0	100	sac.
38	12	5	12	2	0	100	sac.
39	14	8	15	4	0	100	sac.
40	17	7	17	2	0	100	sac.
41	19	4	19	1	0	100	sac.

TABLE 1—(Continued)

NO. OF GEN.	DATE OF BIRTH OF FIRST YOUNG 1910-1911	NO. OF YOUNG IN FAMILY	DATE OF PARENT OF FOL. GEN. 1910-1911	NO. OF IND. IN FAMILY SERIES	PERCENT		TYPE
					♂	♀	
42	Jan. 21, 1911	7	Jan. 21, 1911	1	0	100	sac.
43	23	10	23	1	0	100	sac.
44	25	13	25	1	0	100	sac.
45	26	4	26	1	0	100	sac.
46	29	5	30	4	0	100	sac.
47	Feb. 1	10	Feb. 2	3	0	100	sac.
48	4	7	5	4	0	100	sac.
49	8	6	8	1	0	100	sac.
50	11	9	12	2	0	100	sac.

TABLE 2

*Mass culture derived from individuals of Series A and D*

NO. OF INITIAL GEN.	SERIES	TIME OF ISOLATION 1910	TIME OF APPEARANCE OF HUMPED IND. 1910-1911		REMARKS
9	A	Nov. 3	Nov. 3, 1910		Born as humped ind.; produced humped young
9	A		3	3	Produced humped; young males
5	D	Oct. 25	Oct. 28		Parent became cannibal; humped young; males
5	D		27	30	Only one humped young produced
7	D		26	30	Many humped ind.; males; discontinued 11-10
17	D	Nov. 23	Jan. 2, 1911		Saccate and humped type present for four weeks
17	D		23	none	All saccates; discarded 1-12-'11
3	D	Oct. 18		none	All saccate; disc. 11-3
5	D		31	none	All saccate; disc. 11-4
5	D		23	none	All saccate; disc. 11-4
5	D		24	Nov. 4, 1910	Humped type present; males
15	D	Nov. 20		none	All saccate; disc. 12-23
17	D		23	none	All saccate; disc. 1-12



It will be seen that a transition to the humped type did occur in some of these mass cultures. This indicates the possibility that a tendency toward the mutation-like change is inherent in all individuals, although the presence of some unfavorable condition or the absence of some necessary stimulus may in some way suppress it. But the discovery of such conditions or such stimulus is not easy, for the factors that suggest themselves—the motor stimulus due to the mere influence of numbers, sociality, so to speak; the accumulation of metabolic products; or the recurrent rhythm of transformation after the birth of a given number of generations, from the resting egg—these and all other obvious factors were essentially alike in all the cultures, yet the results are at variance.

In the hope of finding a clue to some external or internal factor, especially of the nature of a physiological rhythm, plots were made of the series *A* and *D*. The number of generations was used as the abscissa and the number of offspring in each generation was taken as the ordinate. The resultant curve is shown in chart 1.

This curve does demonstrate a marked rhythm in reproduction. Both series *A* and *D* show cycles which are in the main independent of each other and therefore also independent of external conditions. Both, however, show secondary curves, which seemingly are related and which therefore probably are the result of external conditions. The influence of these external conditions appear definite though they last only for a short time. It is hardly probable that these influences can be responsible for the larger and more pronounced cyclic changes. These must be due rather to internal factors. They are evidently parallel to the metabolic pulses or physiological rhythm which Calkins and others have recorded for certain Protozoa.

Assuming, then, certain periods or generations in which the rotifer is in a higher physiological state than others, it is natural to predict that this rhythm will be one of the factors to cause the transition from the saccate to the humped type. However, these isolation cultures prove conclusively that this factor is not adequate in itself to produce the transition.

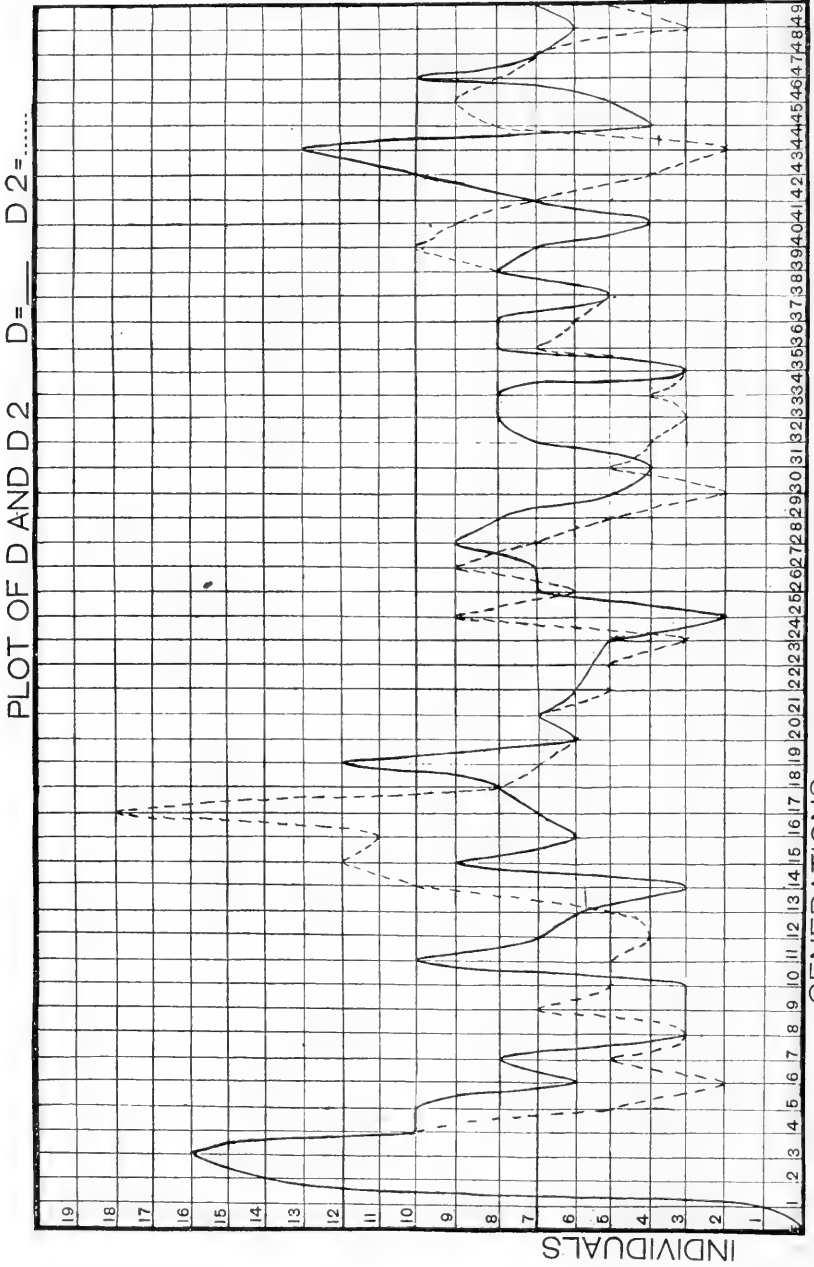


Chart 1

*Results in series bred from different members of a single family*

Early in the experiments the question arose whether, in the selection of individuals as parents for pedigree lines, a physiological difference might exist between the earlier and later individuals produced by a single parent. This seemed the more probable in that such differences have been claimed to be important in the reproduction of certain minor crustacea. To determine whether such differences existed, a number of sister individuals, from the fourth generation of *D*, were allowed to produce series of five generations each. These constituted separate minor pedigree series. They showed no variation in the type of individuals produced. One of these series bred from the ninth sister in a family of ten and termed series *D 2*, was continued further to the seventieth generation under conditions parallel to the general series *A* and *D*; its history is given in table 3. The direct object of this added series was to disclose, if possible, any latent weakness or difference resulting even in the remoter generations of a series derived from the later progeny. This series also furnishes another general control to *A* and *D*. We shall later show that differences do exist between the earlier and later members of a single family. But the results in this series show conclusively that such differences do not manifest themselves under the conditions present throughout these experiments.

*Influence of size of parent on offspring*

The next possibility tested was as to whether the size of the parent in any way influenced the size, form, or any other characteristic of the progeny. From the eighth generation of series *D*, young of different sizes were isolated. When they had developed to maturity four of the smallest and four of the largest were brought together into two parallel mass cultures. In order that all the conditions throughout the experiments should remain identical in the two cultures, partial exchanges of fluid were made between them from time to time. After three weeks under these conditions comparison of individuals from these two cultures showed that the initial size difference had disappeared, the

TABLE 3  
*History of pedigree Series—D2*

NO. OF GEN.	DATE OF BIRTH OF FIRST YOUNG 1910-1911	NO. OF YOUNG	DATE OF PARENT OF FOL. GEN. 1910-1911	NO. OF IND. IN FAMILY SERIES	PERCENT ♂♀	PERCENT ♀♀	TYPE
4	Oct. 18, 1910	10	Oct. 20, 1910	9	0	100	sac.
5	22	5	24	4	0	100	sac.
6	26	2	27	2	0	100	sac.
7	28	5	28	1	0	100	sac.
8	31	3	31	3	0	100	sac.
9	Nov. 2	7	Nov. 2	3	0	100	sac.
10	4	5	4	1	0	100	sac.
11	7	5	7	1	0	100	sac.
12	9	4	10	2	0	100	sac.
13	12	5	12	1	0	100	sac.
14	16	10	16	1	0	100	sac.
15	17	12	17	1	0	100	sac.
16	19	11	19	1	0	100	sac.
17	20	18	20	2	0	100	sac.
18	22	8	22	1	0	100	sac.
19	24	7	24	1	0	100	sac.
20	26	6	26	1	0	100	sac.
21	28	7	28	1	0	100	sac.
22	Dec. 1	5	Dec. 1	1	0	100	sac.
23	3	5	3	1	0	100	sac.
24	5	3	5	1	0	100	sac.
25	8	9	9	3	0	100	sac.
26	11	6	12	4	0	100	sac.
27	15	9	15	1	0	100	sac.
28	16	7	18	5	0	100	sac.
29	20	5	22	2	0	100	sac.
30	24	2	24	1	0	100	sac.
31	26	5	26	1	0	100	sac.
32	29	4	29	1	0	100	sac.
33	31	3	31	2	0	100	sac.
34	Jan. 4, 1911	4	Jan. 4, 1911	1	0	100	sac.
35	8	3	8	2	0	100	sac.
36	10	4	10	2	0	100	sac.
37	12	6	12	1	0	100	sac.
38	14	5	14	1	0	100	sac.
39	17	8	17	1	0	100	sac.
40	19	10	19	1	0	100	sac.
41	21	9	21	1	0	100	sac.
42	23	5	23	1	0	100	sac.
43	25	4	25	1	0	100	sac.
44	27	2	27	2	0	100	sac.
45	29	8	30	2	0	100	sac.
46	Feb. 1	9	Feb. 2	2	0	100	sac.
47	4	8	5	2	0	100	sac.

two cultures had become approximately the same. Again no transition to the humped type had occurred. These facts point to the conclusion that individual variations in parental size have little direct influence upon the size and character of the offspring.

These internal factors having proved themselves inadequate to produce the mutation-like change, a number of experiments were conducted in which external agents—temperature, food, and cultural media—were variously modified, in the hope that among these the requisite factor or factors would be found. The following experiments may be instanced.

#### *Influence of lowered temperature*

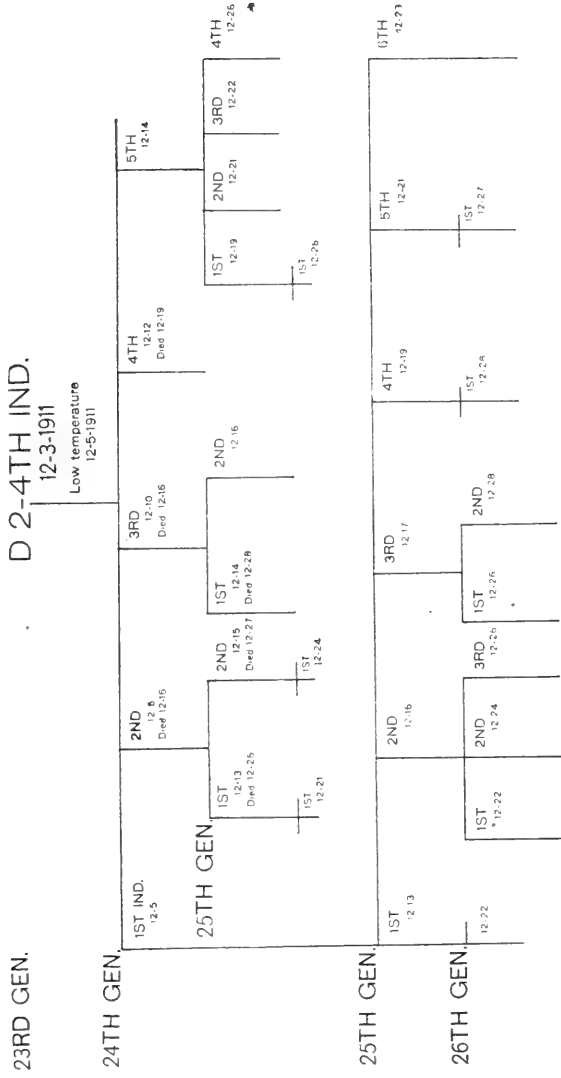
An individual, the fourth member of the twenty-third generation of *D 2*, was transferred on December 5, after two days of life at room temperature (20°C.), into a temperature varying between 8 and 12°C. Two well developed young were born directly after the change, but no more were born until after five days. This as well as the slow rate of development through a period of about a month during which but four generations were produced may undoubtedly be ascribed to the lowered temperature. Not only the rate of reproduction but the activity of the rotifers was greatly decreased. The history of this series is recorded in chart 2.

On January 1, a failure of the heating plant resulted in the complete freezing of these cultures which were consequently discontinued. Freezing, however, does not necessarily kill *A. amphora*, for some which were frozen in solid ice were slowly thawed and continued to live. Moreover, the experiment demonstrated that a reduction in temperature does not cause the mutation-like change.

#### *Influence of a fluctuating temperature*

Still further to test the influence of temperature change, an individual, sister to the one used in the preceding experiment, was allowed to reproduce under repeated fluctuations of temperature. A room temperature of 20°C. was followed by cold (5°) which was then raised again to 20°, and finally maintained at 27

LOW TEMPERATURE EXPERIMENT



Frozen 1-1-1912

Chart 2

to 28°C. The individual was allowed to remain about seven hours under each of these temperatures in turn. The result was a retarded reproduction similar to that at first shown in the preceding experiment. But again there was no transition. Examination of table 4 will show that a considerable continuation of this line of experiment upon the progeny of this individual was equally negative in result.

*Quantitative control of food*

A necessarily important external factor is food. In order that the general effect of high and low nutrition might be studied, a number of experiments were conducted in which the quantity of food supplied to each individual was controlled. Individuals

TABLE 4  
*Influence of a fluctuating temperature upon single series*

NO. OF GEN. IN SERIES D	NO. OF GEN. IN EXPER.	VARIATION IN TEMP. DEGREES C.	CONSTANT TEMP., IN HOURS	DATE OF BIRTH OF FIRST YOUNG 1911	NO. OF YOUNG IN FAMILY SERIES	TYPE
24	1	20	8	Dec. 6	7	sac.
		5	10			
		20	8			
		27	20+			
25	2	20	10	9	9	sac.
		5	15			
		20	5			
		27	25+			
26	3	19	15	12	6	sac.
		5	18			
		19	10			
		28	15+			
27	4	20	20	15	12	sac.
		5	25			
		15	15			
		28	20+			
28	5	20	10	19	8	sac.
		4	20			
		17	10			
		26	20+			
29	6	18	12	22	5	47
		4	10			
		20	8			
		27	16+			
				Discarded	Total ind.	
				26	53	

were placed in cultures containing a moderate supply of Paramecia. They ingested these organisms until their stomachs became noticeably distended. This was followed by a slight increase in the size of the individuals over those in less favored cultures and also by an increase in the number of simultaneously developing embryos, as many as eight or ten being sometimes counted. But these young were not as large, when born, as were those born of parents that contained but one or two. This latter fact is doubtless a mere consequence of the larger number produced at one time; the smaller young were, however, healthy and developed into normal saccate individuals, showing no tendency toward transition.

#### *Excessive food*

To a number of cultures Paramecia were added until the medium became an almost milk-colored broth. The rotifers, however, did not thrive under these conditions; their all but empty digestive tracts indicated that little food was eaten, and they assumed a vertical position in the fluid, with their coronas open wide against the surface. Even when returned to normal medium this new reaction was repeatedly manifested for a period of four to five hours. This experiment, therefore, did not accomplish its aim; the results indicated a general decrease in metabolism.

#### *Effects of low nutrition and starvation*

An individual was isolated in the normal amount of culture medium which had, however, been filtered. To this was added the smallest number of Paramecia which experiment had indicated would serve for the minimum food supply capable of maintaining life and at the same time some rate of reproduction. Such young as were born were immediately isolated in like culture media. Under these conditions the total number of young decreased in each generation, until in the fifth but one was produced. The same conditions, continued to the ninth generation, showed similar low rates of reproduction. Upon resuming a normal food supply in the ninth generation (table 5) a normal



rate of reproduction was resumed. But the results of low nutrition and of mere quantitative food change were again negative so far as producing the humped type was concerned.

TABLE 5  
*Starvation of race*

NO. OF GEN. IN GENERAL SERIES D	NO. OF GEN. IN PRESENT EXPER.	DATE OF BIRTH OF FIRST YOUNG	NO. OF YOUNG IN FAMILY	DATE OF PARENT FOR FOL. GEN. 1910	NO. OF IND. IN FAMILY SERIES	TYPE	REMARKS
27	1	Dec. 10, 1910		Dec. 10	1	sac.	
28	2	11	5	11	1	sac.	
29	3	14	3	14	1	sac.	
30	4	16	4	17	3	sac.	
31	5	20	1	20	1	sac.	starved
32	6	22	4	22	1	sac.	
33	7	24	3	26	2	sac.	
34	8	28	4	28	1	sac.	
35	9	30	4	31	3	sac.	
36	10	Jan. 1, 1911	8	killed			

*Effect of pure starvation and subsequent feeding*

To test the effect of temporary but entire starvation, about one-hundred-and-fifty individuals, taken shortly after birth, were isolated in the customary medium to which no food was added. After about ten hours they were fed. The result was a retarded reproduction, although after food was again given, the number of young born approximated the normal. Again no humped individuals were produced, the saccate type remaining constant.

*Reproductive capacity in sisters*

Although an earlier experiment (tables 1 and 3) indicated that, under normal conditions, the progeny of earlier and later sisters and even the series bred from them, were essentially similar, yet, in the above experiment on total starvation during the growth period, certain observations suggested a different method by which possible differences could be demonstrated between such sisters. It was noted that in some young individ-

uals under starvation, embryos were developed and might even be born before feeding had begun. Such development and birth must obviously depend upon the stored nutritive supply which the young mother had derived from its parent. Thus the degree of such development or the number of young produced under conditions of low nutrition offers a practical measure of the initial vitality or original nutritive supply, and this measure may be applied to the successive individuals produced by any one mother. To test this, twelve individuals were placed in cultures in which food was scarce although sufficient to maintain life. The young born were then isolated in similar cultures of low nutritive content. One individual, usually an early member of the family, was put, as a control, into a culture well supplied with food. In some cases where the food supply was very scarce actual starvation occurred. But the general results as shown in table 6 and chart 3 indicate that in the starved families, the central members of a family receive more nutrition than do either earlier or later members. It is true that under such conditions of low nutrition which were deemed on the whole the most favorable for this experiment, chance variations in feeding cannot wholly be pre-

TABLE 6

*Reproduction of sisters. Individuals marked ( ) heavily fed; all others starved or on minimum nutrition*

NO. OF GEN. IN SERIES D FROM WHICH IND. IS DERIVED	NUMBER OF PROGENY BORN TO SISTERS (1ST, 2ND, ETC.) COMPRISING FAMILY								TOTAL NUMBER IN FAMILY
	1st	2nd	3rd	4th	5th	6th	7th	8th	
10	(8)	3	5	6	8	9	6	7	8
21	(6)	4	7	9	5	0	0		7
27	5	(11)	6	9	3	8	3		7
37	(5)	3	4	6	2	2			6
22	5	(9)	6	4	6	3			6
38	8	8	(9)	7	2				5
31	(4)	4	6	4	1				5
34	(4)	3	4	2					4
32	(4)	3	3	2					4
33	(4)	1	4	1					4
36	0	0	(8)	1					4
34	(2)	1	1						3

NUTRITION RECEIVED FROM PARENT

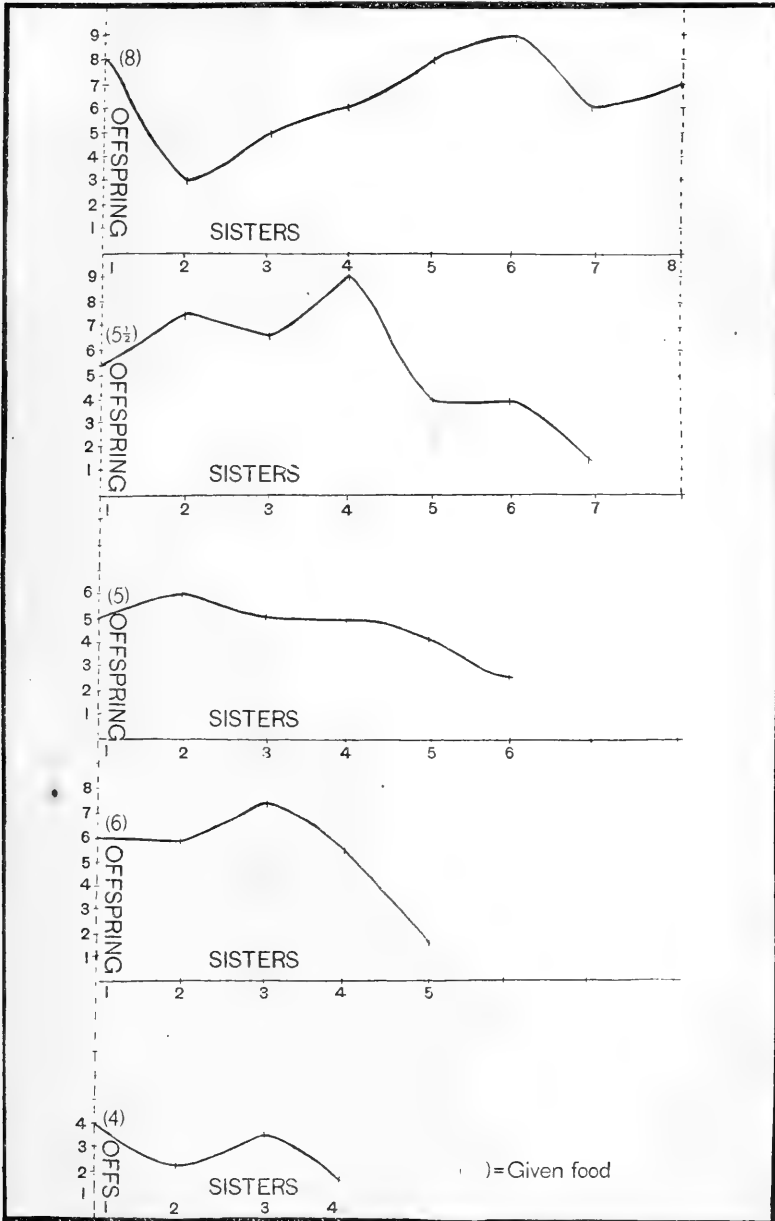


Chart 3

vented and in accord with this the results show some irregularity. Additional evidence, however, has been accumulated in the course of the pedigree series. In many instances in these series other than the first individuals have necessarily been used because of the phenomenon above noted, that the first members of ill-fed families seemed peculiarly lacking in vitality. These enforced shiftings of the sisters used for the pedigree families are responsible for some of the slight irregularities in result shown in table 1 and chart 1.

#### *New cultures*

After the cultivation of numerous lines of the saccate *Asplanchna* for three-and-a-half months, and, obtaining, in some instances, nearly forty generations without a single transition to the humped form, it was thought that possibly the strain of rotifer used was somehow unsatisfactory, due, perhaps, to initial weakness or to something artificial in the treatment of the initial stock of resting eggs. These eggs had rested for but a very brief period before hatching and had also been neither frozen nor dried, processes which are probably typical in nature. It was deemed best therefore, to start new lines from eggs freshly collected in January at the borders of ponds where it was known the species had flourished and where it was known that at least a portion of these eggs had been subjected to both freezing and drouth. No difficulty was experienced in hatching young *Asplanchna* from such material; many had emerged within five days after adding tap water. The precaution was taken, however, of hatching single individuals only from small portions of the débris in order to assure the starting of series from the first generation. Twenty-four such individuals were isolated, twenty-two of which were allowed to reproduce in as many mass cultures, while the other two were made the parents of series *E* and *F*. The histories of the twenty-two mass cultures are given in table 7.

Reference to the table will show that the results are conflicting; in ten cultures humped individuals were developed, while in twelve there were saccates only. The conflicting results were without explanation, although it was recorded in every case

where the humped type developed that the rotifers were larger and more active than in the other cultures. They were also good feeders and their food was usually all eaten at each feeding time; while in the other cultures an excess remained. However, quantitative changes in the food supply did not modify the results.

From the other two individuals *E* and *F*, were developed, as before stated, pedigree lines. From *E* were bred twenty-two generations, while from *F* thirty-eight were bred. Their histories are given in tables 8 and 9 respectively.

TABLE 7

*History of mass cultures*

NO.	DATE OF ISOLATION	DATE OF FIRST BIRTH OF HUMPED TYPE	DATE OF FIRST MALES	DATE OF RETURN TO SACCATE	REMARKS
	1911	1911	1911	1911	
1	Jan. 26				Died
2					All saccate
3		Jan. 27	Feb. 25	Mar. 4	Continuous humped type
4					All saccate
5					All saccate
6		30	4		Discarded as humped
7		Feb. 4		Feb. 9	No males produced; weak
8					All saccate
9		4	9	16	
10		Jan. 29	10	18	
11		Feb. 7		9	Only two humped individuals found
12	27				All saccate
13		4	6	15	
14		10	18	25	
15					All saccate
16		12		21	No males formed
17					All saccate
18					All saccate
19		10	23	Mar. 10	
20					All saccate
21					All saccate
22					All saccate

In the early generations of *E*, the saccate type was constant and will be discussed later on. In the second generation of *F*, however, a transition occurred which was followed by three generations of the humped form. But this soon reverted to the saccate type. This change had occurred in the earlier generations of a series and it seemed well to start other series to ascertain whether the causal factor we were seeking rested in the first generation as such. Hence the following experiment.

TABLE 8

*History of Series E*

S = saccate type; H = humped type; I = intermediate stage

NO. OF GEN.	DATE OF BIRTH OF FIRST YOUNG 1911	NO. OF YOUNG IN FAMILY	DATE OF PARENT OF FOL. GEN. 1911	NO. OF IND. IN FAMILY SERIES	TYPE	PER CENT ♂ ♀	PER CENT ♀ ♀
1	Jan. 28				sac.	0	100
2	31	4	Jan. 31	1	sac.	0	100
3	Feb. 4	3	Feb. 4	1	sac.	0	100
4	6	3	8	2	sac.	0	100
5	9	8	10	3	sac.	0	100
6	13	3	13	2	sac.	0	100
7	16	21	16	2	sac.	0	100
8	20	4	21	4	sac.	0	100
9	23	6	23	1	sac.	0	100
10	25	7	25	2	sac.	0	100
11	28	1	28	1	sac.	0	100
12	Mar. 2	9	Mar. 2	1	sac.	0	100
13	4	8	4	1	sac.	0	100
14	6	7	6	1	{ 1, 2, 3, 4 = S 5, 6, 7 = H }	0	100
15	8	5	8	1	{ 1 = S; 2, 3 = I 4, 5 = IH }	20	80
16	9	6	9	1	humped	0	100
17	11	6	11	1	humped	0	100
18	13	3	13	1	humped	0	100
19	16	13 <sup>1</sup>	16	1	humped - I	50	50
20	18	8	18	1	I - S	0	100
21	21	5	21	1	sac.	0	100
22	23	9	discarded		sac.	0	100

<sup>1</sup> One individual died.

TABLE 9

*History of Series F*

S = saccate type; H = humped type; I = intermediate stage

NO. OF GEN.	DATE OF BIRTH OF FIRST YOUNG 1911	NO. OF YOUNG IN FAMILY	DATE OF PARENT OF FOL. GEN. 1911	NO. OF IND. IN FAMILY SERIES	PER CENT		TYPE
					♂ ♀	♀ ♀	
1	{ Egg 26 Jan. 27	1			0	100	S
2	28	1	Jan. 28	1	0	100	H
3	30	10 <sup>1</sup>	30	1	11	89	H
4	Feb. 1	2	Feb. 1	1	0	100	H
5	3	8	3	1	0	100	S
6	4	6	4	1	0	100	S
7	5	8	5	1	0	100	S
8	6	9	6	1	0	100	S
9	7	11	7	1	0	100	S
10	8	9	8	1	0	100	S
11	10	5	10	1	0	100	S
12	12	10	12	1	0	100	S
13	14	7	14	1	0	100	S
14	15	12	15	1	0	100	S
15	20	8	20	1	0	100	SIHS
16	22	7	22	1	0	100	IH
17	24	17 <sup>1</sup>	24	1	19	81	H
18	26	12 <sup>2</sup>	26	1	10	90	H
19	27	6	27	1	16	84	H
20	Mar. 1	8	Mar. 1	1	0	100	H
21	2	10	2	1	0	100	HI
22	4	6	4	1	16	84	S
23	5	9	5	1	11	89	S
24	7	14	7	1	50	50	IH
25	8	13	8	1	8	92	I
26	9	15	9	1	26	74	IH
27	10	11	10	2	0	100	S
28	12	8 <sup>2</sup>	12	2	50	50	SIH
29	16	8	16	2	12	88	H
30	18	8 <sup>1</sup>	18	1	59	41	H
31	20	6	20	2	0	100	HS
32	22	7	23	2	0	100	S
33	25	8	25	1	25	75	SHIS
34	27	11	27	1	0	100	IS
35	29	8	29	1	0	100	SI
36	30	5	30	1	0	100	S
37	Apr. 2	4	Apr. 2	2	0	100	S
38	6	4 <sup>2</sup>			100	0	SH

<sup>1</sup> One individual died.<sup>2</sup> Two individuals died.

*New races from D*

In a mass culture that had developed from the fourth individual of the forty-second generation of *D*, mutation had occurred, males had been produced, followed by resting eggs. One hatched on the sixteenth, and from it pedigree series *G* was bred, consisting of twenty generations; its history is found in table 10.

Here again as in *F*, the mutation occurred in the second generation. This repetition, together with mass culture results, would seem to indicate a tendency to mutate in the early generations.

TABLE 10  
*History of Series G*  
Interm. = intermediate

NO. OF GEN.	DATE OF BIRTH OF FIRST YOUNG 1911	NO. OF YOUNG IN FAMILY	DATE OF PARENT FOP FOL. GEN. 1911	NO. OF IND. IN FAMILY SERIES	TYPE	PERCENT	
						♂ ♀	♀ ♀
1	Feb. 16				sac.	0	100
2	18	7	Feb. 20	3	sac. and hump.	14.3	85.7
3	24	4	24	1	{ hump. sac.		
4	26	5	26	1	interm.	0	100
5	Mar. 1	2	Mar. 1	1	interm.	0	100
6	4	4	4	2	sac.	0	100
7	6	4	6	1	sac.	0	100
8	8	8	8	1	hump.	0	100
9	9	1			hump.	0	100
10	11	9 <sup>1</sup>	11	1	hump.	25	75
11	13	4	13	2	hump.	0	100
12	16	7	17	3	hump.-sac.	0	100
13	18	4	19	2	{ hump. interm.		
14	21	4	21	1	sac.	0	100
15	23	9	23	1	sac.	11	89
16	25	9	26	2	sac.	0	100
17	28	6	28	1	sac.	0	100
18	30	7	30	1	sac.	0	100
19	Apr. 1	4	Apr. 1	1	sac.	0	100
20	4	2	4	1	sac.	0	100
21			mass		H - males	Resting eggs	

<sup>1</sup> One individual died.



This agrees with many observations made upon these rotifers by Dr. Powers. The period before the transition is variable in length. As to the cause of the mutation at this stage, it will be discussed further in another portion of this paper.

*Influence of qualitative food change*

Owing to a temporary decline in our *Paramecium* food culture an effort was made, in the case of the pedigree series *F*, to substitute *Oxytricha*, which were at hand in large numbers. At first the rotifers ignored this new food, their digestive tracts remaining entirely empty. No young were produced in the generation thus fed for a period of three days and it was feared that the race would be lost. But on the fourth day, that is, on February 19, they suddenly began to gorge themselves with the new food, their stomachs expanding until they resembled white balls. On February 20 a saccate individual was born in the direct line, followed by individuals transitional towards the humped type. The young were likewise fed on *Oxytricha* and they, in turn, produced young of the full humped type. The size of each individual as well as the total number of progeny produced were both greatly increased.

At this time the supply of *Oxytricha* began to run low and after ten days *Paramecia* were again resorted to for food. The humped individuals bred true for a number of generations; but their size, as well as the number of young, gradually decreased until on March 2, or seven days after the supply of *Oxytricha* ran out, the saccate type reappeared. This experiment indicated the possibility of qualitative changes in nutrition as a factor determining the sudden transition in type which we were seeking to explain.

*Influence of Euglena upon Series F and G*

On March 6 a supply of *Euglena viridis* was obtained and it was determined to try this flagellate as food, in order to determine whether mutation could be again induced by a qualitative change in diet. As in the *Oxytricha* experiments, one to two drops of this culture, green with the flagellates, was added to the culture

medium of *F*. This series, then in the twenty-third generation, had assumed the humped type during the *Oxytricha* experiments (generations fifteen to twenty-one inclusive), but had returned to the saccate type two generations previous to the *Euglena* experiment. The influence of the new diet was immediate; for in the first generation of young the mutation had occurred. That such an abrupt transition should have occurred is indeed remarkable. The size of the adult offspring became double that of the parent, while the number of young produced, as shown by table 9, was also nearly doubled. In like manner the new food was supplied to series *G*, now saccate and in its seventh generation, but which had been humped in its second and third generations. In this series also the transition followed in the next generation after the food change.

#### *Influence of Euglena upon E*

In strong contrast to series *F* and *G*, series *E* had proved a weak race with slow and sparing reproduction (fourteen generations of *E* to twenty-three of *F*) and had developed only saccate individuals. *Euglena* was therefore given to this race to determine whether it also had the ability to develop the humped type. That the *Euglena* were injected was proven by examination of stomach contents. The transition did indeed occur, and a glance at table 8 will reveal how marked it was; but it will also be observed that there was no corresponding increase in the number of offspring. Nevertheless, the experiment proves that the ability to mutate is inherent even in individuals of weak races, though dependent for its manifestation upon favorable diet or, more probably, favorable food change.

#### *Influence of Euglena on the old Series D and D 2*

Changed diet having thus shown a powerful influence over the preceding series, it was next applied to the old series, *D* and *D 2*, which had shown at this time an unbroken succession of fifty-seven and fifty-nine generations respectively of the pure saccate type. Here no less than in the previous briefer series the transition was complete and immediate (tables 11 and 12).

TABLE 11

*Continued history of Series D1*

S = saccate type; H = humped type; I = intermediate stage

NO. OF GEN.	DATE OF BIRTH OF FIRST YOUNG 1911	NO. OF YOUNG IN FAMILY	DATE OF PARENT OF FOL. GEN. 1911	NO. OF IND. IN FAMILY SERIES	PER CENT ♂ ♀	PER CENT ♀ ♀	TYPE
50	Feb. 11	9	Feb. 12	2	0	100	sac.
51	14	5	16	3	0	100	sac.
52	20	4	23	4	0	100	sac.
53	25	2	26	2	0	100	sac.
54	28	5	28	1	0	100	sac.
55	Mar. 2	6	Mar. 2	1	0	100	sac.
56	3	6	3	1	0	100	sac.
57	6	4	6	1	0	100	sac.
58	7	7	7	1	0	100	I
59	9	16 <sup>2</sup>	9	2	28	72	SIH
60	11	6	11	1	16	84	H
61	14	4	14	1	25	75	H
62	17	7	17	1	14	86	H
63	20	17	20	1	35	65	H
64	22	6	22	1	0	100	H
65	24	12	24	1	25	75	H
66	25	16	25	1	0	100	HIS
67	26	16	26	1	14	86	IHI
68	29	2	29	1	0	100	S
69	31	8	31	1	0	100	S
70	Apr. 1	4	Apr. 1	1	0	100	S
71	6	11	6	1	0	100	SIH
72	8	9	9	2	44	56	SI
73	11	17 <sup>2</sup>	11	3	26	74	H
74	13	16	13	1	21	79	H
75	14	16 <sup>1</sup>	14	1	40	60	H
76	16	15	16	1	33	67	H
77	17	8	17	1	12.5	87.5	H
78	19	6	19	1	33	67	H
79	21	13 <sup>1</sup>	21	1	50	50	H
80	22	8 <sup>1</sup>	22	1	14	86	H
81	24	6 <sup>1</sup>	24	1	20	80	H
82	26	8 <sup>1</sup>	26	1	0	100	H
83	28	7 <sup>1</sup>	28	1	0	100	H
84	30	6	30	1	0	100	HI
85	May 2	10	May 2	1	40	60	HI
86	4	2	5	2	0	100	HI
87	6	5	6	1	0	100	HI

<sup>1</sup> Two individuals died.<sup>2</sup> One individual died.

TABLE 11—(Continued)

NO. OF GEN.	DATE OF BIRTH OF FIRST YOUNG 1911	NO. OF YOUNG IN FAMILY	DATE OF PARENT OF FOL. GEN. 1911	NO. OF IND. IN FAMILY SERIES	PER CENT ♂ ♀	PER CENT ♀ ♀	TYPE
88	May 8	4	May 8	1	0	100	HI
89	9	5	9	1	20	80	HI
90	10	6	10	1	0	100	HI
91	11	8 <sup>1</sup>	12	2	20	80	HIS
92	13	5	14	4	0	100	HI
93	17	3	17	1	0	100	H
94	18	8	18	1	12	88	H
95	19	5 <sup>2</sup>	19	1	0	100	H
96	20	2	20	2	0	100	HI
97	22	2	22	2	0	100	S
98	24	4	24	1	0	100	H
99	25	7	25	1	0	100	H
100	26	2			0	100	H

<sup>1</sup> Three individuals died.<sup>2</sup> Two individuals died.

TABLE 12

*Continued history of Series D2*

NO. OF GEN.	DATE OF BIRTH OF FIRST YOUNG 1911	NO. OF YOUNG IN FAMILY	DATE OF PARENT OF FOL. GEN. 1911	NO. OF IND. IN FAMILY SERIES	PER CENT ♂ ♀	PER CENT ♀ ♀	TYPE
47	Feb. 4	8	Feb. 5	2	0	100	sac.
48	7	7	7	3	0	100	sac.
49	11	3	11	1	0	100	sac.
50	13	5	15	5	0	100	sac.
51	?	?	?	?	0	100	sac.
52	?	?	?	?	0	100	sac.
53	25	?	25	?	0	100	sac.
54	26	3	26	1	0	100	sac.
55	28	6	28	1	0	100	sac.
56	Mar. 2	12	Mar. 2	1	0	100	sac.
57	4	9	4	1	0	100	sac.
58	6	7	6	1	28	72	SIH
59	8	6	8	1	33	67	I
60	10	8	10	1	37	63	H
61	12	5	12	1	40	60	H
62	17	13	17	1	0	100	HI
63	20	3	20	1	0	100	H
64	22	6	22	1	0	100	S
65	24	8	24	1	0	100	S
66	26	6	26	1	0	100	IH
67	28	3	28	1	0	100	HS
68	30	9	30	2	0	100	SI
69	Apr. 1	8	Apr. 1	1	0	100	S
70	3	4	died				S

In each of the above instances, when the *Euglena* was first given to a single individual rotifer, sister individuals were invariably kept as controls and fed on the customary *Paramecium* diet. In no case did mutation occur among the controls. Each series continued to reproduce the saccate form only.

The above cases of immediate mutation following upon changed nutrition, even after long periods of pure saccate generations, and also in generations varyingly removed from the resting egg, offer excellent evidence that mutation depends chiefly, if not wholly, upon food conditions, rather than upon internal cyclic changes or any of the other factors which we had previously investigated. Especially noteworthy is the fact that the addition of but a single drop of the new food culture was capable of producing the full transition between these markedly dissimilar types of the species.

*Possible influence of matter dissolved in the water of the  
Euglena culture*

To determine whether the above mutations were due wholly to a change in food or whether they might be influenced as well by substances dissolved in the water of the *Euglena* culture, some of this medium was passed through a common filter paper, which separated out the *Euglena* and particles of foreign matter. The resulting filtrate was examined and found to contain no Protozoa. Drops of this were added to a series of individual cultures; but in no case was a mutation produced. Additional quantities were again added with the same negative result. Hence it appears safe to say that the factors which produce the mutations are really in the ingested food and are not chemical substances dissolved in the culture medium.

*Interchange of culture medium*

In order, however, to test still more conclusively the possible influence of culture medium and its dissolved contents, the fluid from ten mass cultures containing only saccate individuals and from ten cultures containing the humped type was filtered in each case and interchanged, culture for culture. In one instance only

was this exchange of fluid followed by mutation of the saccate to the humped type, and in this case the transition did not occur until after five days. This instance at least is thus not comparable to those transitions caused by qualitative food change, for these latter almost always follow within one to two days after the new stimulus. It is more probably to be compared to the occasional mutation incidental in mass cultures throughout the course of experimentation. These instances will be further discussed as a whole later in the paper.

#### *Return to Paramecium feeding*

After about two weeks of feeding with *Euglena* the supply of this organism had so decreased that its use had to be discontinued in all cases except the pedigree series *D*. Lines *D 2*, *E*, *F* and *G*, were returned to the *Paramecium* diet. Within one to four generations all had returned to the saccate type. In some cases transitional individuals had intervened; in others the change was immediate (tables 8, 9, 10, and 11). But all continued to produce only saccates for several generations of *Paramecium* feeding, or until they were discontinued. In line *D*, in which *Euglena* feeding was continued, the humped type was reproduced without exception during this period. Nevertheless, after another ten days, the supply of *Euglena* quite gave out. At this period, however, an effort was made to mingle other and varied Protozoa with the regular diet of *Paramecium* in the hope that this might prevent the backward mutation to the saccate type. This was successful only in part, for although in the linear series the species remained constant at least as far as the ninety-sixth generation, yet many of the sisters not included in the pedigree lines produced the saccate type. These latter were usually the later born individuals of the older parents. Thus for twenty-five generations following the mutation which occurred as the result of *Euglena* feeding Series *D* continued in the humped form. A single temporary return to the saccate type occurred in the ninety-seventh generation but this, as table 11 shows, again reverted to the humped type in the following generation. Table 11

gives the history of *D* to its one-hundredth generation, at which point the records were discontinued though the series was continued for a number of generations before discarding.

*Observations on mutations occurring in mass cultures fed on Moina*

In a large mass culture, into which all discarded *A. amphora* were thrown, the crustacean *Moina paradoxa* developed. Some of the *Asplanchna* succeeded in ingesting the young *Moina* despite their large size. This diet was followed by a mutation to the humped type. Although it was not demonstrated by actual observation that *Moina* feeding caused the transition in the culture, yet a few minor experiments not mentioned elsewhere in this paper, in which this type of food was employed proved conclusively that this food change is also capable of producing the transition. There resulted an interesting selection: a few of the smaller humped rotifers and many of the saccates were unable to capture and ingest the crustacean and as the amount of food decreased, died. The average size of the individual of the culture was consequently increased. A period of male production followed, with resting eggs. This again lessened the number of individuals in the culture, and as the *Moina* also decreased in number it finally died out.

*Observation on mutations occurring in mass cultures where Hydatina and Brachionus were present*

To a number of ordinary mass cultures, fed as usual on *Paramecium*, the rotifers, *Hydatina senta*, *Brachionus bakeri*, and *B. urceolaris* were introduced. The *Asplanchna*, after a time only, began to eat these smaller rotifers and, as in the former experiments with *Euglena*, the customary mutation occurred, and males were produced followed by large numbers of resting eggs. These two smaller rotifers furnished a more regular and lasting food supply than did *Moina* and the cultures were continued for some time, the humped type remaining constant throughout, with the exception of a few individuals of the third type of the species.

*Production of the third or campanulate type of Asplanchna amphora*

Although not previously mentioned except in the case of the last culture, we need to add that the third or campanulate type of the *A. amphora* appeared in a number of the mass cultures. Examination of their stomach contents showed them to be cannibals. They reproduced either their own type, males, or the humped type, using young of their own species as food. Pedigree experiments were begun with this type; however, the delicacy of the feeding technique and the amount of time required caused us to abandon them after five generations.

*Male production*

In view of the unsettled question of sex determination and of the light which our experiments might throw upon it, careful records were kept in all pedigree lines and mass cultures of the male production, but a full discussion of the data thus obtained is left for another paper in the course of preparation. Nevertheless we may well add the interesting facts that males are rarely produced by the saccate form of the species, throughout however many generations it is cultivated. On the other hand, they are frequently produced by both the humped and campanulate types. There is an undoubted difference in physiological level which is registered by the transition from one of these forms to another and this furnishes a new and valuable instrument for attacking the problem of sex determination, work upon which is now under way.

## DISCUSSION OF GENERAL RESULTS

One general and striking result in the foregoing experiments is the relative stability under many conditions of the smaller saccate *A. amphora*. Instead of being a transient developmental form all but inevitably giving place to the humped rotifer within a few generations, the experiments point strongly toward the conclusion that this form may propagate itself indefinitely without marked morphological change. The greater stability of this form adds to the interest of the phenomena as not unrelated to



the process of species-making. It also tends decidedly to justify the application of the concept of mutation to the sudden changes which have been here described.

It needs hardly to be pointed out after the above experiments, that a change in food organism or a qualitative nutritional difference constitutes the major factor which is instrumental in producing the sudden changes. All other factors turn out negative, or at least negligible in results, as compared to food change.

The most important factor is plainly a qualitative change in diet; yet it seems probable that a quantitative change, a mere alternation of hunger with high feeding, is an effective though less potent factor. Thus it was observed that in mass cultures, fed on *Paramecium* in which the mutation took place, the food supplied was quickly eaten, the digestive tracts of the rotifers again becoming empty before the next meal. A similar condition occurs as a matter of course in feeding upon the larger organisms, *Hydatina*, *Brachionus*, and *Moina*. A single such organism furnishes a large and nutritious meal for the *Asplanchna* and the digestion of this meal is by no means always followed immediately by another. In other words, the feeding upon these large and active organisms inevitably means an irregular food supply in which hunger or sheer starvation alternate in the most varied ways with over-nutrition. This is strictly true with *Moina* feeding, individuals in starvation and depletion always being found in such cultures. It is unfortunate, however, that in our effort at periodic feeding in isolation cultures no positive results were secured. The truth is that such feeding simply does not succeed.

In regard to mass cultures, however, it should be pointed out that qualitative food change as a cause of mutation can never be wholly excluded. This is because of the tendency towards at least occasional cannibalism which the species always manifests, the ingestion of a young *Asplanchna* by an older individual obviously constituting a marked change of diet. This change of diet has been assigned by Dr. Powers in the paper above mentioned as the cause of the second mutation, from the humped to the campanulate type.

The one peculiar instance of the sudden change in type which does not at first thought seem interpretable as due to quantitative or qualitative nutritional change, is the tendency towards mutation shown by the early generations following the resting egg. But, upon second thought, this instance groups itself readily with the others. The newly hatched *Asplanchna* grows first upon the stored nutrition of the egg and frequently continues to develop to a point of starvation before food is taken. Thus, in the first and second generations, especially if the food organism be a different one from that upon which the parent fed, we have really a definite instance of changed nutrition, a change, as said, which is always quantitative and may well be qualitative also.

#### DISCUSSION OF LITERATURE

In general, little experimental work has as yet been done upon rotifers, in regard to their morphological characteristics, which need here be considered. Beside the paper of Dr. Powers mentioned at the beginning of this article and which need not here be discussed, there remains the brief series of experiments by von Lange upon the very closely allied rotifer which he designates *A. sieboldii*. Von Lange's effort was to substantiate Daday's contention of dimorphism in the rotifer, and this he was easily able to do, rearing from the resting egg the saccate form, which passed, during the third to the seventh generation, into a humped *Asplanchna*. He was unable to carry his experiments beyond the eighth generation. What most concerns us here is his purely speculative suggestions as to the cause of the transition from the saccate to the humped type. He assumes that the appearance of the humps is due to an increase in vitality—"Steigerung der Vitalität"—but, curiously enough, he attributes this increase in vitality to the mere act of parthenogenesis as such, evidently deeming that the development through two to six generations of parthenogenesis is in itself the cause of the transition to the humped type. It is obvious that such an assumption cannot stand in the light of our experiments, which show that parthenogenesis in and of itself possesses no inherent tendency towards

an increase in vitality, but rather, that it is an indifferent though a vacillating, rhythmic process leading to retrogressive as well as progressive changes.

In addition to this meager amount of experimentation certain observations have been made upon variation in nature and certain theories have been brought forward to interpret them which here deserve consideration.

L. A. Jägerskiöld (94) has observed new pelagic varieties of the rotifers *Anuraea cochlearis* and *A. aculeata* in the Baltic Sea. The substance of his explanations of these varieties are contained in the following quotations.

Wahrscheinlich ist wohl, dass diese ich möchte fast sagen Tendenz, mit gekrümmten und auswärts gebogenen Hörnern zu variieren, die bei den oben beschriebenen Formen vorhanden ist, auf das pelagische Leben dieser Tiere zurückgeführt werden muss und als gewissermassen mit dem Analog zu bezeichnen ist, was man bei den pelagischen Diatomaceen gefunden hat.

In addition, Jägerskiöld has found intermediate forms between the typical species of *Anuraea* and these varieties.

We would call attention to the indefiniteness of ascribing these varieties to pelagic life as a whole without effort to analyse its factors; and we raise the question whether it is not possible that the variation is here again due to the food changes which would all but inevitably follow from the transfer from fresh to salt water.

On this subject of variation in *Anuraea cochlearis* another scientist, R. Lauterborn, has made extensive observations. In an article published in 1904 on "Die cyklische oder temporale Variation von *Anuraea cochlearis*," he considers the many variations of this rotifer as closely related to changes in temperature. In the spring and summer months types are present which are entirely absent during the winter months. On page 589, he says:

Wir wenden uns nun zu einer speziellen Betrachtung derjenigen Gewässer, in welchen *A. cochlearis* durch die *Tecta*-, *Hispeda*-, und *Irregularis*-Reihen vertreten ist. Hier trat uns schon früher wiederholt der bestimmende Einfluss vor Augen, den der jährliche Gang der Temperatur auf die Grösse des Panzers der Gesamtart ausübt. Wir dürfen

darum auch eine Korrelation zwischen der jährlichen Variationskurve der Grösse des Panzers und der jährlichen Temperaturkurve erwarten.

May it not be well at least to point out that the correlation which Lauterborn has shown to exist between the temperature of the water, and the form, size, and so forth, of *Anuraea* may quite as well be an indirect as a direct relation. We believe that nowhere in his article does Lauterborn consider the question of the feeding habits of the organism which he has studied so extensively; yet nothing would seem more natural than that a change in thermic conditions may give rise to changed and varied food supplies, which in their turn may modify *Anuraea* as we have found such changes to modify *Asplanchna*. If observation or experiment should show this latter supposition to be correct, we should at once have an explanation for certain facts which, as Lauterborn himself states, are not explicable upon the hypothesis of direct influence of temperature. These facts are, namely, that with the advent of spring there appears in *Anurea* not one line of mutation or line of variation, but instead, several definite lines diverge simultaneously. Such results may not be in contradiction to the theory of direct effect of temperature but they at least seem more in harmony with the assumption of varied habits of feeding as the definite causes of the changes.

Victor Langhans and Wesenberg-Lund have also made interesting observations upon marked variations which occur in *Asplanchna priodonta*. Their observations are essentially similar though their interpretations differ. This species is a fairly close relative of the species which we have studied. The facts recorded are essentially as follows: The size of the species varied enormously. One form, which is spherical and found only in winter, is about one-third as large as the huge elongated summer form. The jaws (Langhans), though of the same general type, differ in many minor respects. Since the appearance of these types follows definite seasons of the year, Wesenberg-Lund ('98) at first concludes that the variation depended upon internal factors. To quote, he says (p. 209):

Da ich ausserdem alle Zwischenstufen jener zwei Formen von Weibchen gefunden habe, ist in der Tat auch keine andere Erklärung dieser merkwürdigen Erscheinung möglich, als dass es eine Wachstumsercheinung sei, die entweder so zu erklären ist, dass alle runde Weibchen zu den gestreckten heranwachsen und grössere und schlauchförmigere Junge hervorbringen, je grösser und schlauchförmiger sie selbst werden, oder so, dass nur bestimmte Generationen der parthenogenetischen Brut diese Fähigkeit besitzen. . . . Verhält es sich so, dass nur bestimmte Bruten die Fähigkeit besitzen zu gestreckten Weibchen heranzuwachsen, dann liegt es auch nahe, dass jene zwei Weibchen verschiedene Reproductionsverhältnisse darbieten.

Later, however, he evidently discarded this view. In his article published in 1910, he considers in general the changes of certain of the morphological characteristics of a number of animals, such as various rotifers (including *A. priodonta*) Daphnids, and Protozoa. He states (p. 18):

Weil nun die höchste Entwicklung der Schwebearparate unstreitbar mit der Zeit im Jahr zusammenfällt, wo die Fallbewegung die rascheste ist (bei höchster Sommertemperatur) greift die Ansicht mehr und mehr um sich, dass die Temporalvariationen der Planktonorganismen (wenigstens der perennierenden) als eine Reaktion auf die periodischen Änderungen im physikalischen Zustand des Wassers aufzufassen sei. Variationen der Viskosität, wie des spezifischen Gewichtes einerseits und Temporalvariationen andererseits verhalten sich zu einander wie Ursache und Wirkung.

That such a result should be ascribed to such a causative factor seems perhaps plausible; yet before it is accepted beyond question, the facts plainly demand experimental analysis. It is well known that the viscosity of water varies between 0° and 25°C., almost inversely with the temperature. The effect upon the morphological characters, we should expect at least would correspond, step by step, with viscosity. However, this is not the case, as Wesenberg-Lund observes (p. 22): "Jetzt aber, da wir wissen, dass sie in der so kurzen Zeit von ca. 3 Wochen der Hauptsache nach vollendet ist, wird uns die Sache viel begreiflicher." The entire variation occurs in three weeks at the most. He even adds later, in speaking of *Hyalodaphnia cucullata*: "Man hat oft gelegenheit während der 3 oben erwähnten Wochen, in der Bruträumen der plumpen, rundköpfigen Winterformen Bruten mit spitziger Kopfform zu finden." The

transition may thus occur in a single generation and in the embryo, just as we have found the sudden effects of nutrition to occur in *Asplanchna*. On the other hand, the changes in external conditions assumed as the cause are slow and gradual. This continuity of the assumed cause may not preclude the possibility of the production of discontinuous effects; but we think it leads one to question their probability.

In the article of Langhans ('06) in which the morphological characteristics of *A. preodonta* alone are considered, the influence of the viscosity of the water is mentioned along with certain other important factors. He states (pp. 461 and 462): "Es haben sich bei der *Asplanchna preodonta* grosse Variationen . . . . in der Grösse der Tiere gezeigt," and

Die Erscheinung der temporalen Grössenvariation wird bedingt durch das Eintreten des quantitativen Maximums und ist weder auf eine Anpassung an die mit der Temperatur wechselnde Dichte des Wassers, noch auf eine Anpassung an die mit der Temperatur wechselnde innere Reibung des Wassers zurückzuführen. Sie dient also nicht der Regulierung der Schwebefähigkeit, sondern ist eine direkte Folge der mehr oder minder günstigen Nahrungsverhältnisse.

Langhans' conclusions, though theoretical, are thus more or less in general agreement with our own. They, however, need experimental confirmation and we would conjecture that especially the progressive changes observed will be found to be due, not merely to 'günstige Nahrungsverhältnisse,' but to sudden qualitative changes in food supply.

In thus emphasizing the value of the nutritive factor and laying weight upon the outcome of our own experiments we do not wish to belittle the possibility that other factors—possibly primary, very probably secondary—may be experimentally discovered which produce in rotifers, either fluctuating variation or mutational change.

Meanwhile, the demonstration of a single definite primary factor is of value in itself and offers, we think, a useful instrument for further research along the lines of variation, sex-determination, and heredity.

## CONCLUSIONS

1. The saccate type of *Asplanchna amphora* reproduces its own type indefinitely when reared under uniform conditions.
2. A marked physiological rhythm is present in the successive development of generations.
3. In the young successively produced by one individual under uniform conditions, the mid-members of the family receive more nutrition from the mother than do the earlier or later members.
4. The size of the parent in lines with common ancestry and without mutation does not directly influence the size of the offspring.
5. Change in temperature, in itself, has no direct influence in producing morphological change.
6. Starvation of the saccate type does not cause mutation.
7. Starvation of the humped type causes retrograde mutation.
8. Alternate feeding and starvation in isolation cultures does not cause mutation.
9. Alternate feeding and starvation in mass cultures probably does cause mutation.
10. Change of food, as from *Paramecium* to *Oxytricha*, *P.* to *Euglena*, *P.* to *Moina*, *P.* to *Brachionus*, and *P.* to *Hadatina* produces mutation.
11. Substances dissolved in culture medium or in the water of the food culture do not have a direct influence toward producing a mutation.
12. Production of males is rare in the saccate and abundant in the humped and campanulate types.

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# SOME SPECIFIC DIFFERENCES AMONG PROTOZOA WITH RESPECT TO THEIR RESISTANCE TO HEAT

ROBERT H. HUTCHISON

*The Zoölogical Laboratory, University of Pennsylvania*

## TWO FIGURES

It is well known that different species of bacteria show marked differences in their ability to withstand high temperatures, such differences being of considerable practical importance in bacteriological work. A temperature of 30°C. is fatal for some forms, while others, including most pathogenic forms, are not killed until 50°C. is reached, and finally there are the so-called thermophilic bacteria, forms whose death temperature is 70° or over. Similar differences are known to exist among the Protozoa, many pathogenic forms living normally at temperatures which are fatal to their free living relatives. In all such cases the question of adaptation must play an important part. The powers of adaptation of unicellular organisms to new environments are high, as is shown by the experiments of Dallinger ('87) on acclimatization of Protozoa to high temperatures. The question arises whether the thermal resistance of any given organism is merely a response to the temperature of its environment, or whether it is something more fundamental, depending upon the nature of the animal itself. If the thermal resistance is intimately connected with the original protoplasmic constitution of the animal, one would expect to find considerable and constant differences even among forms living under approximately the same conditions. Is such the case? If so, how much variation is shown within the species? Are the descendants of a single individual less variable than a population selected at random?

Do different 'races' or 'biotypes' of the same species differ from each other in their resistance to high temperatures, as they have been shown to do in many other respects? Under what conditions may the resistance of the race or species be altered? The following experiments, intended to throw light on these points, were undertaken at the suggestion of Dr. M. H. Jacobs and carried out during the winter of 1911-1912 in the Zoölogical Laboratory of the University of Pennsylvania. It is intended to supplement the work reported here with more extensive experiments on the influence of salts upon thermal resistance of Protozoa and to learn to what extent acclimatization may be produced by growth at a comparatively high temperature.

In order to compare the resistance of different species, the time element must be the same for all experiments. It is a matter of common knowledge that the time of exposure has a great deal to do in determining the fatal temperature. Loeb says (*Dynamics of living matter*, p. 107): "It is erroneous to speak of a definite temperature as a fatal one, instead we must speak of a deadly temperature zone. . . . For warm-blooded animals 45°C. is fatal, but 42° is also fatal if maintained for a longer time." Rautman ('09) gives the limits for life for *Paramecium* as 5° to 35°C., but says that it will withstand 45° if exposed for a very short time. According to Jackimoff, *Trypanosoma Brucei* is killed by exposure to 45° for five minutes while it resists a temperature of 43° for twenty-five minutes. The time element is therefore an important factor. Preliminary experiments on the determination of death temperatures with the ordinary warm stage apparatus proved unsatisfactory in that it was not easy to raise the temperature gradually nor to bring about this gradual rise in the same length of time in successive experiments. Another objection to the warm stage was that the number of animals which could be kept under observation was very small and accurate counting was not possible when large numbers were present. Moreover, the temperature at which swimming movements stop is not the same as the death temperature, and in using the warm stage and a binocular dissecting microscope it is not easy to distinguish temporary rigor

from permanent death rigor. The method finally adopted was this: A blood serum oven was used, and on the floor of this oven a number of small glass dishes were arranged and numbered consecutively. Each dish was 5 cm. square and 1 cm. thick and was provided with a hollow ground cavity having a capacity of about 3 cc. In each of these dishes was placed four or five drops of the culture medium containing the species to be examined. Each dish was covered with a thin glass plate to prevent evaporation, and the oven itself was closed in the usual way. Using a medium flame the temperature was raised gradually from the room temperature (ca. 20°) to 42° in about one hour. With a little practice it was possible to bring about this rise in approximately the same time in different experiments; the few minutes variation had no appreciable effect on the results. Not only was there an advantage in making the rise of temperature a very gradual one but each dish was in direct contact with the copper floor of the oven, thus making the loss of heat by radiation quite low. In order to determine as nearly as possible the temperature of the dishes, a thermometer was placed in a horizontal position on the floor of the oven. The thermometer, like the dishes, was not only in contact with the metal upon which it rested but was bathed in the warm air of the oven. Dr. E. F. Phillips, of the United States Department of Agriculture, was kind enough to make a series of thermo-electric determinations, and by introducing a thermo-electric couple underneath the glass cover of the dish, has made possible a comparison of the readings of the thermometer with the real temperature of the culture fluid containing the animals. In the region between 22° and 43° the actual temperature of the fluid was never more than half a degree below that shown by the thermometer. Another advantage of the method used was that the dishes could be removed and allowed to cool before counting, thus giving an opportunity for the recovery of those animals only temporarily affected. In all these experiments at least half-an-hour was allowed for possible recovery. The dishes were removed from the oven as successively higher temperatures were reached. Thus dish no. 1 was removed when it had reached 35°, no. 2 at 36°,

and so forth. After allowing for recovery, the dishes were examined under a Leitz binocular dissecting microscope and the animals present were counted. A compound microscope was necessary only when it was uncertain whether cilia or membranelles were still beating in the case of otherwise motionless individuals. The criterion used in deciding whether a given individual was dead or not was the cessation of all movement in cilia, membranelles, and so forth. This had to be applied carefully in the case of *Blepharisma*, where not much change in the optical appearance of the protoplasm is noticeable, as it is in *Paramecium*. In *Paramecium bursaria* cytolysis invariably occurs at the fatal temperature, and this breaking up of the cell was taken as the decisive point.

In this way a number of different species were examined as they happened to appear in the laboratory cultures. These cultures were hay infusions seeded with some material from a pond. Most of them, except quite old cultures, were slightly acid in reaction. As a rule *Paramecium caudatum* developed most abundantly in them and results of experiments with this species are therefore stated first. A record of each experiment was made as follows:

*Paramecium caudatum* from a three weeks old culture, acid in reaction

NUMBER OF DISH	TEMPERATURE	DEATHS	TOTAL PRESENT IN EACH DISH
	<i>degrees</i>		
1	36	0	135
2	37	0	50
3	38	10	40
4	39	8	25
5	40	55	55
6	41	65	65

Some twelve experiments were performed upon *Paramecium caudatum*, taken from various mixed cultures. The results are summed up in table 1. Tabular results of experiments with other species are given in tables 1 to 6.

To represent these results graphically it is most convenient to use the percentages. For example, from table 5 one may con-

TABLE 1

*Paramecium caudatum* (mixed cultures)

	TEMPERATURE					
	36°	37°	38°	39°	40°	41°
Totals subjected to a given temperature	976	891	907	693	635	556
Number of deaths.....	5	42	114	454	625	556
Percentage dead at given temperature....	0.5	4.7	12.5	65.5	98.4	100

TABLE 2

*Paramecium aurelia*

	TEMPERATURE				
	38°	39°	40°	41°	42°
Totals subjected to each temperature.....	630	453	407	382	437
Number of deaths.....	0	6	168	368	437
Percentage dead at given temperature.....	0	1.3	41.2	97	100

TABLE 3

*Paramecium bursaria*

	TEMPERATURE					
	37°	38°	39°	40°	41°	42°
Totals subjected to each temperature....	256	251	212	144	48	120
Number of deaths.....	0	35	153	140	48	120
Percentage dead at given temperature....	0	15	72	97.1	100	100

TABLE 4

*Blepharisma lateritia*

	TEMPERATURE					
	37°	38°	39°	40°	41°	42°
Totals subjected to each temperature....	547	503	502	519	446	300
Number of deaths.....	0	0	114	278	421	300
Percentage dead at given temperature....	0	0	22.7	54	92	100

TABLE 5  
*Spirostomum teres*

	TEMPERATURE					
	33°	39°	40°	41°	42°	43°
Totals subjected to each temperature....	89	155	95	139	88	84
Number of deaths.....	0	0	0	11	37	84
Percentage dead at given temperature....	0	0	0	8	43	100

TABLE 6  
*Urostyla grandis*

	TEMPERATURE		
	35°	36°	37°
Totals subjected to each temperature.....	15	14	18
Number of deaths.....	0	6	18
Percentage dead at given temperature.....	0	43	100

struct a curve for *Spirostomum teres* in the following way: Between 40° and 41°C. 8 per cent of the deaths occurred. This point is marked on an ordinate halfway between 40° and 41°. Between 41° and 42° C. 43 - 8 per cent or 35 per cent of the deaths occurred and this is marked on an ordinate halfway between 41° and 42°. At a temperature between 42° and 43°C. 100 - 43 per cent or 57 per cent of the deaths occurred. This number 57 is represented on an ordinate halfway between 42° and 43°. Lines drawn connecting these points give the curve for *Spirostomum* shown in figure 1. The curves for the other forms are constructed in the same manner.

Of these six species *Paramecium caudatum* is seen to be the most variable. The animals experimented with were taken from several different cultures, all of which had been seeded with material from a pond. The curve for *Paramecium aurelia* is based upon figures obtained partly from one pure line culture and partly from one or two mixed cultures. It is probable that experiments with more cultures of this species would give a little more spread to the curve, that is, would show a little more variation within



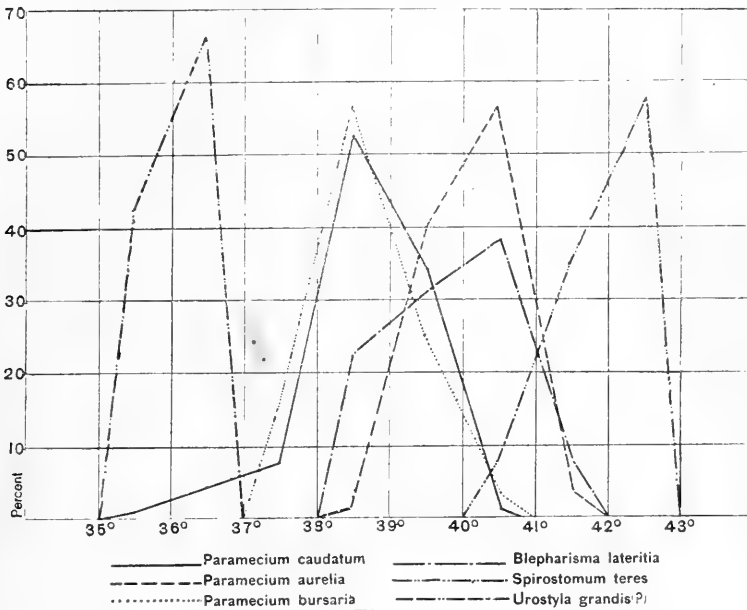


Fig. 1

the species. However, the resistance of *P. aurelia* appears to be distinctly higher than that of *P. caudatum*. The modes of the two curves are two degrees apart. This difference in their resistance to heat, together with the fact that they react differently to carbon dioxide (Jacobs '12), may perhaps be considered further evidence of the distinctness of the two species. In the case of *P. caudatum* and *P. bursaria* it will be seen that the modes of the two curves coincide, but the shape of the curve is quite different. Although the greatest number of deaths occur at the same point, yet there is a characteristic difference in that *P. caudatum* shows more individual variation within the species. This may be due, however, to the fact that a much larger number of individuals from a greater variety of sources were employed than was the case with *P. bursaria*. The curve for *Blepharisma* is peculiar in that there is no one point which stands far above the others, but there are three points comparatively close together giving the curve a flattened appearance. This is accounted for by the fact that in the cultures in which it devel-

oped in abundance at least two distinct forms were easily seen. Some were large, much vacuolated, comparatively sluggish in movement and of a bright pink color. Others were smaller (especially in breadth), vacuoles small and few, more active in their movements and of a duller color. The larger forms were always killed a short time before the others. Spirostomum and Urostyla were found developing in the same culture and both were present in the dishes during the experiments and therefore they were subjected to exactly the same conditions. The surrounding medium, the time of exposure, the time allowed for recovery, and so forth, were identical, yet the two forms are more strikingly different in their ability to withstand heat than any two of the forms experimented on.

Experiments with other forms gave some interesting results, although not extensive enough to warrant the construction of curves. One experiment with Glaucoma showed that it was killed at 35°C., while one species of Stylonychia under the same conditions is more resistant, its death temperature being ca. 40°. In another case Stylonychia were observed alive and active at 40° when all of 95 Paramecia in the same drop were dead. Among the flagellates some specific differences were observed. Peranema is killed at 40°; Chilomonas at 38°; Gonium at about 37° and Synura at some point below 28°. Synura was found but twice and only one experiment was successful. The dishes were removed in order starting at 28°, but even in that one removed at 28° all Synura were dead. The colonies had disintegrated and no recovery or formation of new colonies was observed within a week. The very interesting observations of H. N. Parker ('11) give further evidence of a very low death point. The case of Synura seems to be an unusual one. Grosse-Allerman ('09) mentions one case which is even more unusual. He states that Amoeba terricola is killed by a few minutes' exposure to 25°. By way of contrast, it is rather striking to read of Amoeba limax and Nassula elegans *living* in hot springs at a temperature of 50° or 52° (Issel '10), and of parasitic forms such as Trypanosoma brucei and Trypanosoma elmassani killed only after five minutes' exposure to 45°C. (Jackimoff '09). But leaving such

extreme cases out of consideration, it appears that of the common Protozoa living under the same conditions of temperature and in the same or approximately the same medium, each species has a resistance peculiarly its own. The thermal resistance is quite constant for the species under given conditions. Of the forms studied, *Spirostomum* had the highest resistance ( $42^{\circ}$  to  $43^{\circ}$ ) while *Synura* had the least ( $< 28^{\circ}$ ). The constancy of the thermal resistance makes possible a definite expression in the form of curves. This in contrast with their resistance to carbon dioxide, where it was found "a certain amount of individual and cultural variation may occur which prevents the expression of the resistance of the species in absolute terms" (Jacobs '12).

As to the effects of individual and cultural variation upon the death-temperature curve some experimental evidence can be given here. The curve for *Paramecium caudatum* shown in figure 1 indicates a comparatively large amount of variation. Since different races of *Paramecia* have been shown to differ in certain physiological respects, such as their tendency to conjugate, and so forth (Jennings '10), it is conceivable that they may be different in their ability to withstand heat. If so, the spread of the curve in figure 1 may be explained on the ground that several strains or races were present in the wild cultures examined. To test this point some pure line cultures were started. A large quantity of hay infusion made up at one time, was divided equally among six jars, thus making the medium the same in all. Each jar was seeded with a single individual transferred from an ordinary culture by means of a capillary pipette. Three of these culture were followed with some care for a period of two months at which time some unfavorable condition caused the animals to disappear. The results are summed up in tables 7 to 9. Curves based upon these results are given in figure 2.

It appears that, by mere chance, a strain (Race 5) of comparatively low resistance had been found. It will be observed that the mode of the curve for Race 5 is three degrees below that for Race 1 or Race 6. It must be stated here that all these cultures developed rapidly and were in good healthy condition for about a month. Then some unfavorable conditions, prob-

ably the exhaustion of food supply, gradually arose, which interfered with the development of the animals. Race 5 disappeared entirely at the end of the two months while Races 1 and 6 maintained themselves a few weeks longer. Although Race 5, under the influence of some unknown condition, disappeared much sooner than the others, yet its resistance during its early healthy period of rapid development was just the same as that during the later period of depression. For example, one experiment on

TABLE 7

*Paramecium caudatum* Pure line no. 1

	TEMPERATURE				
	37°	38°	39°	40°	41°
Totals.....	370	688	618	545	480
Number of deaths.....	0	3	103	375	480
Percentage dead at given temperature.....	0	ca.0.5	16.6	68.9	100

TABLE 8

*Paramecium caudatum* Pure line no. 5

	TEMPERATURE				
	35°	36°	37°	38°	39°
Totals.....	30	174	411	363	268
Number of deaths.....	0	32	280	342	268
Percentage dead at given temperature.....	0	18.4	68.1	94.2	100

TABLE 9

*Paramecium caudatum* Pure line no. 6

	TEMPERATURE					
	36°	37°	38°	39°	40°	41°
Totals.....	181	455	485	485	569	292
Number of deaths.....	0	0	0	62	419	292
Percentage dead at given temperature....	0	0	0	13	73.5	100

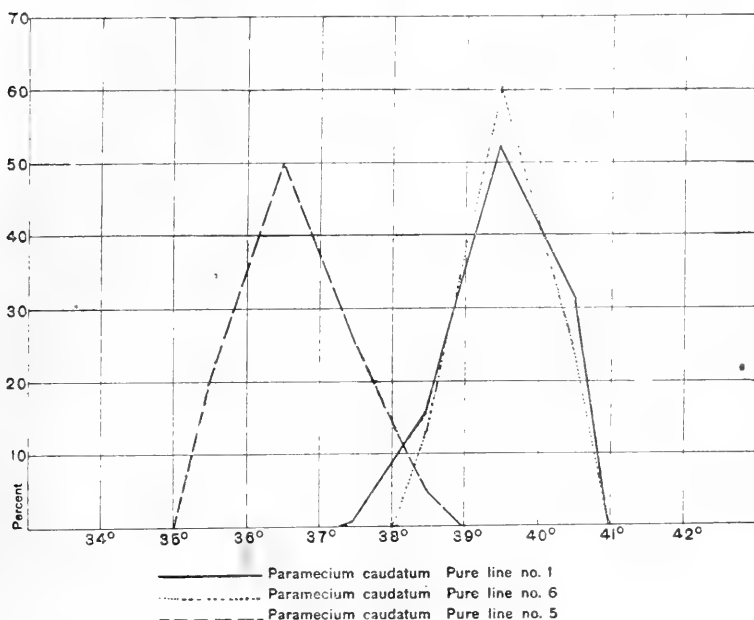


Fig. 2

February 17 (just three weeks after the culture was started) gave these results:

- At 36° there were 8 deaths in a total of 76
- At 37° there were 35 deaths in a total of 120
- At 38° there were 130 deaths in a total of 130

In another experiment on March 30 (during the period of depression):

- At 36° there were 5 deaths in a total of 40
- At 37° there were 11 deaths in a total of 28
- At 38° there were 19 deaths in a total of 19

One may see the effect of the depressing influence by comparing the totals present in the same quantity of the medium on these dates. Evidently, whatever factor influenced this race in such a way as to interfere with its development, had no effect on its resistance to heat. In spite of unfavorable medium, the curve for Race 5 represents the normal condition of a distinct race or biotype, and one is led to infer that different biotypes of *P. caudatum* may have different powers of resistance to heat. At

least, to the question: Are the descendants of a single individual less variable than a population selected at random? an affirmative answer can be given.

The effect of cultural variation on thermal resistance is not appreciable in amount. There are several important changes which take place in a hay infusion during the course of growth, namely, changes in acidity, increase in the amount of alkali and of inorganic substances (Peters '07) (Fine '12). Yet in all the cultures whose history was followed out for any length of time, there seemed to be no change in the thermal resistance of the forms present. The figures given above for Pure line no. 5 illustrates this. In fact all the pure line cultures agree in this respect. As further evidence of this point two experiments with a pure race of *Paramecium aurelia* are recorded here. The first set of figures were obtained when the culture was ten days old, straw colored and acid in reaction. The second set of figures were obtained when the culture was two months old, and of a dark brown color:

CULTURE 10 DAYS OLD			CULTURE 2 MONTHS OLD		
Temperature	Deaths	Totals	Temperature	Deaths	Totals
<i>degrees</i>			<i>degrees</i>		
38	0	110	38	0	21
39	0	140	39	0	23
40	28	95	40	8	32
41	140	140	41	37	37

It appears therefore that the effect of the ordinary changes which occur in the medium is not profound enough to change the thermal resistance of the forms present.

The influence of the acidity of the medium deserves special mention, because the amount of acid present changes more than any other known ingredient. In a hay infusion the acidity rises rapidly to a maximum within the first few days and then gradually declines (Fine '12). Yet in several cultures which were followed during two, three, and in one case four months of their history, the thermal resistance of the animals was not perceptibly altered. In a few experiments, where *Paramecium caudatum*

and *Blepharisma lateritia* were transferred to an  $n/900$   $H_2SO_4$  solution and then subjected to heat in the usual way, the fatal temperature was not different from that of the animals in the ordinary medium. Fine ('12) states that the acid content of an infusion could be increased seven or eight times before interfering with the development of animals present. There is reason to believe that the acidity of the medium can be increased considerably before any effect is produced on the thermal resistance.

As to the effect of alkalis in modifying the death temperature curve no decided answer can be given as yet. The OH concentration increases during the life of a hay infusion as indicated by the change of color (Peters '07). From statements made above it is plain that the ordinary increase is not enough to affect perceptibly the thermal resistance.

The effect of salts in fairly high concentrations may be quite marked. *Paramecium caudatum* transferred, with as little of the medium as possible, to  $n/50$   $NaNO_3$  and then subjected to heat in the usual way was killed at a temperature two degrees higher than was the case in the unchanged medium. *Paramecium caudatum* from another culture was transferred to  $n/50$   $NaCl$  and also to  $n/50$   $KNO_3$ . In both these cases the whole death temperature curve was shifted two degrees higher on the scale. These salts then have the effect of increasing the resistance to heat. To determine to what extent osmotic and to what extent chemical factors are involved in the above results, a more extensive series of experiments has been undertaken, which will be discussed elsewhere.

#### SUMMARY

1. Of the common species of Protozoa examined each one has a resistance peculiarly its own. Under given conditions its resistant is quite constant and differs more or less from that of other species subjected to the same conditions.

2. The amount of variation within the species may be considerable. Under the conditions employed in these experiments the fatal temperature zone may spread out over as much as six degrees (*Paramecium caudatum*).

3. Different strains or biotypes of *Paramecium caudatum* may have quite different powers of thermal resistance. Each biotype shows considerably less variation than a population selected at random.
4. The thermal resistance of the forms studied was not perceptibly affected by the age of the culture and its accompanying changes.
5. The ordinary changes in the acidity of the medium seemed to have no influence on the resistance of the animals to heat.
6. The thermal resistance of a species may be somewhat modified by raising the salt content of the medium.

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# THE MODES OF INHERITANCE OF AGGREGATES OF MERISTIC (INTEGRAL) VARIATES IN THE POLYEMBRYONIC OFFSPRING OF THE NINE-BANDED ARMADILLO

H. H. NEWMAN

*The Hull Zoölogical Laboratory of the University of Chicago*

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## STATEMENT OF PROBLEMS

After nearly four years' study of the peculiar inheritance situation presented by the armadillo it is now possible to make a fairly definite statement of the problems involved and to attempt a solution of some of them. The principal difficulty encountered during the progress of the work has been one of orienting the somewhat unique problems within the general field of genetics, of definitely relating the data and conclusions to those of standard lines of investigation in heredity and general biology. It would

seem advisable then at the outset to acquaint the reader with what the writer conceives to be the problems peculiar to the material and to indicate some of the main lines of analysis employed in the solution of these problems.

The principal problems may be briefly stated as follows:

1. In what respects are the modes of inheritance of polyembryonic offspring different from those of the ordinary kind? Does the fact that, in the case of the armadillo, four offspring come from a single fertilized egg involve any unusual inheritance features not present in cases where a fertilized egg produces only one offspring?

2. Since the quadruplets derived from a single fertilized egg are, in consequence of their mode of origin, more closely related genetically than brothers or any other blood relatives, are they more closely similar than any other relatives? In other words, to just what extent is the basic assumption of taxonomy, that the degree of resemblance is a function of the degree of blood relationship, borne out in the present material?

3. In view of our knowledge that the originally single blastodermic vesicle divides at a very early period into four distinctly independent embryonic rudiments, to what extent is it possible to determine what characters are predetermined before the separation and what are modified by individual experience during development? In brief, is it possible to estimate in this apparently exceptionally favorable material the relative values of predetermined and epigenetic factors in the development of any given definitive character or set of characters?

4. In view of the fact that extremely trivial personal minutiae seem to be strongly inherited in certain peculiar ways by individuals or sets of quadruplets, what sort of conception of the character of the predetermining germinal basis accords with the facts? What is the nature of the predeterminers of personal minutiae?

5. What is the mode of inheritance of meristic variates or of aggregates of these? Are they inherited in the blended fashion as dimensional variates appear to be, or according to the laws of alternative inheritance after the manner of substantive variations?

6. Is there a real difference in the absolute variability of the two sexes?

7. To what extent is sexual dimorphism a factor in disturbing the inheritance ratios in connection with the characters dealt with in this study?

8. To what extent may resemblances and differences among the individuals of a given set of quadruplets be a product of environmental similarity or diversity?

#### GENERAL INTRODUCTION

In a paper entitled "The limits of hereditary control in armadillo quadruplets" (Newman and Patterson '11) the authors presented a statistical study of the variations of the scutes in the nine bands of armor in twenty sets of armadillo quadruplets. The fact that in each case the four members of a set were known to be the product of the division of a single fertilized egg gave especial interest to a detailed comparison of the members of the various sets. It was assumed that in so far as the four individuals of a given set were alike this similarity was predetermined prior to the separation of the four embryonic primordia, and in so far as they were different their differences were attributable to divergent epigenetic factors operating during or subsequent to the period of segregation. It was possible to show that with respect to the total number of scutes in the nine movable bands the coefficient of correlation for quadruplets was 0.9348, a degree of correlation higher than that determined for any inter-individualistic relation and paralleled only by those obtaining between structures of the right and left hand sides of the same individual. This coefficient of correlation was taken to be an index of hereditary control, in the sense that it indicates just how nearly perfect in operation is the predeterminative mechanism. It was also stated that the alignment of scutes into the respective rows or bands is probably not predetermined to any large extent but is probably due for the most part to mechanical pressures incident to growth, factors purely epigenetic in character, a statement the validity of which is not borne out by the present investigation.

Perhaps the most striking revelations had to do with the occurrence of rare atypical arrangements of bands. In some cases a certain peculiar band arrangement was repeated with remarkable fidelity of detail in two, three or even four individuals of a set, a circumstance seeming to indicate unequivocally that the character in question must have been in some way predetermined before the separation of the embryonic rudiments of the four members of the set. Equally striking and perhaps even more puzzling were the cases in which certain single scutes, peculiar in some respect, were seen to recur in two or more fetuses in almost precisely the same location. Such units of organization were referred to as probably the smallest characters capable of hereditary control and transmission since they are of the same order as hair groups in other mammals.

Throughout the paper attention was repeatedly called to the fact that the fetuses are arranged in pairs, one pair attached to the right hand placental disc and the other to the left, and that the resemblances were closer between the individuals of a pair than between those of opposite pairs. The pairing was explained by assuming that each pair came from one blastomere of the two-cell stage of cleavage, an assumption which now seems unwarranted and unnecessary.

The paper summarized above called forth comment both commendatory and critical. By some the work was considered highly suggestive and worthy further analysis, by others it was relegated to the category of papers that missed the mark and had no especial significance. Among the criticisms and helpful suggestions that have come to me from various sources are the following:

1. A well known biometrical expert writes frankly that he is unable to place any reliance on the accuracy of coefficients of correlation derived from only twenty sets of quadruplets, especially since the probable error of the determination has not been worked out. This objection should be satisfactorily met in the present studies, for nearly seven times the original number of sets of quadruplets are now available. Curiously enough the results of determinations of constants, when this larger number was used, do not differ materially from those obtained from the smaller

number. It is a simple matter to determine the probable error and this deficiency of the former paper will be avoided in the future.

2. It has been objected that the title of the paper is misleading in that it purports to deal with heredity when no data concerning heredity, defined as the genetic relation existing between parent and offspring, is furnished. Although I am unwilling to admit that a study of fraternal correlation is foreign to the science of heredity, I am willing to acknowledge that the failure to deal with the inheritance of these characters from mothers to offspring was a real oversight. The present studies, however, should satisfy to a large extent the demands of the critic in that it is shown that practically all of the characters dealt with in the previous paper are inherited directly from the mothers according to certain laws that will be made clear in the body of the paper.

3. It has been objected that a study restricted to the characters of the banded region of the armor furnishes too limited a basis for reaching conclusions of wide or general application. By way of meeting this objection, I have worked out the data for all of the other regions of the armor and for a number of other types of characters as well. The facts concerning all of these characters are in perfect harmony with those determined for the banded region, and will be presented after a more thorough examination of the banded region has been made.

4. It has been repeatedly insisted by those interested in the work, and to some extent by the writer, that experimental breeding is essential to an analysis of the problems of heredity involved in the present material. That breeding is far less essential than might at first be supposed will be admitted, I believe, by those who take the trouble to note the following considerations. A detailed study of all the phases of inheritance exhibited by the characters thus far studied shows that there is present no appreciable sexual dimorphism. Not only are males and females alike with respect to all somatic characters examined (except in the genitalia and their accessories), but the two sexes inherit equally strongly from the mothers. Since there is no sex-limitation in the inheritance of the characters studied the paternal contribution though un-

known may be assumed to be exactly equivalent to the maternal, which is known. A study of the mothers, therefore, should reveal the essential facts of inheritance, although the problems would be more readily handled if we knew the characters of both parents. Moreover, the present scheme of studying the inheritance from the maternal side alone offers many unique advantages that could not be duplicated under the conditions of experimental breeding. It is possible readily to obtain a large number of sets of offspring, numbers that could be secured only at enormous expense, if at all, were breeding attempted. Again it is possible by the methods employed in this study to obtain, by killing the mothers, a definite orientation of the fetuses in pairs and to preserve these relations by means of their placental attachments. It is hardly probable that one would be willing to kill the mothers used in breeding experiments, for this would involve too great a sacrifice of the time and labor expended in domesticating and bringing about the mating of captive animals. If, moreover, mothers are allowed to give birth to young, all placental attachments are lost and the value of the material is correspondingly lessened. Finally, it has been my experience that, whenever mothers give birth to litters in captivity, they eat or otherwise mutilate some or all of the new-born young, a perversion of maternal instinct that could scarcely be obviated since births seem to occur only at night. On the whole then I am convinced that the present method of collecting pregnant females at the proper season, removing and orienting the advanced sets of quadruplets, preserving the complete armor of the mothers, and carefully tagging mother and fetuses with corresponding numbers, furnishes the only practicable means of obtaining in adequate abundance the necessary material for the study of heredity in this species. Since only the armor of the mothers is preserved the scope of the investigation is limited, but there can be no question as to the many advantages offered by the armor. The structures and arrangements are clearly defined and readily enumerable. They reach a definitive number and alignment comparatively early in fetal life and are so well defined in advanced fetuses that the detailed comparison between mother and young is easy and certain.

During a sojourn of three weeks in the armadillo country I was able to secure 158 new sets of quadruplets, together with the preserved armor of the mothers. Of these 137 sets are sufficiently advanced to admit of an accurate examination and enumeration of the scutes of the banded region. A somewhat smaller number are available for a study of the tail characters and the armor of the cephalic plate, while only about twenty of the most advanced sets are available for work upon the scapular and pelvic shields, in which the scutes are smaller and less well defined than elsewhere.

We have now a somewhat more adequate embryonic background for the study of heredity than was available when the former paper was written. Patterson, continuing the study of the early development of the species begun in collaboration with the present writer, has recently traced the origin of the four fetuses from a previously single blastodermic vesicle. He finds that, soon after the completion of the process of germ-layer-inversion, the inner ectodermic vesicle thickens up in two regions corresponding to the right and left sides of the uterus and from these thickenings grow out two buds of tissue, each of which subsequently undergoes a dichotomous splitting in order to form the primordium of a pair of embryos, each pair being the product of an originally single bud. Genetically the individuals of a pair are more closely related than are those of opposite pairs, and the closer resemblance between individuals of a pair is simply explained. It was shown in our earlier paper (Newman and Patterson '10) that at the primitive streak stage the materials destined to form each of the four embryos is isolated from those of the others, and that, when the circulation is established, both the somatic and placental blood supply of each fetus is entirely separate. It is still impossible to state definitely the exact time at which the physiological isolation of the individual embryonic primordia occurs, but we are safe in assuming that there could be no shifting of the ectodermal elements from one hemisphere to the other after the end of the period of germ-layer-inversion, when the whole ectoderm is a minute body containing perhaps a hundred or so cells. Nature, therefore,

has furnished us with an interesting experiment in developmental physiology. It is as though the experimenter were to be able to cut an early gastrula of a sea-urchin into four equal parts and were to be able to rear each of the four pieces to adult life, under environmental conditions experimentally uniform for the four pieces, and could then compare the four individuals thus produced spine for spine, tube-foot for tube-foot, with the parents and among themselves. The detailed results of such an experiment, were it possible to perform it, would furnish fascinating data for the student of genetics. Human skill, however, could never compass the task of subdividing the gastrula equally or without injury nor would it be possible to ensure strict equality of developmental conditions for the four pieces. Yet this is just what nature has accomplished for us in the armadillo. The experiment is successfully performed every time pregnancy occurs and it is our task to analyze the results and to draw conclusions, a task which has proven to be neither simple nor yet entirely without promise of a certain measure of success.

With this background and with what I consider to be a thoroughly adequate mass of material to work with, it becomes a problem to decide on the most advantageous method of presenting the results of the analysis, to determine what particular phase of the subject should receive first attention. For several reasons it seems advisable to proceed from the general to the particular, from more typical to less typical conditions. Following this scheme I shall present first the data dealing with the inheritance of aggregates of meristic variates and shall reserve for subsequent treatment the facts concerning the inheritance of peculiarities of individual elements of large aggregates.

The material for this study is furnished by an accurately confirmed enumeration of the total numbers of scutes in the nine bands of armor of 56 male and 59 female sets of advanced quadruplets and their mothers. All sets are excluded from this study in which any fetus or mother exhibits atypical band arrangements or in which the carapace of the mother was injured in such a way as to render comparison with those of the offspring difficult. The



work has been primarily statistical in character, but the value of individual cases has not been lost sight of.

A determination, for the aggregate of scutes in the banded region, of the coefficient of fraternal correlation is made separately for males and for females. Following this the degree of correlation existing between mothers and male, and that between mothers and female offspring are determined separately and compared to discover whether there is any sex-limitation in the inheritance of these aggregates. A study is then made of the correlations existing in smaller aggregates, the individual bands, and these are compared with those determined for larger aggregates. After the banded region is thus disposed of the caudal armor is dealt with in a similar fashion and a comparison of the two regions is attempted. Finally, by way of further comparison, the cephalic, scapular and pelvic shields are briefly dealt with.

#### THE INHERITANCE OF AGGREGATES OF SCUTES IN THE NINE BANDS OF ARMOR

This region was dealt with rather extensively in a former paper (Newman and Patterson '11), but at that time only twenty sets of fetuses were available, a number considered by biometricians as inadequate for statistical treatment. Moreover, no attention was paid to the conditions of the mothers, although the scute counts of a few of the latter were given in table 6, pages 874-875. This table will also serve to indicate the distribution of scutes in the separate bands of mothers and offspring and will obviate the necessity of publishing so large a mass of figures as would be involved in a table of the type of that just referred to if 115 sets were included. It will be sufficient for present purposes to give in tabular form the totals for the nine bands of mothers and offspring (table 1, A and B). It seems necessary to publish a considerable amount of the data upon which the subsequent calculations are based in order that it may be possible for anyone interested in so doing to check any errors of calculation, which may have slipped in in spite of a painstaking effort to avoid them. If errors exist they are unquestionably of a minor sort and not of sufficient import to invalidate the significance of the results.

All of the counts herewith presented have been systematically repeated and checked over by a competent assistant, whose services have very materially lightened the necessary drudgery incident to this type of investigation.

#### 1. POLYEMBRYONIC CORRELATIONS IN THE BANDED REGION

In the former paper dealing with this subject the interrelationships of the individuals of a set of quadruplets were referred to as fraternal or intra-fraternal correlations. The relation, however, is not fraternal but one of a decidedly more intimate character, and a special term is required to express it. For lack of a better phrase I have chosen to refer to the general correlation within the set of quadruplets as polyembryonic correlation. These relations are unquestionably equivalent to intra-individual relations in other forms and therefore should be determined before the hereditary relations between parent and offspring are considered.

In determining the coefficients of correlation for the polyembryonic relation the simple method devised by Harris ('10) and used in the earlier study will be adhered to. The method has the advantage of obviating the necessity of plotting correlation tables. One has merely to determine the mean, the square of the standard deviation ( $\sigma x^2$ ) and the square of the positive differences ( $\sigma v^2$ ), since positive and negative differences are equal where each individual is treated as a subject and as a relative in computing correlations. The formula used is as follows:

$$r = \left\{ 1 - \frac{1}{2} \frac{\sigma v^2}{\sigma x^2} \right\}$$

Then the probable error of the determination is derived by the use of the usual formula:  $E_r = \frac{0.6745(1-r^2)}{\sqrt{n}}$

For my own satisfaction I took the trouble to plot a regular correlation table for the polyembryonic relation and found that it required, even when small figures were used, a sheet of paper 2 feet square, and capable of reproduction only in the form of a much folded chart, the value of which would scarcely warrant its publication.

For the sake of brevity the results of an intricate series of arithmetical computations are set down in a few short paragraphs as follows:

a. *Polyembryonic correlation for 56 sets of male quadruplets*

$$\text{Mean} = 558.62 \pm 0.65 \text{ scutes}$$

$$\sigma x^2 = 236.18$$

$$\sigma v^2 = 33.36$$

Substituting for the general formula, we have:

$$r = \left(1 - \frac{1}{2} \frac{33.36}{236.18}\right) = 0.9294 \pm 0.0057$$

b. *Polyembryonic correlation for 59 sets of female quadruplets*

$$\text{Mean} = 559.05 \pm 0.57 \text{ scutes}$$

$$\sigma x^2 = 169.95$$

$$\sigma v^2 = 33.05$$

Substituting for the general formula, we have:

$$r = 1 - \left(\frac{1}{2} \frac{33.05}{169.95}\right) = 0.9129 \pm 0.0059$$

c. *Polyembryonic correlation for the 20 sets of quadruplets used in the former paper*

$$\text{Mean} = 558.48 \pm 2.51 \text{ scutes}$$

$$\sigma x^2 = 257.62$$

$$\sigma v^2 = 33.5$$

Substituting, we get:

$$r = \left(1 - \frac{1}{2} \frac{33.5}{257.62}\right) = 0.9348 \pm 0.0111$$

A survey of these results brings out the following noteworthy points:

1. The coefficients of correlation for the new collection of quadruplet sets are sensibly equivalent to those determined for the original 20 sets, indicating that 20 sets consisting of 80 individuals are enough for statistical purposes, the statement of experts to the contrary.

2. Males as a sex would seem to show a greater absolute variability as attested by the higher standard deviation and the greater actual differences among individuals of a set, but, relatively to their standard deviation, their differences are small and this makes males appear to be a little more closely correlated than females. The difference is slight, though greater than the combined probable errors, and may not be significant. Subsequent determinations seem to contradict the earlier finding as to the higher degree of variability of males as a sex.

3. *It may be considered as established that the coefficient of correlation for the polyembryonic relation, as determined for the banded region, is the equivalent of that determined for intra-individualistic relations, and is of a totally different order from that shown to obtain for any inter-individualistic relation previously ascertained.*

## 2. INTRA-PAIR CORRELATIONS IN THE BANDED REGION

Relations even more intimate than those just presented, obtain between the individuals of a pair. Since there are two pairs in each set of quadruplets this intra-pair relation is an intra-polyembryonic relation. The two individuals of a pair have been shown to be derived from a common bud of early embryonic tissue and to retain their paired relation through their placental connections, one pair being attached to the right hand placental disc and the other pair to the left. There can now be no doubt that these placental relations serve as valid criteria of genetic relationships within the set. We should then expect, a priori, a higher degree of correlation between these 'twins' than obtains for the quadruplets.

In brief the calculation based on the data in table 1, A and B, give the following results:

a. Intra-pair correlation for 56 sets of male quadruplets is  $0.9333 \pm 0.0082$

b. Intra-pair correlation for 59 sets of female quadruplets is  $0.9182 \pm 0.0097$

No other pairing of individuals gives even so high a correlation as that obtaining for the quadruplets, while for both males and females it is plain that the intra-pair relation is more intimate

than the general polyembryonic one. This goes to emphasize further the validity of the law that degrees of resemblance are in direct proportion to degrees of blood relationship.

Knowing the mode of origin of the quadruplets as we do the real problem, it seems to me, is to explain not so much why the individuals of a set are so similar but *why they are not exactly alike*. How are the differences effected and what are the developmental factors producing divergent characters among the individuals derived from a single fertilized egg? Are the differences simply the product of the inequalities in the environic factors incident to growth or is there an unequal distribution of inheritance materials? These are questions that will inevitably recur as we proceed with the presentation of data and that can not be discussed to advantage until all the available facts are systematically arrayed before the reader.

### 3. CORRELATIONS BETWEEN MOTHERS AND OFFSPRING

This phase of heredity was neglected in the previous paper owing to lack of data concerning the mothers. None of the shells of the mothers were preserved and in only a few cases were scutes of the banded region counted before the carcasses were destroyed. This oversight was the result of a failure, until too late, to appreciate the opportunities afforded by the material for the study of certain unique phases of heredity. Our attention was focused on the problem of polyembryony to the exclusion of its consequences and of the bearings of the latter on the broader problems of genetics. The present collection of material, however, makes good the earlier deficiency in that the complete armor of each mother is permanently preserved and a detailed comparison can be made between parent and offspring.

Male and female sets are dealt with separately and comparisons are thus made possible between the indices of heredity in the two sexes. In some few cases the shells of the mother were too badly scarred or otherwise damaged to afford an accurate scute count and in such cases it was necessary to exclude both mother and offspring from the tables. In other cases the fetuses were either too

small for accurate work or one or more of the fetuses had undergone degeneration. Such cases were also ruled out. Finally, all cases in which either mother or offspring showed any atypical band arrangements, such as fusions or splittings, the whole set was put aside for special treatment in a subsequent paper. There remain then only 115 sets that are normal and perfect in every way, of which 56 are males and 59 females. The scute totals of these, together with that of their mothers, are given in table 1, A and B.

The method of calculating the coefficient of correlation is that devised by Pearson for such cases as this. The general formula used is:

$$r_{xy} = \frac{\sigma x^2 + \sigma y^2 - \sigma v^2}{2 (\sigma x \sigma y)}$$

Without going into details of calculation the results may be given as follows:

*a. Coefficient of correlation between 56 sets of male quadruplets and their mothers (data from table 1, A)*

Mean of mothers ( $x$ ) = 559  $\pm$  1.26 scutes

Mean of offspring ( $y$ ) = 558.62  $\pm$  0.65 scutes

Standard deviation of mothers ( $\sigma x$ ) = 14.02  $\pm$  0.89 scutes;

$\sigma x^2 = 196.64$

Standard deviation of offspring ( $\sigma y$ ) = 15.36  $\pm$  0.48 scutes;

$\sigma y^2 = 236.18$

Square of the average difference between mothers and offspring

( $\sigma v^2$ ) = 195

Substituting for the general formula we get:

$$r_{xy} = \frac{196.64 + 236.18 - 195}{2 (14.02 \times 15.36)} = 0.5522 \pm 0.0625$$

*b. Coefficient of correlation between 59 sets of female quadruplets and their mothers (data from table 1, B)*

Mean of mothers ( $x$ ) = 559.8  $\pm$  1.34 scutes

Mean of offspring ( $y$ ) = 559.05  $\pm$  0.57 scutes

$\sigma x$  of mothers = 15.34  $\pm$  0.95 scutes;  $\sigma x^2 = 235.37$

$\sigma y$  of offspring = 13.03  $\pm$  0.41 scutes;  $\sigma y^2 = 169.95$

$\sigma v^2$  of mothers and offspring = 180.29

Substituting, we get:

$$r_{xy} = \frac{235.37 + 169.95 - 180.29}{2(15.34 \times 13.03)} = 0.5638 \pm 0.0597$$

An analysis of these results leads to the following conclusions:

1. The degree of correlation between mothers and offspring is, on the average, very low as compared with that which obtains for sets of offspring. Taking into account the probable errors, that of both males and females approximates 0.5, which may be taken to mean that, lumping all sets together, half of the inheritance of the characters in question comes from the mothers and presumably the other half from the fathers. This does not necessarily mean that in each set there is an even blend between the scute counts of the two parents, for an examination of individual cases shows that such is not the case. Correlations between one parent and the offspring, closely equivalent to that determined above, have been shown to obtain for characters that are known to be inherited in the alternative fashion. For example, we note that according to Pearson and Lee ('00) the character of eye color in man runs from 0.55 to 0.44 (averaging almost exactly 0.5); again, the character of coat color in thoroughbred horses shows a degree of correlation, between sire and foal, of 0.517 and, between dam and foal, of 0.527. A survey of individual cases goes far to bear out the conclusion here suggested that aggregates of meristic variates may be inherited in the alternative fashion. Such a survey is attempted in the next section of the paper.

2. There appears to be practically no difference between the degree of correlation for mothers and males and that for mothers and females. In other words, there is no sexual dimorphism or sex limitation in the inheritance of the characters in question. Indirectly this goes to support the assumption that the fathers and mothers have an equal influence on the determination of the scute numbers and hence that a study of the modes of inheritance based solely on the mothers is likely to reveal all the fundamental facts of inheritance. What little difference exists between the coefficients of correlation of males and mothers and that of females and mothers is in favor of the latter, females being a little more like

their mothers than males are. In view of the fact, however, that the difference between the coefficients of uniparental correlation for males and females is considerably less than the probable error of the determination it may well be that the difference is unreal.

#### 4. EVIDENCES OF ALTERNATIVE INHERITANCE OF AGGREGATES OF MERISTIC VARIATES

The statistical method, if applied to the exclusion of the method of dealing with individual cases, is apt to cover up some of the most significant facts and to reveal only partial truths. A study of the individual cases given in table 1 indicates strongly that sets of quadruplets or individuals of a set tend to resemble one or other parent to the exclusion of the other in the matter of the total numbers of scutes in the banded region. Complete identity between individual offspring and the mother are common, too common to be mere matters of coincidence. Many cases exist in which the four quadruplets fluctuate only very slightly about the condition of the mother; while an equally large number of cases are found in which none of the individuals of a set are at all close to the mother in scute counts. These sets presumably fluctuate about the scute count of the fathers. A third class of cases exists in which one, two or three fetuses may be said to inherit the maternal scute number, while the remainder presumably inherit that of the fathers. From this it may be concluded that the germ plasm of the mother is in some cases prepotent or dominant for the entire set with respect to the factors that determine the numbers of scutes, in other cases that of the father dominates in the same fashion. It is not difficult either to understand that, since every cell of the body is a hybrid product, maternal influences might gain more or less complete ascendancy in some fetuses of a set, and might be subservient to the paternal influences in others, so that there might be a very real segregation within sets of quadruplets of maternal and paternal characteristics. An attempt might be made to classify the 115 sets of fetuses into three classes according to whether they show pure maternal, pure paternal or



mixed characters, but such a classification could at best be only an approximation of the truth. In certain cases it may well be that the conditions of father and mother are very much alike and in such cases it would be impossible to tell where to class the set. A few typical cases will serve to exemplify the conditions.

Set C 4 may be taken as a type of sets in which all four fetuses, in their scute numbers, fluctuate about the maternal condition. The mother has 574 scutes, and the fetuses respectively 574, 579, 571, 576 (average 575). Those that are not exactly like the mother cannot be said to be a blend between the number of the mother and that of the father, for some of the numbers are higher and one lower than that of the mother. Other sets showing the same condition are C 23, C 31, C 96, and so forth.

Set C 47 is a good example of the type of set in which there is a total lack of resemblance between mother and all the members of a set, and in which the paternal number is evidently dominant. The mother has 581 scutes and the four fetuses respectively 554, 559, 550, 559. Probably the father had a scute count of about 556. Other cases of this sort are C 20, C 21, K 25, and so forth.

Set C 29 shows the type of mixed set in which one individual closely resembles the mother and the other three depart widely from the latter and presumably closely resemble the father. The mother has 561 scutes and the fetuses, respectively, 549, 560, 547, 546. Fetus II is almost identical with the mother and I, III and IV fluctuate about the number of the father whose scute number was probably about 547. Other cases of this sort are seen in sets C 73, K 10, C 34, and so forth.

Set K 13 is an example of the type of set where one of the natural pairs closely resembles the mother and the other presumably the father. The mother has 565 scutes. The pair of fetuses III and IV have each 563 scutes, while the other pair, I and II, have respectively 575 and 570. Other cases of this kind are to be seen in sets C 75, K 9, C 27, and so forth. There are numerous cases in which one of each pair resembles the mother and the others do not. Set K 18 exemplifies such a condition. The mother has 559 scutes, and the fetuses, respectively, 559, 574, 559, 566. It might be conjectured that fetus II, with 574 scutes closely re-

sembles the father, while fetus IV, with 566 is almost exactly a blend between 574 and 559. Careful examination shows that the scute complex of the two sides are not equal, but that the right side is like that of the mother and the left side like the father. That there is a real intra-individual segregation of the tendencies of the two parents, following the line of bilateral symmetry will be conclusively shown in a future paper in connection with the inheritance of atypical peculiarities.

Set C 73 is a case in which only one of the four fetuses resembles the mother, while three of them probably fluctuate about the number of the father. The mother has 544 scutes and the fetuses, respectively, 555, 553, 551, 544. The father had presumably about 553 scutes. Other cases of this type are seen in sets C 79, K 77, K 89, and so forth.

Then, finally there are not a few cases where the individuals of a set differ considerably but none of them resemble the mother. For example let us consider set K 57. The mother has 552 scutes and the offspring, respectively, 536, 533, 540, 540. If we suppose for the sake of discussion that 533 or thereabouts represents the scute number of the father 544 would be an exact blend between the numbers of the two parents. The number 540 may be considered as an approximation of such a blend. It is highly probable that this apparent blending is due to a bilateral segregation of maternal and paternal tendencies so that in each of the fetuses III and IV one-half resembles one parent and the other half the other. I am unable to make myself fully clear by the use of the present material that there does exist a pronounced tendency for such a bilateral segregation of parental characters, but trust that I shall be able to offer convincing evidence of its reality in connection with the inheritance and distribution of double and split bands and scutes.

I am convinced, after much study of the data, that these large aggregates of meristic variates are inherited, as are many substantial characters, according to the laws of alternative inheritance. There are so many evidences of practically complete dominance of one or the other parent and so little evidence of any true blending that I am inclined to believe that, except in so far as some regions may be dominated by the maternal tendency and others by the paternal, there is no such phenomenon as blending.

At best an unreal appearance of blending might be produced by the fact that a large region, conceived of as a mosaic of pure maternal or pure paternal territories, might as a whole strike a kind of spurious average between the numbers characteristic of the two parents. As yet, however, I see no necessity of positing any intra-individual segregation more elaborate than that involved in the dominance of the paternal influence on one lateral half and that of the maternal on the other half. That the right and left half of the armor element are rather independent regions from the standpoint of their origin is clearly appreciated when we realize that before the invagination and closure of the neural tube the regions of embryonic ectoderm destined to form the right and left sides of the shell are widely separated from each other and, only on the closure of the neural tube and the healing together of the two wings of ectoderm, is a single shield produced. Since unquestionably the hereditary potentialities of these ectodermal regions was settled long before the period of embryonic development in question, it is not difficult to understand that one part might be in its tendencies quite different from the other; that one might be dominated by the maternal forces and the other by the paternal.

##### 5. POLYEMBRYONIC AND UNIPARENTAL CORRELATIONS IN INDIVIDUAL BANDS

In order further to test the validity of conclusions expressed in the earlier paper to the effect that, although the total number of scutes in the banded region is very rigidly predetermined, their alignment into bands is a mechanical process not subject to hereditary control, I shall herewith present further data derived from the new collection of material. It does not seem worth while to take up the large amount of space that would be needed for tabulation of the scute counts by bands of the 115 sets dealt with in table 1. Instead I shall use only 20 male and 20 female sets comprising a large part of the collection of fetuses marked 'C.' Forty sets are considered ample for our present purposes for the reason expressed above that even the original twenty sets give results almost identical with those determined for the much larger number dealt with in previous paragraphs. The reader is referred

to table 6 (pages 874-875) of our former paper (Newman and Patterson '11) for a good sample of the way in which scutes are distributed into the individual bands. It will suffice to present here a mere outline of the calculations used to determine the various coefficients of correlation that obtain for three of the bands (1, 5 and 9) taken as samples.

*a. Polyembryonic correlations for bands 1, 5 and 9 in 20 male and 20 female sets of quadruplets*

Band 1:  $\sigma x^2 = 3.524$

$$\sigma y^2 = 3.766$$

$$r = 0.4657 \pm 0.0576$$

Band 5:  $\sigma x^2 = 3.488$

$$\sigma y^2 = 4.45$$

$$r = 0.3625 \pm 0.0649$$

Band 9:  $\sigma x^2 = 4.624$

$$\sigma y^2 = 3.888$$

$$r = 0.5796 \pm 0.0482$$

*b. Parental correlations for bands 1, 5 and 9 in 20 male and 20 female sets of quadruplets*

Band 1: Mean of mothers ( $x$ ) = 62.35 scutes

Mean of offspring ( $y$ ) = 62.06 scutes

$$\sigma x^2 = 3.025$$

$$\sigma y^2 = 3.524$$

$$\sigma v^2 = 3.593$$

$$r_{xy} = 0.4542 \pm 0.0598$$

Band 5: Mean of mothers ( $x$ ) = 61.32 scutes

Mean of offspring ( $y$ ) = 61.13 scutes

$$\sigma x^2 = 3.269$$

$$\sigma y^2 = 3.488$$

$$\sigma v^2 = 4.65$$

$$r_{xy} = 0.3114 \pm 0.067$$

Band 9: Mean of mothers ( $x$ ) = 64.18 scutes

Mean of offspring ( $y$ ) = 64.23 scutes

$$\sigma x^2 = 4.288$$

$$\sigma y^2 = 4.624$$

$$\sigma v^2 = 6.742$$

$$r_{xy} = 0.5964 \pm 0.0434$$

The polyembryonic and parental correlations of the three bands will be more readily compared from the following tabulation:

	BAND 1	BAND 5	BAND 9
Polyembryonic 'r'.....	0.4657 $\pm$ 0.0576	0.3625 $\pm$ 0.0649	0.5796 $\pm$ 0.0482
Parental 'r'.....	0.4542 $\pm$ 0.0598	0.3114 $\pm$ 0.067	0.5964 $\pm$ 0.0434

In none of three bands dealt with is the difference between the polyembryonic and the parental correlation as great as the probable error and in one of the three bands (9) the parental correlation is a little larger than the polyembryonic. It will be further noted that the average uniparental correlation is 0.492, which is very close to that determined for the banded region as a whole. This must be true for the nine bands for it is these bands that make up the banded region.

#### 6. SUMMARY FOR THE BANDED REGION

1. Polembryonic correlation for 56 sets of male quadruplets is  $0.9294 \pm 0.0057$ ; that of 59 sets of female quadruplets is  $0.9129 \pm 0.0059$ . There is little real difference in this respect between the two sexes and the degree of correlation of both is of the same order as that determined for the antimerically paired organs of the same individual.

2. The evidence here presented does not confirm the earlier statements regarding the relative variability of the two sexes, for there appears to be no sensible difference between males and females in their degree of variability or closeness of polyembryonic correlation.

3. Males and females also inherit equally strongly from the mothers, showing that there is no sexual dimorphism in connection with the armor and no sex limitation in the inheritance of armor characters. The coefficient of uniparental correlation for both sexes is very close to 0.5, a coefficient which is to be expected in all cases of alternative inheritance.

4. That these aggregates of integral variates are inherited in the alternative fashion is strongly indicated by an examination of individual sets. There is little evidence of blending.

5. A study of the correlations, polyembryonic and uniparental, as determined for individual bands, taken in conjunction with a consideration of the conditions revealed by individual sets, leads to an abandonment of the earlier conclusion "that the process of scute alignment is largely mechanically determined and hence beyond the limits of hereditary control." The apparent lack of hereditary control is due to the fact that approximately half of the offspring inherit the number of scutes in a given band from the mother and the remainder presumably inherit the paternal number. The statistical method makes it appear that hereditary control is much weaker for the individual band than it is for the whole banded region, while an examination of individual sets reveals the opposite state of affairs. Thus does the statistical method, if relied upon too trustingly, lead to error.

#### INHERITANCE OF AGGREGATES OF SCUTES IN THE CAUDAL ARMOR

In order to determine whether the conditions found in the banded region are peculiar to that part of the armor or are of more general application, a study of other well defined regions has been made by way of comparison. In many ways the rings of the tail shield are as favorable for our purposes as are the bands of the mid-body region. In addition certain new features are introduced, in that there is a steady decrease in the size of the rings from anterior to posterior and that the rings completely surround the tail instead of covering only the dorsal side of it as is the case with the bands.

One of the less favorable features of the tail region for the study of heredity has to do with the fact that the region is much more liable to injury than are other regions and that, in consequence, a large percentage of the mothers brought in by the hunters have mutilated tails, some being chewed by dogs and others broken off by hunters in their attempt to pull the animals out of their burrows by the only handle that is within reach. Furthermore, a

considerable portion of the mothers in my collection 'K' were skinned and prepared for shipment in my absence and the tails were cleaned in such a fashion that an accurate scute count could not be made. It has been possible, however, to get together a collection of 40 sets (20 male and 20 female) in which the mothers had normal and uninjured tails and these constitute a sufficiently large collection for our purposes.

The number of well-defined caudal rings varied from 10 to 13 and is usually 11 or 12, most frequently the latter. For convenience, and because several specimens have no more than 10 rings, I have chosen to deal with only the first ten rings in all cases, just as in the banded region the first nine bands were always taken for tabulation although some specimens showed a tenth band. Although there are three rows of scutes to a band in some bands and only two or one in others, there is only one large prominent row bordering the posterior margin of each band. This is the only row counted for a ring. Scute counts, ring by ring, are given in table 2.

#### 1. POLYEMBRYONIC CORRELATIONS IN THE CAUDAL ARMOR AS A WHOLE

As was done in the case of the nine movable bands taken as a whole, I shall deal first with the total number of scutes in the caudal region. As before only the bare outline of the calculations is presented.

*a. Correlation for the total number of scutes in the 10 tail rings for 20 sets of male quadruplets (78 individuals)*

Mean = 166.6 scutes

$\sigma x = 6.072$ ;  $\sigma x^2 = 36.87$

$\sigma v^2 = 7.728$

Substituting, we get:

$$r = 1 - \frac{1}{2} \frac{7.728}{36.87} = 0.8939 \pm 0.013$$

*b. Correlation for the total number of scutes in the 10 tail rings for 20 sets of female quadruplets (79 individuals)*

Mean = 167 scutes

$\sigma x = 7.095$ ;  $\sigma x^2 = 50.35$

$\sigma v^2 = 7.2307$

Substituting, we get:

$$r = 1 - \frac{1}{2} \frac{7.095}{50.35} = 0.9282 \pm 0.0093$$

## 2. UNIPARENTAL CORRELATIONS IN THE CAUDAL ARMOR AS A WHOLE

*a. Correlation for the total number of scutes in the 10 tail rings between 20 sets of male fetuses and their mothers*

Mean of mothers = 168.7 scutes

$\sigma x$  of mothers = 6.604;  $\sigma x^2 = 43.61$

$\sigma v^2$  between mothers and offspring = 72.32

$$r_{xy} = \frac{43.61 + 36.87 - 72.32}{2(6.604 \times 6.072)} = 0.1018 \pm 0.0642$$

*b. Correlation for the total number of scutes in the 10 tail rings between 20 sets of female fetuses and their mothers*

Mean of mothers = 169.9 scutes

$\sigma x$  of mothers = 7.38;  $\sigma x^2 = 54.49$

$\sigma v^2$  between mothers and offspring = 60.59

$$r_{xy} = \frac{54.49 + 50.35 - 60.59}{2(7.38 \times 7.095)} = 0.4261 \pm 0.0558$$

These results bring out the following facts:

1. Polyembryonic correlation for the tail armor is of the same order as that shown to obtain for the banded region, the former averaging for males and females 0.9109, the latter 0.9211. Later it will be shown that this same high order of correlation obtains for all armor regions and for certain other characters of an entirely different sort.



2. There is a small sexual difference in the matter of the number of scutes, females being a little more closely correlated polyembryonically than males. In the banded region, it will be remembered, the males were a little more closely correlated than the females. Probably there is no real sexual difference in this matter that would hold good for any collection of quadruplets.

3. With regard to uniparental correlation, however, there is a very pronounced difference between the two sexes (at least in the 40 sets dealt with here). The correlation between mothers and males, especially if we take into account the probable error, is so low as to amount to practically no correlation. This would seem to mean that, in this collection at least, the males inherit much more strongly from the fathers than from the mothers. In the female sets, however, the correlation coefficient is not far from 0.5, and in that respect about the same as that which obtains for both sexes in the banded region. Probably the statistical treatment in this case conceals some of the truth as will be brought out in the next paragraph.

## 2. FURTHER EVIDENCE OF ALTERNATIVE INHERITANCE OF GROUPS OF MERISTIC VARIATES

A glance down the 'totals' column of table 2, A, will show that in practically all cases the numbers of scutes of the four quadruplets either fluctuate about that of the mother, or else are quite unlike that of the mother and are presumably inherited from the father. The following may be said to be maternal: C 21, C 23, C 46 (mixed), C 76, C 91, K 64, K 90. The rest are to be considered as paternal. In this collection of 20 sets of male quadruplets it happens that the proportion of sets like the mother to those like the fathers is  $6\frac{1}{2}$  to  $13\frac{1}{2}$  (counting the mixed set as belonging equally to the two classes). This great predominance of sets resembling the fathers is undoubtedly responsible for the almost total lack of correlation between the male offspring and the mothers. I do not believe that such a condition would be found to hold generally, but that in a small collection of 20 sets one is very apt to have a disproportion between sets inheriting the scute numbers of the mother and those inheriting them from

the father. In another collection we would probably find the disproportion swinging the other way.

A similar survey of the figures shown in table 2, B, shows the same state of affairs, except that there is a more equal distribution of maternal and paternal sets. The following sets may be classed as maternal: C 27, C 32 (mixed), C 43, C 45, C 63 (mixed), C 66, C 68 (mixed), C 75 (mixed), C 79 (mixed), C 80, C 95 (mixed), K 48 (mixed), K 82. The others are classed as paternal. Counting the mixed sets, which have some maternal and some paternal individuals as one-half each, we have  $9\frac{1}{2}$  cases maternal and  $10\frac{1}{2}$  paternal. This slight preponderance of paternal sets accounts for the coefficient of correlation between females and mothers being somewhat below the expected 0.5. It is actually  $0.4261 \pm 0.0558$ .

It will be noted that this data shows even fewer evidences of blended inheritance than that given for the banded region and more striking evidences of a very typical sort of alternative or exclusive inheritance. If blending occurs at all it plays only a very minor rôle in the inheritance of these scute aggregates.

### 3. CORRELATIONS IN INDIVIDUAL TAIL RINGS

Little need be said by way of preface to tables 3 and 4 which give respectively the polyembryonic and the uniparental correlations computed from the data given in table 2.

Analysis of table 3 shows substantially the same conditions that were brought out in connection with the individual bands in the banded region. A comparison between the two regions may readily be made by placing together this table and table 10 (page 882) of the former paper which I have referred to several times. Although in both the bands and the rings the polyembryonic correlation is very high (0.9211 for the bands and 0.9109 for the rings) when considered in the aggregate, that of individual bands or rings falls markedly lower, that of the bands on the average being 0.6459 and that of the rings 0.6263. The resemblance between the two types of correlation in these two widely separated regions of the armor is remarkably close and probably represents a rather general relation that will be found to apply even more

widely. In the banded region the coefficient of polyembryonic correlation for individual bands ranges from 0.5051 to 0.7458; in the caudal region that of individual tail rings ranges from 0.5241 to 0.7454. Here again there is close agreement between the two regions of armor.

This striking similarity must mean that in both regions the same factors are in operation and that what is true for one region is true for the other. Already it has been stated more than once that the comparatively low coefficient that obtains for individual bands means that there is practically no predetermination with respect to the alignment of scutes into individual rows. Although this may be true for groups or averages I think I can show that the statistical method once more conceals a considerable part of the truth; for it is quite clear, as we shall see in the following paragraphs, that with respect to individual rings of individual members of sets of quadruplets there is a strong tendency for an exact repetition of the maternal scute numbers, which means not only germinal predetermination but also exclusive or alternative inheritance.

In table 4 certain peculiar conditions come to light. In some of the rings the uniparental (maternal) correlation is almost as high as the polyembryonic correlation, in others—the probable error being taken into account—there is practically no correlation between mothers and offspring, while in the remaining rings the coefficient of correlation falls about midway between no correlation and that which obtains for the polyembryonic relation. This state of affairs does not in the least comply with my notion of a consistent scheme of blended inheritance, but does strongly indicate at least a strong predominance of some sort of alternative inheritance. In some bands the resemblance between mother and offspring is almost as strong as that which exists among the quadruplets; in others there is practically no resemblance to the mother and we must assume that the paternal influence is dominant. On this basis one would expect, if the influence of both parents is equal, an average uniparental coefficient of correlation of just half that of the average polyembryonic coefficient of correlation for the ten rings, which is 0.3131. The actual average maternal

coefficient of correlation for the ten bands is 0.3470 which is very close to expectation especially if we consider that there is an average probable error of  $\pm 0.04$ .

When one comes to study individual cases he is still more forcibly impressed by the predominance of the alternative mode of inheritance as opposed to the blending mode. One might take almost any set at random to illustrate this point. In set C 23 (table 2, A) for example, we find the following rows with exactly the same numbers of scutes as the mother:

Fetus I	Rings 3, 6, 7, 8, 9, 10
Fetus II	Rings 5, 7, 9
Fetus III	Rings 5, 7, 8, 10
Fetus IV	Rings 1, 2, 3, 5, 7, 8, 9, 10

In the forty rings of the four fetuses 21 are exactly like the corresponding rings of the mother and 19 are unlike those of the mother and presumably like those of the father. This interpretation of the situation receives a set-back when we note that in ring 1 and also in ring 2 three numbers of scutes appear, of which one is identical with that of the mother. Of course both of the other numbers cannot be identical with those of the father, hence there must be a small amount of blending or striking a compromise between the conditions of the two parents or else there is a certain amount of incomplete dominance. Only two rings out of forty break the rule and we must conclude that the amount of blending, incomplete dominance, is of small and possibly of questionable significance. This set just considered was classed as a mixed set on the basis of the totals in the ten rings and it shows the admixture of the alternative maternal and paternal characters in the individual bands. Let us now examine a set in which there is little or no mixture of the two parental tendencies. Such cases are common enough and we may take as a type set C 30 in which only 2 rings out of 40 are identical with those of the mother. As a type where the maternal influence is predominant we might cite C 90 where 31 out of 40 rings are identical with those of the mother. These are only samples and one can select many other cases equally well illustrating the point.

Perhaps, however, the best evidence of the existence of alternative inheritance is found on the examination of the conditions in individual offspring. The nearest approach to complete identity between one parent and an individual offspring is seen in set C 95 in which *fetus II* has nine out of its ten rings identical with those of the mother. There are several cases in which there is complete identity for 8 rings, and still more for 7 rings, between mother and individual offspring. Such cases are surely good examples of a sort of dominance of the tendencies of one parent over those of the other and are in that sense to be considered as belonging to the category of characters inherited according to the laws of alternative inheritance.

#### COMPARATIVE STUDY OF THE INHERITANCE OF SCUTE AGGREGATES IN THE FIVE MAIN ARMOR SHIELDS

In order that the present study may be complete for the armor it is necessary to bring in for comparison the data for the other three shields, cephalic, scapular and pelvic. The labor involved in counting the scutes in the two last named shields, which possess an average of 2000 scutes to the individual, is so great that it is necessary to limit the number of sets studied to 20, 10 of each sex. These 20 sets were chosen solely on the basis of the size of the fetuses, since it is necessary to use the most advanced stages in order to obtain accurate counts in all regions. This is especially true for the scapular and pelvic shields where the scutes are very small and are not arranged regularly in rows. It is necessary to mark with ink spots the scutes as enumerated and much time is consumed in the process. The cephalic shield is the least favorable for study on account of the fact that its scutes are not sharply marked off from those of the face and nose. It is only by setting up certain artificial boundary lines that one is able to secure even a reasonably accurate comparison between parent and offspring. So far as individuals of a set are concerned, however, this difficulty is not serious, for one can readily decide before beginning the process of counting whether or not certain doubtful border-line scutes are to be included in the count.

It will be shown in this section of the paper that, although in one region of the armor, for example the banded region, the maternal scute number may be dominant, other large sections of the armor may show a dominance of the paternal scute number. In brief there may be a mosaic of maternal and paternal regions of dominance involving larger regions than any that have been dealt with in the earlier parts of the paper. The data upon which this study is based is given in full in tables 5, A and B.

#### 1. POLYEMBRYONIC CORRELATIONS FOR THE FIVE MAIN ARMOR SHIELDS

The results in bare outline as derived from a statistical treatment of the data given in extenso in table 5 are presented in comparative form in table 6. A perusal of this table brings out the fact that the polyembryonic coefficient of correlation for every one of the five armor shields is in excess of 0.9 and that there is very little difference between the degree of correlation determined for the various regions. One might conclude that correlations of this magnitude are of very general occurrence in connection with polyembryony. Probably the highest coefficient of correlation ever determined is that which obtains for the 20 sets just dealt with in the caudal shield, namely, 0.9898. This is remarkably close to absolute correlation and will probably hold the record for such coefficients indefinitely. Further comment on the table is unnecessary as the figures speak for themselves.

#### 2. UNIPARENTAL CORRELATIONS FOR THE FIVE MAIN ARMOR SHIELDS

In table 7 are given all of the figures used in calculating the coefficients of correlation that obtain between mothers and offspring for the five armor shields, except those already presented in table 6. It will be noted that, with the exception of the caudal shield, the coefficients average approximately 0.5. In the case of the caudal shield, however, there is scarcely any appreciable correlation between mothers and offspring. We are therefore justified in assuming that, in this particular lot of fetuses, the

inheritance is predominantly paternal. Probably another lot of 20 sets would show either a more even balance between the maternal and paternal tendencies or a dominance of the maternal characteristics. It need scarcely be reiterated that such a condition as this favors the idea that inheritance is largely of the alternative rather than of the blended type.

### 3. A SURVEY OF INDIVIDUAL SETS

Reference to table 5, A and B, will show that there are sets of quadruplets in which the maternal influence is predominant, or at least evident, in all of the five shields. Set C 23, for instance, shows in the cephalic shield an admixture of maternal and paternal tendencies, fetuses III and IV being maternal and the others paternal; the scapular, banded and pelvic shields show dominance of the mother in all four fetuses; and the caudal shield shows the same two fetuses maternal that are maternal for the cephalic shield while the others are presumably paternal. Fetuses III and IV show a dominant maternal influence in all five shields, while fetuses I and II are maternal in three out of five shields. The grand total of the mother for the five shields is 2897, while that of the four fetuses averages 2904, a difference of only 7 scutes. On the whole then the paternal influence must have been very slight indeed.

In set C 15 is seen a somewhat similar state of affairs. In the cephalic shield I is maternal, in the scapular shield, banded region and pelvic shield all fetuses are maternal, while in the caudal shield all are paternal, or at least not strongly maternal. Yet the grand total of the mother, 3019, is identical with the average grand total of the four offspring.

As examples of sets in which the maternal influence is largely or wholly recessive we may cite sets C 28, C 47 and C 81. In these sets none of the five shields shows scute counts at all closely similar to those of the mother and are presumably pure paternal. Then there are several sets in which there is a fairly equal distribution between the two parents as to their dominance over the different shields. Such sets are C 21 and C 92 in which two shields are maternal and three paternal.

Although it is much more common to find all four fetuses maternal or paternal for any particular shield there are many sets in which a more complex distribution of parental dominance exists. C 95 is a good example of such a set, for it is clear that in the cephalic shield all fetuses are paternal, in the scapular shield II, III and IV are maternal, in the bands II and IV are maternal, in the pelvic shield I is maternal, while in the caudal shield I and II (and possibly III and IV) are maternal. Of the 20 regions of the four fetuses 10 are maternal in scute counts and the other 10 are presumably paternal. Such a state of affairs as this is probably the result of the union of two parents of rather similar scute characters, so that there was no very pronounced opposition of scute numbers in any of the regions. The balance between the influences exerted by the two parents is so equal that there is a sort of alternating dominance even in a given shield, in that some fetuses are, with respect to any given shield, maternal and others paternal. When two parents are sharply opposed, however, either with respect to the whole armor or with respect to any shield, one or the other parental tendency dominates in all fetuses. It is impossible to determine what factors may be responsible for the dominance of one or the other parental tendencies. Sometimes the larger number dominates the smaller and sometimes the opposite is true. We may have to do with the comparative vigor of maternal and paternal germ cells or to mere chemical differences in the materials whose activities result in the production of scutes. The result is a typical case of alternating dominance.

An interesting correlation with regard to parental resemblances exists between certain of these largest aggregates of scutes in that it is very common to find that the three shields comprising the body armor (scapular, bands and pelvic) behave in inheritance as though they were a single region, all three being either maternal or paternal. Such a state of affairs is found in sets C 15, C 23, C 21, C 76, and so forth. The division of the body armor into these three regions is after all really arbitrary and is due to the fact that a certain mid-body region has been secondarily broken up into movable bands. Ancestrally the whole shield was doubtless rigid and evolution has been in the direction of developing



more and more bands, which give greater freedom of movement without any loss of protection. Frequently, however, the body armor fails to act as a single unit in inheritance, for there are cases in which the bands are maternal and the other shields paternal or vice versa.

#### CONCLUSIONS AND SUMMARY

What facts of importance have we succeeded in bringing to light after an exacting and laborious statistical study of the data presented? Have the results justified the time and labor and printer's ink necessary for their publication? In reply to these inquiries it may be answered (and the answer will appeal forcibly, I believe, to the reader) that the unique character of the material challenges the investigator's curiosity to such an extent that he is impelled to attempt to unravel the mystery. The student of genetics has a right to his own opinions as to the value of these results and may be able to add much by way of interpretation. Whatever differences there may be regarding the theoretical bearings of these results the data stand for themselves and are offered to the expert for further analysis.

No doubt the conclusions, for the most part, might have been anticipated on the grounds of what we know of inheritance in general and of the peculiar mode of reproduction of the armadillo in particular. Yet it is impossible to know in advance whether material will yield richly or poorly, whether we shall be rewarded with striking results or only commonplace findings.

To my mind the points that are distinctly worth the labor expended in their determination are as follows:

1. The coefficient of correlation for the polyembryonic relation has been shown to be very much the same for all of the larger region of the armor, in each case exceeding 0.9. We have here evidence of the rather general occurrence of this unparalleled approach to identity between these closest of blood relatives. This finding strongly supports the taxonomic assumption that degrees of resemblance are in direct ratio to degrees of blood relationship.

2. Within single sets of quadruplets there is a sort of segregation or distribution of the parental influences, so that some indi-

viduals in some or all of their regions resemble one parent closely and others resemble the other parent. This probably means that the personality of each of the quadruplets was not determined at the time of fertilization but that there is a struggle from the beginning of life until all growth processes are at an end between the two opposed parental tendencies, and whether for any region one or other parental tendency predominates depends on certain internal or external factors incident to growth.

3. Probably no other material is so well adapted for the study of the inheritance of meristic variates as are the scute complexes of the armadillo armor. No detailed study of the inheritance of such intricate and complex groups of characters has ever been attempted. For the most part workers in the field of genetics have chosen for study rather simple substantive characters because they give simpler and more readily analyzable results. Of the comparatively small amount of genetic work that has been done with meristic variates as a basis most of it has had to do with dimensional variates, in spite of the fact that fluctuations in dimensions are well known to be to a large extent influenced by nutritive factors and are little if at all matters of inheritance. The present material is such that the definitive condition of the scute aggregates is reached in the uterus long before birth and is uninfluenced by the only variable external condition that seems to be present, namely, nutrition. The justification for this statement is contained in the observation that differences of size, weight and stage of development (which are unquestionably the obvious results of inequalities of nutrition) in no way affect the degrees of resemblance existing between the members of sets of quadruplets. There are not a few cases in which there are marked discrepancies between the members of a single set with respect to size, and so forth, and no corresponding difference in scute counts. There is one case, for example, in which one pair of fetuses appears to be fully a month further along in development, than the other pair; yet all four are strikingly alike in all characters having to do with scute numbers and arrangement. Other cases occur in which one or three retarded fetuses are in the same cho-

tion with much more advanced fellow fetuses, but there is no unusual difference as to scutes. Such dimensional differences within sets are no doubt due to accidental interferences with the food supply of a more or less radical character. The fact that these very pronounced inequalities in growth conditions fail to disturb the inheritance ratios proves in the most unequivocal way, I believe, that the resemblances and differences that we have studied in connection with the scutes and their aggregates are purely matters of heredity and as such are ideal for our purposes.

4. Unless the writer is totally misled by the data, which are given in extenso so that the reader may have the opportunity of making his own judgment, the mode of inheritance of larger and smaller groups or aggregates of scutes is primarily alternative with only a minor degree of blending. It is highly probable that blending would disappear entirely if smaller aggregates than those treated were to be dealt with, for the apparent blending in the larger armor regions may be merely the result of an averaging of the scute numbers belonging to the mosaic of minor inheritance regions of which some are maternal and others paternal in character. There is doubtless a considerable degree of incompleteness in dominance, just as there is in practically all cases of alternative inheritance, which would account for the smaller fluctuations of the four quadruplets about the maternal scute number or, presumably, the paternal. It is interesting, however, to note that in the smaller aggregates, such as the tail rings this incompleteness in dominance disappears to a large extent and we find in the offspring a very large number of rings with exactly the same number of scutes as occur in the corresponding rings of the mother. Presumably this is also true for the father. Incomplete dominance is the rule even in the case of the simplest characters and hence we must expect the same or a greater degree of incompleteness in the case of such complex characters as those which form the basis of the present study. And we would expect also to find that the more precise are the methods of comparison between parent and offspring the less complete would be the dominance. No such precise and searching comparison of genetically related individuals

has ever been attempted as that here presented, and it should be a matter of some surprise that there is as close an approach to complete dominance as there appears to be.

In a subsequent paper the writer intends to carry out a study of inheritance of smaller groups of integral variates and of single units. The results of this study might be anticipated to the extent of saying that they strongly support the conclusions expressed in the present paper, and especially those regarding the alternative character of the inheritance of meristic variates.

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TABLE I A

*Showing the total number of scutes in the nine bands of 56 sets of male fetuses and of their mothers*

SET NO.	MOTHER	FETUS				SET NO.	MOTHER	FETUS			
		I	II	III	IV			I	II	III	IV
C 4	574	574	579	571	576	K 21	576	548	549	553	562
C 5	539	554	548	562	558	K 24	544	554	548	560	553
C 20	566	543	549	547	549	K 25	560	526	531	531	537
C 21	566	539	538	533	538	K 29	540	566	572	562	564
C 23	575	576	572	580	574	K 32	575	584	588	589	586
C 28	565	574	577	582	574	K 38	570	566	569	564	563
C 29	561	549	560	547	546	K 39	539	547	544	547	543
C 30	558	548	554	557	553	K 41	558	554	559	561	556
C 31	570	567	576	566	567	K 47	550	550	544	539	547
C 34	560	570	564	572	560	K 49	575	567	572	567	575
C 44	564	547	543	548	552	K 50	555	546	538	545	544
C 47	581	554	559	550	559	K 51	558	550	552	554	553
C 64	541	541	547	542	546	K 53	548	554	554	554	553
C 71	549	549	556	555	552	K 54	565	565	576	569	568
C 73	544	555	553	551	544	K 56	554	576	586	579	575
C 76	569	560	552	554	558	K 58	546	541	531	546	541
C 91	570	583	586	579	582	K 61	555	542	542	546	544
C 92	590	589	584	585	579	K 64	557	570	577	567	570
C 96	553	557	550	553	551	K 65	533	563	561	555	560
K 1	575	573	568	578	581	K 67	560	557	556	555	555
K 7	580	578	589	585	583	K 70	539	525	528	530	531
K 10	539	547	539	549	544	K 73	541	521	518	523	520
K 12	537	548	555	552	554	K 74	543	572	561	567	562
K 13	565	575	570	563	563	K 76	583	572	573	571	576
K 14	583	573	571	574	579	K 81	548	568	572	569	572
K 15	562	557	548	551	549	K 83	558	564	556	559	554
K 17	581	570	573	576	578	K 86	554	526	531	527	537
K 18	559	559	574	559	566	K 90	552	546	552	556	550

TABLE 1 B

*Showing the total number of scutes in the nine bands of 59 sets of female fetuses and of their mothers*

SET NO.	MOTHER	FETUS				SET NO.	MOTHER	FETUS			
		I	II	III	IV			I	II	III	IV
C 9	577	558	558	559	553	K 27	574	578	570	578	575
C 15	584	581	576	582	584	K 28	577	565	564	559	568
C 18	554	555	552	550	549	K 31	542	548	552	556	557
C 22	587	591	594	596	596	K 34	541	538	540	541	549
C 24	575	564	566	560	558	K 35	585	568	570	563	576
C 26	568	560	558	567	558	K 36	569	565	565	563	563
C 27	565	577	559	567	565	K 37	561	562	554	570	558
C 36	564	580	579	581	578	K 42	541	534	528	529	536
C 42	554	534	539	535	543	K 43	582	587	583	577	585
C 43	541	569	566	562	562	K 44	565	552	549	548	548
C 62	559	549	551	553	556	K 45	531	565	565	563	563
C 65	543	561	563	573	573	K 46	562	555	551	548	553
C 66	564	538	537	545	540	K 48	589	577	571	582	574
C 67	535	556	556	555	555	K 52	567	572	569	571	568
C 68	544	571	569	564	561	K 55	551	554	561	555	549
C 72	539	562	565	558	553	K 57	552	536	533	540	540
C 75	557	548	544	555	555	K 59	576	563	575	566	569
C 79	535	537	550	541	548	K 60	536	547	543	542	549
C 81	556	565	566	574	567	K 62	553	540	538	546	541
C 89	570	560	553	558	558	K 63	564	547	543	547	545
C 95	564	556	565	557	562	K 68	545	540	536	542	544
K 3	560	562	559	562	560	K 72	544	541	542	547	545
K 5	566	557	556	561	558	K 75	561	564	561	561	557
K 6	550	564	566	569	561	K 77	549	557	549	554	558
K 9	555	554	556	561	563	K 79	568	577	570	573	570
K 11	548	542	539	544	541	K 82	557	559	553	559	555
K 19	572	563	560	573	573	K 84	609	572	573	569	584
K 20	572	565	573	576	579	K 85	565	556	558	558	561
K 23	539	551	554	550	553	K 89	563	551	559	562	550
K 26	551	566	565	559	569						

TABLE 2A

Showing the numbers of scutes in the first 10 rings of the tail in 20 male fetuses and their mothers

SERIES NO.	FETUS OR MOTHER	NUMBER OF TAIL RING										REMARKS	
		1	2	3	4	5	6	7	8	9	10		Total
C 21	I	27	24	22	19	17	16	14	14	12	12	177	
	II	28	25	21	19	17	16	14	14	12	12	178	
	III	27	25	22	19	17	16	14	14	12	12	178	
	IV	27	24	22	18	17	16	14	14	12	11	175	
	M	28	25	22	18	18	16	15	13	12	11	178	
C 23	I	26	25	21	19	16	15	14	12	12	11	171	
	II	27	23	20	19	17	16	14	13	12	11	172	
	III	26	23	20	19	17	16	14	12	11	10	168	
	IV	25	22	21	19	17	16	14	12	12	10	168	
	M	25	22	21	18	17	15	14	12	12	10	166	
C 28	I	28	23	20	17	16	14	14	13	11	10	164	
	II	25	23	21	18	17	14	14	12	12	10	166	
	III	24	22	20	18	16	15	14	12	12	10	163	
	IV	26	23	19	19	16	14	14	12	11	10	164	
	M	27	24	22	19	17	15	14	13	12	10	173	
C 29	I	24	22	18	17	14	14	12	11	10	10	152	
	II	24	22	19	17	14	13	12	11	10	10	152	
	III	26	21	18	16	15	14	13	12	11	10	156	
	IV	24	21	18	16	15	14	13	12	11	10	154	
	M	27	22	20	18	15	15	14	12	11	10	164	
C 30	I	25	22	20	18	17	14	14	13	11	9	163	
	II	26	22	20	18	16	14	14	12	11	10	163	
	III	26	22	20	18	16	15	13	12	11	10	163	
	IV	25	22	20	18	16	14	14	12	11	10	162	
	M	27	24	21	19	17	16	15	13	12	11	175	
C 31	I	24	22	20	17	16	14	13	11	11	10	158	
	II	26	22	19	18	16	15	12	12	10	10	160	
	III	24	22	20	17	16	15	13	12	10	10	159	
	IV	25	22	18	17	15	14	13	12	11	10	157	
	M	28	23	21	18	17	16	14	13	12	10	172	
C 44	I	24	24	20	18	17	15	13	12	11	10	164	
	II	25	23	20	18	16	14	14	12	11	10	163	
	III	25	22	20	18	16	14	14	12	11	10	162	
	IV	26	23	19	19	15	15	14	12	11	10	164	
	M	25	21	19	18	16	14	13	12	11	10	159	
C 46	I	27	25	20	19	16	16	14	12	12	11	172	
	II	26	25	22	19	16	16	14	13	11	10	172	
	III	27	23	19	18	16	15	14	13	11	11	167	
	IV	27	22	20	18	16	14	14	12	12	11	166	
	M	27	24	21	18	18	15	14	13	12	11	173	

TABLE 2 A—Continued

SERIES NO.	FETUS OR MOTHER	NUMBER OF TAIL RING										REMARKS	
		1	2	3	4	5	6	7	8	9	10		TOTAL
C 47	I	26	23	19	17	16	15	14	13	12	10	165	
	II	27	23	19	18	16	15	13	12	11	10	164	
	III	27	25	21	18	16	15	13	12	11	10	168	
	IV	27	23	20	19	16	14	14	12	12	11	168	
	M	29	23	22	19	18	16	16	13	12	11	179	
C 71	I	25	23	19	18	17	16	14	12	12	10	166	
	II	26	23	20	18	17	15	14	13	12	10	168	
	III	25	21	19	18	16	15	14	12	12	10	162	
	IV	24	22	20	18	16	15	14	13	12	10	164	
C 76	M	29	22	21	18	17	15	14	13	12	10	171	
	I	26	23	19	18	16	14	13	12	11	10	162	
	II	26	24	19	18	16	14	13	12	10	10	162	
	III	25	22	19	18	17	15	14	12	11	10	163	
	IV	26	22	19	18	16	15	13	12	10	10	161	
C 91	M	27	23	20	18	16	14	12	11	10	10	161	
	I	27	21	19	18	17	15	14	13	12	10	166	
	II	26	23	19	17	16	16	14	12	12	10	165	
	III	27	22	20	18	16	15	14	13	11	11	167	
	IV	26	24	22	19	17	16	14	13	12	11	174	
C 92	M	27	22	21	18	17	16	15	14	12	10	172	
	I	26	26	20	18	16	14	13	13	12	10	168	
	II	25	23	21	19	16	14	13	13	11	10	165	
	III	27	24	20	18	16	15	14	12	11	10	167	
	IV	26	23	19	17	17	15	13	12	11	10	163	
C 93	M	29	26	22	19	17	15	15	13	12	11	179	
	I	26	25	22	19	17	14	14	14	12	10	173	Only 3 fetuses
	II	27	25	22	19	17	15	14	12	12	10	174	
	III	26	24	22	18	17	15	15	13	12	10	172	
	M	26	22	19	17	16	14	13	12	12	10	161	
C 94	I	28	26	22	19	17	16	14	13	12	11	178	Only 3 fetuses
	II	27	25	22	19	17	16	14	13	12	11	176	
	III	26	25	21	19	18	16	14	13	12	11	175	
	M	26	21	20	17	16	15	14	12	12	10	163	
	I	27	25	21	19	16	15	13	12	12	10	170	
C 96	II	26	23	21	18	16	15	14	13	11	10	167	
	III	26	24	21	18	16	15	14	12	12	10	168	
	IV	26	24	21	18	17	16	14	12	12	10	170	
	M	26	23	19	18	15	14	13	12	11	9	160	
	I	28	24	21	19	17	16	14	13	12	11	175	
K 16	II	28	25	22	19	18	16	14	13	12	10	177	
	III	28	24	21	19	16	16	14	14	12	11	175	
	IV	29	24	21	19	18	15	14	13	11	10	174	
	M	28	24	21	19	18	15	14	14	12	11	176	



TABLE 2 A—Continued

SERIES NO.	FETUS OR MOTHER	NUMBER OF TAIL RING										REMARKS	
		1	2	3	4	5	6	7	8	9	10		TOTAL
K 24	I	27	23	20	18	17	16	14	13	12	11	171	
	II	27	23	22	20	17	15	14	14	12	11	175	
	III	27	23	21	19	17	16	14	13	12	11	173	
	IV	27	24	20	19	16	15	14	14	12	11	172	
	M	26	23	20	18	16	14	14	12	11	10	164	
K 64	I	25	22	19	18	16	14	13	12	11	10	160	
	II	26	22	20	18	16	15	13	12	11	10	163	
	III	27	22	20	18	17	15	13	12	11	10	165	
	IV	26	21	19	19	16	14	14	12	11	10	162	
	M	26	22	20	18	17	15	13	12	11	10	164	
K 90	I	26	23	20	17	16	14	13	12	11	10	162	
	II	26	23	20	18	15	14	14	12	11	10	163	
	III	26	23	21	18	16	14	13	12	11	10	164	
	IV	26	25	21	18	16	14	13	12	11	10	166	
	M	26	23	20	19	16	14	13	12	11	10	164	

TABLE 2 B

Showing the numbers of scutes in the first 10 rings of the tail in 20 female fetuses and their mothers

SERIES NO.	FETUS OR MOTHER	NUMBER OF TAIL RING										TOTAL
		1	2	3	4	5	6	7	8	9	10	
C 15	I	27	25	21	20	17	16	15	13	13	11	178
	II	28	25	21	19	17	16	14	14	12	11	177
	III	27	23	22	19	18	16	15	14	13	11	178
	IV	28	25	20	20	17	16	14	14	12	11	177
	M	29	26	23	20	19	17	16	14	14	13	191
C 18	I	26	25	20	19	17	16	14	13	11	10	171
	II	26	25	21	19	18	16	14	13	12	11	175
	III	26	25	22	19	17	17	14	13	11	10	174
	IV	27	25	20	20	17	16	14	13	11	11	174
	M	23	23	21	18	16	15	13	12	11	10	162
C 22	I	29	25	22	19	17	16	14	13	12	11	178
	II	29	25	22	21	17	15	14	14	12	11	178
	III	26	25	21	19	18	16	15	13	12	11	176
	IV	29	25	22	19	16	16	14	13	12	11	177
	M	25	23	20	17	17	16	14	13	11	11	167
C 27	I	26	22	21	18	17	15	14	13	11	10	167
	II	28	22	20	20	16	14	14	12	12	10	168
	III	25	23	20	19	17	15	14	12	12	10	167
	IV	26	23	20	18	17	14	14	13	12	10	167
	M	27	24	19	18	16	15	13	12	12	10	166
C 32	I	26	22	19	18	17	15	14	13	12	11	167
	II	27	23	22	19	16	15	14	12	11	10	169
	III	27	23	21	19	17	14	14	12	12	11	170
	IV	27	24	21	19	17	16	14	12	12	11	173
	M	26	22	20	18	16	15	14	13	12	10	166
C 43	I	27	26	24	20	18	17	15	14	12	10	173
	II	27	27	22	20	18	17	15	14	12	10	172
	III	27	25	23	21	18	16	16	14	12	10	172
	IV	27	26	22	20	18	17	15	14	12	10	171
	M	27	25	22	21	18	17	15	14	12	10	171
C 45	I	24	22	20	18	16	16	14	12	12	11	165
	II	25	23	18	17	16	15	13	12	12	11	162
	III	25	21	20	18	16	14	14	12	12	11	163
	M	26	22	21	18	16	15	14	12	12	11	167
	I	26	23	19	16	15	15	13	12	11	10	160
C 62	II	25	21	19	16	16	14	13	12	12	11	159
	III	24	23	19	16	15	14	14	11	11	11	158
	IV	24	21	20	17	15	14	14	12	11	10	158
	M	28	25	21	18	18	16	14?	14	12	12	178

TABLE 2 B—Continued

SERIES NO.	FETUS OR MOTHER	NUMBER OF TAIL RING										TOTAL
		1	2	3	4	5	6	7	8	9	10	
C 63	I	25	24	20	18	16	14	14	12	11	10	164
	II	26	23	20	18	16	14	13	12	11	10	163
	III	25	23	20	18	17	14	13	12	11	10	163
	IV	25	23	21	17	15	14	13	11	10	10	159
	M	26	24	20	17	16	15	13	12	12	10	165
C 65	I	26	22	20	18	16	14	13	12	11	10	162
	II	25	23	19	18	16	14	13	12	11	10	161
	III	27	22	19	18	16	15	13	12	11	10	163
	IV	25	23	19	18	16	14	13	12	11	9	161
	M	26	24	23	19	17	15	14	12	11	10	171
C 66	I	25	23	19	20	17	16	14	13	11	11	169
	II	27	24	21	18	16	14	14	13	12	11	170
	III	27	24	21	18	16	15	14	12	11	10	168
	IV	27	23	21	19	18	16	14	13	11	10	171
	M	27	23	21	18	18	15	14	13	12	11	172
C 68	I	30	23	21	18	17	16	14	14	12	11	176
	II	28	23	19	18	18	15	14	13	12	11	171
	III	27	22	20	20	17	16	15	13	12	11	173
	IV	27	24	20	18	16	15	14	14	12	11	171
	M	27	24	20	18	17	16	14	13	12	10	171
C 75	I	26	21	19	17	14	13	13	12	11	10	156
	II	25	22	19	17	15	14	13	12	11	10	158
	III	24	21	18	16	15	14	12	12	11	10	153
	IV	24	21	18	16	15	13	12	12	11	10	152
	M	25	22	19	17	15	14	13	12	11	10	158
C 79	I	27	26	24	21	17	17	15	14	13	12	176
	II	27	26	22	20	18	16	14	14	12	11	180
	III	26	26	21	18	18	17	14	14	12	12	178
	IV	27	27	23	20	18	15	14	14	12	11	183
	M	25	26	21	19	18	15	14	14	12	11	175
C 80	I	26	23	19	18	17	15	14	12	11	10	165
	II	25	24	19	18	16	15	14	12	11	11	165
	III	25	21	19	18	16	15	14	13	12	11	163
	IV	24	22	20	18	16	14	14	12	12	10	162
	M	26	23	19	18	16	14	14	12	11	10	163
C 81	I	25	24	20	19	17	16	15	14	12	11	173
	II	27	25	22	18	17	16	14	13	12	11	174
	III	27	24	21	20	17	16	14	13	12	11	175
	IV	25	24	22	19	17	15	14	13	11	11	171
	M	27	23	18	17	16	14	14	14	12	10	165
C 90	I	26	25	21	19	16	15	14	13	12	10	171
	II	27	23	20	19	17	16	14	13	12	10	171
	III	27	23	20	19	16	14	14	13	12	10	168
	IV	28	23	20	18	17	15	14	13	12	10	170
	M	28	26	23	19	18	17	14	13	12	11	181

TABLE 2 B—Continued

SERIES NO.	FETUS OR MOTHER	NUMBER OF TAIL RING										TOTAL
		1	2	3	4	5	6	7	8	9	10	
C 95	I	22	23	21	19	16	15	14	12	11	10	163
	II	25	23	19	18	16	14	14	12	11	10	162
	III	24	24	21	18	16	15	14	12	12	10	166
	IV	25	24	20	19	17	14	14	12	12	10	167
	M	25	23	20	18	16	14	14	12	11	10	163
K 48	I	27	25	21	20	18	17	15	14	12	11	170
	II	28	25	23	20	19	16	16	14	13	12	176
	III	26	25	22	20	19	17	15	14	12	11	171
	IV	26	25	22	20	18	17	14	14	12	11	169
	M	28	24	21	19	18	17	16	14	12	11	170
K 82	I	27	23	22	20	18	16	15	14	12	11	178
	II	26	24	20	19	17	15	14	14	12	12	173
	III	27	22	21	19	18	16	14	14	12	11	174
	IV	27	24	21	19	17	16	14	14	12	12	176
	M	27	23	22	20	18	15	14	14	12	11	176

TABLE 3

*Polyembryonic correlation coefficients and other determinations for the scutes of the individual rings of the caudal armor of 40 sets of quadruplets (20 male and 20 female).*

NO. OF RING	MEAN IN SCUTES	$\sigma x^2$	$\sigma y^2$	$r$
1	26.14	1.5617	1.3766	0.5587 $\pm$ 0.0304
2	23.33	1.9094	1.3203	0.6542 $\pm$ 0.0253
3	20.31	1.5254	1.2164	0.6012 $\pm$ 0.0282
4	18.42	1.0452	1.0255	0.6539 $\pm$ 0.0254
5	16.52	0.8165	0.5627	0.6560 $\pm$ 0.0250
6	15.07	0.8980	0.6233	0.6530 $\pm$ 0.0254
7	13.82	0.4482	0.3376	0.6234 $\pm$ 0.0270
8	12.54	0.6971	0.3549	0.7454 $\pm$ 0.0198
9	11.54	0.3820	0.3636	0.5241 $\pm$ 0.0315
10	10.44	0.3095	0.2510	.5945 $\pm$ 0.0286
Average .....				0.6263

TABLE 4

*Uniparental correlation coefficients and other determinations for the numbers of scutes in the individual rings of the caudal armor of 40 sets of quadruplets (20 male and 20 female) and their mothers*

NO. OF RING	MEAN OF MOTHERS	$\sigma_{x^2}$ OF MOTHERS	$\sigma_{y^2}$ OF MOTHERS AND OFFSPRING	$r_{xy}$ BETWEEN MOTHERS AND OFFSPRING
1	26.67	1.7194	3.1655	0.0353 $\pm$ 0.065
2	23.35	1.7326	2.4200	0.3336 $\pm$ 0.0477
3	20.67	1.3689	2.7324	0.0558 $\pm$ 0.0535
4	18.27	0.6852	1.2484	0.2855 $\pm$ 0.0493
5	16.82	0.9919	1.2548	0.3020 $\pm$ 0.0488
6	15.40	0.8900	1.0572	0.5709 $\pm$ 0.0362
7	14.25	1.1875	0.8407	0.5412 $\pm$ 0.0388
8	12.75	0.6875	0.6556	0.5518 $\pm$ 0.0374
9	11.70	0.4100	0.4713	0.4030 $\pm$ 0.0450
10	10.42	0.4843	0.4904	0.3918 $\pm$ 0.0458
Average .....				0.3470

TABLE 5 A

*Showing numbers of scutes in the five shields of armor of 10 sets of male quadruplets and their mothers*

SERIES NO.	FETUS MOTHER	CEPHALIC SHIELD	SCAPULAR SHIELD	BANDED REGION	PELVIC SHIELD	CAUDAL SHIELD	GRAND TOTAL
C 21	I	132	1029	539	862	177	2739
	II	137	1025	538	869	178	2747
	III	136	1011	533	874	178	2729
	IV	136	1004	538	849	175	2702
	M	133	1085	566	1002	178	2964
C 23	I	154	1080	576	936	171	2917
	II	157	1091	572	927	172	2921
	III	151	1087	580	923	168	2889
	IV	146	1081	574	920	168	2889
C 28	M	148	1082	575	926	166	2897
	I	132	1086	574	935	164	2891
	II	137	1061	577	955	166	2896
	III	134	1080	582	937	163	2896
	IV	141	1089	574	941	164	2909
C 30	M	129	954	565	835	173	2656
	I	128	1006	648	854	163	2699
	II	122	1023	554	843	163	2705
	III	126	1009	557	858	163	2713
	IV	129	1006	553	853	162	2703
C 31	M	123	1113	558	857	175	2826
	I	145	1096	567	889	158	2855
	II	148	1099	576	887	160	2870
	III	148	1103	566	876	159	2852
	IV	145	1106	567	882	157	2858
C 46	M	134	964	570	913	172	2753
	I	149	1051	558	883	172	2823
	II	143	1054	550	900	172	2819
	III	148	1048	554	923	167	2840
	IV	147	1059	556	949	166	2874
C 47	M	147	1108	562	759	173	2749
	I	130	999	554	873	165	2721
	II	133	972	559	906	164	2734
	III	134	979	550	883	168	2714
	IV	132	994	559	883	168	2736
C 76	M	112	1087	581	942	179	2901
	I	133	1110	560	867	162	2832
	II	129	1112	552	887	162	2842
	III	131	1094	554	883	163	2825
	IV	138	1093	558	864	161	2825
C 92	M	124	1139	569	916	160	2908
	I	138	1052	589	866	168	2813
	II	136	1039	584	863	165	2887
	III	137	1011	585	881	167	2781
	IV	134	1023	579	884	163	2793
C 96	M	136	978	590	809	179	2692
	I	138	1037	557	869	170	2771
	II	136	1042	550	867	167	2762
	III	137	1046	553	846	168	2750
	IV	134	1037	551	853	170	2745
	M	136	974	553	810	160	2633

TABLE 5 B

Showing numbers of scutes in the five shields of armor in 10 sets of female quadruplets and their mothers

SERIES NO.	FETUS MOTHER	CEPHALIC SHIELD	SCAPULAR SHIELD	BANDED REGION	PELVIC SHIELD	CAUDAL SHIELD	GRAND TOTAL
C 15	I	143	1142	581	996	178	3040
	II	134	1131	576	977	177	2995
	III	135	1142	582	973	178	3010
	IV	132	1174	584	945	177	3012
	M	143	1140	584	961	191	3019
C 18	I	166	1061	555	860	171	2813
	II	164	1068	552	833	175	2792
	III	167	1072	550	851	174	2814
	IV	169	1105	549	874	174	2861
	M	147	1027	554	831	162	2721
C 22	I	161	1147	581	920	178	2997
	II	163	1160	594	904	180	3001
	III	155	1158	597	930	176	3015
	IV	158	1141	596	940	177	3012
	M	140	1134	587	936	167	2964
C 32	I	146	1007	558	846	167	2724
	II	145	1003	554	839	169	2710
	III	141	1007	564	868	170	2750
	IV	148	1011	557	848	173	2737
	M	148	1094	556	871	166	2835
C 62	I	119	1032	549	927	160	2787
	II	124	1026	551	909	159	2769
	III	117	1044	553	890	158	2762
	IV	120	1010	556	920	158	2764
	M	119	1146	559	1013	178	3015
C 65	I	130	1000	561	926	162	2799
	II	135	1010	563	915	161	2784
	III	135	1021	573	939	163	2831
	IV	136	978	573	946	161	2794
	M	125	991	543	927	171	2757
C 68	I	145	1146	571	1013	176	3051
	II	146	1150	569	1017	171	3053
	III	140	1129	564	1008	173	3014
	IV	142	1118	561	1007	171	2999
	M	121	1054	544	877	169	2765
C 80	I	129	896	530	855	165	2575
	II	129	882	528	872	165	2576
	III	129	950	541	845	163	2628
	IV	128	902	532	852	162	2576
	M	129	953	553	851	163	2648
C 81	I	129	987	565	756	173	2610
	II	132	1000	566	759	174	2631
	III	132	979	574	777	175	2637
	IV	130	975	567	765	171	2608
	M	124	920	556	801	165	2566
C 95	I	145	1049	556	802	164	2716
	II	150	1064	565	843	163	2776
	III	148	1073	557	827	166	2771
	IV	153	1070	562	835	167	2797
	M	140	1068	564	808	162	2742

TABLE 6

*Showing polyembryonic correlation, and so forth, for the five main regions of the armor*

SHIELD OF ARMOR	MEAN NUMBER OF SCUTES	$\sigma_x^2$	$\sigma_y^2$	$r$
Cephalic.....	138.6	836.92	17.07	0.9376 $\pm$ 0.0074
Scapular.....	1052.0	3723.21	371.23	0.9502 $\pm$ 0.0059
Banded.....	562.7	235.26	36.26	0.9329 $\pm$ 0.0079
Pelvic.....	878.0	3118.40	378.43	0.9394 $\pm$ 0.0072
Caudal.....	168.0	36.68	7.32	0.9898 $\pm$ 0.0012

TABLE 7

*Showing uniparental correlation, and so forth, for the five main regions of the armor*

SHIELD OF ARMOR	MEAN OF MOTHER	$\sigma_x^2$ OF MOTHERS	$\sigma_y^2$ BETWEEN MOTHERS AND OFFSPRING	$r_{xy}$ BETWEEN MOTHERS AND OFFSPRING
Cephalic.....	131.95	121.14	123.20	0.5268 $\pm$ 0.054
Scapular.....	1050.50	5199.50	5061.00	0.4389 $\pm$ 0.061
Banded.....	564.40	174.95	197.10	0.5251 $\pm$ 0.058
Pelvic.....	882.00	4730.80	4751.03	0.4036 $\pm$ 0.063
Caudal.....	169.00	58.67	64.35	0.1662 $\pm$ 0.073



## BIPARENTAL INHERITANCE OF SIZE IN PARAMECIUM

H. S. JENNINGS AND K. S. LASHLEY

*The Zoölogical Laboratory of The Johns Hopkins University*

In a recent paper ('13) we have shown that there is biparental inheritance of the rate of fission in *Paramecium*—the two lines of progeny descended from a pair of conjugants being more alike in their rates of fission than would be the case if their parents had not conjugated. The present paper examines the question whether there is likewise biparental inheritance in size as a result of conjugation.

The material for this examination was derived from the extensive Experiment 16, described in our previous paper. In this experiment 482 lines of propagation, derived from the two members, *a* and *b*, of 241 pairs, were cultivated side by side, under uniform conditions, for forty-seven days. In addition to the main slide cultures, employed for the study of the rate of fission, there was kept a small mass culture of each line. These mass cultures were likewise kept under uniform conditions; they furnished for measurement large numbers of specimens of each line.

On April 17, 1913, twenty-five days after conjugation, and nineteen days after the mass cultures were set in progress, a considerable number of specimens from each line were killed in Worcester's fluid and preserved for measurement. Later, 2687 of these specimens were measured. Those measured belonged to 86 diverse lines, derived from 43 diverse pairs of conjugants; thus on the average a trifle above 31 specimens were measured from each of the 86 lines. The numbers measured varied, however, from 16 to 66 for given lines (table 3).

It would of course have been of interest if descendants from a larger number of pairs could have been included, but the details of conducting so large an experiment as this one, concerned mainly with the study of the rate of fission, are so laborious that we did

not find it possible to measure a larger number. It appears hardly probable that even so large a number will soon again be measured, under the necessary conditions; moreover, the results reached from the study of these 2687 specimens seem clear.

Before proceeding to the study of these descendants of the pairs, we must first examine the original wild culture, to determine what the similarity is between the members of the pairs, owing to assortative mating. Pearl ('07) and the senior author ('11) have shown that there is a considerable degree of assortative mating with respect to size, in *Paramecium*; larger specimens tend to mate with larger; smaller with smaller. This tendency is measured by the coefficient of correlation in size for the two members, *a* and *b*, of the pairs. In the senior author's extensive study of

TABLE 1

*Measurements in length of 180 non-conjugants taken at random from the culture from which were derived the conjugants employed in the experiment. The unit of measure is 4 microns.*

	LENGTH																												
	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69
No. of specimens	1	1	1	1	1	2	5	4	6	8	8	13	9	18	18	11	9	11	12	11	8	10	4	1	5	3	1	1	1

the matter ('11) it was found that in 'wild' cultures of *Paramecium caudatum*, such as we are here dealing with, the coefficient of correlation in size due to assortative mating averages about 0.380.

In the culture from which our conjugants of the present Experiment 16 were derived, there were measured 92 conjugating pairs (184 specimens), and 180 non-conjugants. The results of these measurements are given in tables 1 and 2. The unit of measurement was 4 microns.

The non-conjugants varied in length from 164 to 280 microns, the mean length being  $222.890 \pm 1.020$  microns, with a standard deviation of  $20.299 \pm 0.722$  microns and a coefficient of variation of  $9.099 \pm 0.323$  per cent.

The conjugants showed, as always, less variation than the non-conjugants; the length varied from 168 to 236 microns.

TABLE 2

Correlation table for the measurements in length of the members of 92 pairs of conjugants (184 individuals). The larger individual of each pair is called *a*, the smaller *b*. The unit of measurement is 4 microns (so that to obtain the lengths in microns, the measurements given are to be multiplied by 4).

	Length of <i>a</i>																
	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	
Length of <i>b</i>					1												1
42																	
43	1	2			1												4
44		2	2	1													5
45				2	1	1		1									5
46				4	4	3	1										12
47				3	8	3	2	1	1	2							20
48					4	4	4	4	3							1	20
49						4	5	3	3								15
50							1	3	1	1							6
51									2		1						3
52												1					
53										1							1
	1	4	2	11	18	15	13	12	10	4	1					1	92

The mean size is  $193.283 \pm 0.475$  microns; the standard deviation  $9.550 \pm 0.336$  microns (Shepard's correction being employed), and the coefficient of variation is  $4.941 \pm 0.174$  per cent.

The important point, for present purposes, is the coefficient of correlation in length between the two members (*a* and *b*) of the pairs. This turns out, as computed from table 2, to be  $0.3881 \pm 0.0422$ . This signifies that if we select members having a certain length, when the length of these selected members (*a*) is changed by one unit, the length of the mates *b* is on the average likewise changed in the same direction, but that there is sufficient variation so that the average change for these unselected mates *b* is but 0.3881 of the change in the selected members *a*.

Now the question which interests us at present is this:

When we obtain progeny from the two members, *a* and *b*, of the pairs, will these progeny likewise show a similarity in size and hence a coefficient of correlation? And will this similarity, as measured by the coefficient, be less than that of the parents; or equal to it; or greater than that of the parents?

If the progeny show a likeness in size not greater than that of the parents, then of course there is no indication of biparental inheritance—such similarity as exists being fully accounted for by the assortative mating between the parents. If there is actual inheritance from both parents, *the coefficient of correlation between the progeny will be greater than that between the parents.*

The parents at the time of conjugation are adults, of a medium size; they do not include specimens that are small because young, nor specimens that are large in preparation for fission; this is fully set forth in the paper by the senior author ('11). The progeny of these conjugants, on the other hand, include, as we find them, young and old. If, therefore, we take a single specimen of each line at random, we shall often obtain a young small specimen of *a*, an old large specimen of *b*; the similarity in size that may actually exist between adults of average size being thus quite lost. Such random selection would, therefore, give a much reduced coefficient of correlation, as compared with that between adults of average size.

How can this difficulty be met? Evidently, the proper method of procedure is to obtain by measurement of a considerable number of specimens the approximate mean for each line; then to measure the correlation between these means, for the two lines (*a* and *b*) of each pair.

This is what we have done for the 86 lines with which the present paper deals. The means for each line, together with the number of specimens measured, are given in table 3.

The coefficient of correlation between the means of the lines belonging to a single pair was computed from this table, employing each mean to the second decimal place. The coefficient in such a case is best computed without the formation of a table. It is to be remembered that we are in the present case dealing with *like* variates as members of the pairs; hence the methods employed with symmetrical tables are to be used. The coefficient was computed both by the product method and by the difference method. These give the same result, a coefficient of 0.5703. Making use of Shepard's correction, in connection with the product method,

TABLE 3

Mean lengths for 86 lines of *Paramecium caudatum* (2687 individuals), derived from the two members (a and b) of 43 pairs; together with the number of individuals measured for each line. The lines are designated by the same numbers used for these particular lines in table 51 of the authors' former paper ('13). The lengths are given in units, each of which is equal to 4 microns; so that to obtain the lengths in microns, each is to be multiplied by 4.

PAIR	a		b	
	Number measured	Mean length	Number measured	Mean length
1	32	39.656	31	39.516
2	50	47.720	22	38.000
3	42	42.786	24	36.167
4	41	46.244	37	45.865
5	31	40.936	34	40.971
6	29	39.276	33	40.212
9	36	38.306	45	40.244
10	28	39.429	29	35.207
12	36	45.278	35	46.429
14	28	39.893	35	38.114
15	18	37.111	28	36.714
16	28	39.821	37	39.838
19	34	42.794	32	45.844
20	27	40.593	37	43.081
21	27	36.407	34	34.824
22	32	37.094	24	35.583
23	30	37.167	34	35.382
24	27	37.111	38	40.974
25	38	48.711	30	42.367
27	26	39.538	30	41.533
28	33	39.788	66	38.894
29	32	39.125	34	38.588
30	33	45.091	34	38.294
31	30	43.367	33	40.030
32	29	38.828	29	39.448
34	33	43.333	16	41.688
35	29	40.138	28	38.214
40	28	39.107	30	39.033
41	22	38.000	31	37.935
42	33	33.758	36	35.778
43	26	39.115	30	39.267
44	35	40.286	27	40.222
45	25	38.440	28	40.679
47	28	51.893	36	48.833
56	35	37.686	28	39.036
57	28	37.500	33	42.333
58	29	47.172	32	41.000
59	30	39.467	29	37.724
60	29	41.310	32	38.813
61	28	39.536	26	36.038
62	29	42.483	26	42.308
63	25	38.520	27	42.111
64	26	43.192	32	42.344

the correct coefficient of correlation is found to be  $0.5744 + 0.0487$ . This coefficient is considerably higher than that found in the senior author's extensive study of the matter ('11), to exist between the conjugants in any of the eight wild cultures examined. The mean of all the 86 means is found to be 40.33 units (161.32 microns); the standard deviation of the means is 13.60 microns; their coefficient of variation 8.43 per cent.

The question may be raised as to whether the proper method of procedure for determining the coefficient of correlation would not be, to mate in the correlation tables every specimen of *a* of a given pair, with every specimen of *b*, of the same pair, thus giving a very large number of pairings (in this case 41,900), from which to compute the coefficient. This method of work might be useful for certain purposes, but our precise object is to determine whether the mean sizes of the lines *a* and *b* of the pairs are correlated and how much; this the method suggested would not do. By including among the matings those of young, small specimens of *a* with old, large specimens of *b* (and vice versa) the coefficient would be much reduced; so that even if the mean sizes of the members pairs were perfectly correlated (mean size of *a* and *b* equal in each pair), this method might give a low coefficient of correlation. We have worked out the coefficient by this method; mating each of the descendants of *a* of each pair with those of the corresponding *b* (41,900 pairings) gives a coefficient of correlation of 0.2871—almost exactly half that of the correlation between the means of *a* and *b*.

Our result, then, is that in consequence of conjugation, the coefficient of correlation of 0.3881, due to assortative mating, is increased to 0.5744—an increase of 48 per cent. This increase can be attributed only to biparental inheritance. As a result of conjugation, the progeny of the two members of the pairs become more alike in size—just as our previous study had shown them to become more alike in their rate of reproduction.

It may be of interest to compare the similarity in size of these members of pairs, with the similarity in rate of fission of the same lines. The lines were taken for measurement merely as they happened to come to hand, without any knowledge of their rates of fission. In table 3 each line is given the same number as in table 51 of our previous paper ('13), so that any comparison desired can be made. We will select for examination the number of fissions for each *a* and *b* of table 3 for the first twenty days of the

experiment (as shown in table 51 of the previous paper). The correlation of the two members of the pairs (*a* and *b*) in number of fissions for twenty days is found to be  $0.4439 \pm 0.0584$ . This is almost exactly the same coefficient as that found for the entire 358 lines that lived through the twenty days; this, as shown in our former paper ('13), was  $0.4793 \pm 0.0275$ . The agreement between this value found for the 86 lines considered in the present paper, with that for the 358 lines of the entire experiment indicates that these 86 lines form a typical sample of the population, and that therefore the value found for the correlation of *a* and *b* in length would not have differed significantly if we had included representatives of a larger number of pairs. We may, therefore, conclude with confidence that the correct value of the correlation in mean size between the descendants of pairs for this case is close to 0.5744.

#### SUMMARY

As a result of conjugation the progeny of the two individuals that have conjugated become more alike in their average length, so that biparental inheritance occurs in respect to body size (as well as in respect to rate of fission). The members of pairs in the culture examined showed, owing to assortative mating, a coefficient of correlation in body length of 0.3881; this was increased as a result of conjugation to such an extent that their progeny showed a coefficient of 0.5744—an increase of 48 per cent.

April 4, 1913

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# FURTHER EXPERIMENTS ON NATURAL DEATH AND PROLONGATION OF LIFE IN THE EGG<sup>1</sup>

JACQUES LOEB

*The Rockefeller Institute for Medical Research, New York*

1. The unfertilized egg dies in a comparatively short time, while the act of fertilization gives rise to a theoretically at least unlimited number of generations. The death of the unfertilized egg may be called a case of "natural" death of a cell; it is perhaps the only case in which we can feel sure that death is caused by internal "natural" causes and not by avoidable injuries. The act of fertilization is the only one known to prevent natural death.

The velocity with which the unfertilized eggs die differs for the eggs of various species; the mature egg of the starfish dies much more rapidly than the egg of the sea urchin. The writer pointed out that this difference might be connected with a difference in the rate of oxidations in the two kinds of eggs, since he had been able to show that the suppression of oxidations by the withdrawal of oxygen from the sea water or by the addition of a trace of KCN prolongs the life of these eggs. Last year, Loeb and Wasteneys were indeed able to prove a difference in the relative rate of oxidations between the eggs of these two kinds of animals in the sense which is demanded by our hypothesis. The mature unfertilized egg of the starfish has a rate of oxidations which equals that of the fertilized egg;<sup>2</sup> while the rate of oxidations in the unfertilized mature egg of the sea urchin is only about one-fourth or one-sixth of that of the fertilized egg of the same species.

<sup>1</sup> Loeb, Maturation, natural death and the prolongation of life, etc. Biol. Bull., vol. 3, p. 295, 1902. The mechanistic conception of life, 1912.

<sup>2</sup> Loeb and Wasteneys, Arch. f. Entwicklungsmechanik, vol. 35, p. 555, 1912.

2. Our analysis of the process of the causation of development has led us to the result that it requires generally the coöperation of two factors (or substances); one which calls forth the membrane formation (or the change in the cortical layer of the egg); and a second factor which saves the egg from the disintegration with which it is usually threatened after membrane formation. This second—corrective—factor is most conveniently supplied in the form of a short treatment of the egg with a hypertonic solution.<sup>3</sup> The question therefore presented itself to which of these two factors the life-saving effect of the act of fertilization was due.

The natural inference from our former experiences would have been to ascribe the life-saving effect of the act of fertilization to the second corrective factor for the following reason. If we cause artificial membrane formation in the unfertilized egg of the sea urchin, we do not prolong its life but on the contrary we shorten it. Such an egg dies at room temperature in a few hours while without the membrane formation it may live at least for a few days. The artificial membrane formation alone (if it is not followed by the second corrective factor) hastens the death of the unfertilized egg. We can understand the reason for this since the artificial membrane formation accelerates the rate of oxidations in the sea urchin egg to exactly the same amount as the entrance of a spermatozoon (Warburg,<sup>4</sup> Loeb and Wasteneys<sup>5</sup>). By the way of exclusion this seemed to restrict the life-saving effect of the act of fertilization as well as of artificial parthenogenesis to the second corrective factor.

3. Observations which the writer made this winter, however, show that this conclusion is not correct. In his earlier work he had already found that the hypertonic solution is just as effective as a corrective factor if it precedes the artificial membrane formation as if it follows it. The only difference between the two cases is a difference in the time of exposure required. When the artificial membrane formation is called forth first and the eggs are exposed to the hypertonic solution (50 cc. sea water + 8 cc.

<sup>3</sup> Loeb, *Die chemische Entwicklungserregung des tierischen Eies*, Berlin, 1909. *The mechanistic conception of life*, 1912.

<sup>4</sup> Warburg, *Zeitschr. physiol. Chemie*, vol. 66, p. 305, 1910.

<sup>5</sup> Loeb and Wasteneys, *Journ. Biol. Chemistry*, vol. 14, p. 469, 1913.

2.5 m NaCl) afterwards, the eggs of *purpuratus* need remain in the solution for only from forty to sixty minutes. If, however, the eggs are put into the hypertonic solution first and submitted to the treatment for membrane formation (e.g., butyric acid treatment) afterwards, they must remain in the hypertonic solution from 90 to 150 minutes. In the case of the egg of *Arbacia* this treatment in itself would induce artificial parthenogenesis, but in the egg of *Strongylocentrotus purpuratus* it does in most cases leave the eggs either intact or causes them to segment once or a few times and then to go into a state of rest again. When such eggs are afterwards treated with butyric acid they will develop.

The reason why the eggs must remain longer in the hypertonic solution when this treatment precedes the artificial membrane formation, than when it follows, seems clear if we consider the fact that the corrective effect of the hypertonic solution is weakened or inhibited if we inhibit or diminish the oxidations in the egg. This indicates that the corrective effect is in some way connected with the formation of a product of oxidation in the egg which is not formed in normal sea water. Since the rate of oxidations is from four to six times as great in the egg after the membrane formation as it is before, we can understand why the hypertonic solution brings about the corrective effect so much more quickly in the egg after membrane formation than before.

4. Working on the idea that the corrective effect of the hypertonic solution was due to the formation of a specific oxidation product in the egg it occurred to the writer to test whether the corrective effect of the hypertonic solution was reversible or permanent. Unfertilized eggs of *S. purpuratus* in which no membrane formation had been called forth were treated with a hypertonic solution and then portions of these eggs were treated after varying intervals of from one hour to three days with butyric acid. In all cases the butyric acid treatment now sufficed to call forth in these eggs a normal development at any time. This shows that the corrective effect produced by the hypertonic solution is irreversible and lasts in the egg as long as the latter lives.

The description of an experiment will illustrate this point. The unfertilized eggs of one female *S. purpuratus* were put for  $\frac{1}{2}$ , 1,  $1\frac{1}{2}$ , 2,  $2\frac{1}{2}$ , and  $3\frac{1}{4}$  hours into hypertonic sea water (50 cc. sea water + 9 cc. 2.5 m NaCl + KCl + CaCl<sub>2</sub>). None of these eggs developed into larvae and none of the eggs that had been up to  $1\frac{1}{2}$  hours in the hypertonic solution segmented. Of the eggs that had been 2 hours in the hypertonic solution 0.5 per cent went into the two-cell stage but did not develop beyond this; of the eggs that had been  $2\frac{1}{2}$  and  $3\frac{1}{4}$  hours in the hypertonic solution 10 per cent segmented into the two- or as far as the four-cell stage. Practically all the eggs were intact the next day.

Twenty-two hours later part of each lot of these eggs was treated  $1\frac{1}{2}$  or 3 minutes with propionic acid (2.8 cc.  $\frac{N}{10}$  acid to 50 cc. sea water) to induce membrane formation. All formed tight fitting membranes. The result was as follows. The eggs that had been treated with the hypertonic solution  $\frac{1}{2}$  and  $1\frac{1}{2}$  hours disintegrated after the artificial membrane formation. The exposure to the hypertonic solution had been too short to produce a corrective effect. Among those treated for  $1\frac{1}{2}$  hours one swimming larva was found the next day. Of those that had been in the hypertonic solution for 2 hours, 25 per cent segmented normally after the artificial membrane formation and developed into perfect blastulae; of those treated with hypertonic solution for  $2\frac{1}{2}$  and  $3\frac{1}{4}$  hours practically all developed but many of the blastulae were sickly, a sign that the eggs had been a little too long in the hypertonic solution.

Forty-eight hours after the treatment with hypertonic solution a second portion of the eggs that had been exposed  $2\frac{1}{2}$  hours to the hypertonic solution was caused to form membranes by the treatment with propionic acid. The eggs had a tendency to stick to the glass but all segmented regularly and developed into blastulae which appeared normal.

Three days after the treatment of the eggs with the hypertonic solution another lot (those that had been two hours in the hypertonic solution) was treated with propionic acid. Those eggs that were still alive formed tight fitting membranes and all began to segment regularly. A few developed into normal blastulae.

On the fourth day all the eggs which had not been treated at all or had only been treated with the hypertonic solution were dead. Those eggs that had been treated with both the hypertonic and the propionic acid were developing and alive.

This experiment was repeated quite often with the same result. It proves that the corrective effect of the hypertonic solution is irreversible and remains in the egg as long as it lives.

5. One word should be said in regard to the fact that the exposure of the eggs to hypertonic sea water which leads in *Arbacia* quite frequently to the origin of larvae, behaves so differently in the eggs of *S. purpuratus*. The writer has repeatedly pointed out this difference. His recent investigations have led him to think that this is due to a typical difference in the reactions of the eggs of the two species to the influence of the hypertonic solution. In both species the eggs of only part of the females can be induced to develop by the treatment with the hypertonic solution alone, but the number of females with susceptible eggs is much greater in *Arbacia* than in *S. purpuratus*. This year the writer has investigated what constitutes this difference in the behavior of the eggs of different females of *purpuratus* and found the following fact. The eggs of different females were treated with the same hypertonic solution at the same temperature and for the same time. The eggs of the majority of the females behaved as described in the preceding chapter of this paper. The eggs of some of the females of *purpuratus* developed into larvae through the mere treatment with a hypertonic solution. The difference between the two groups was this. The eggs which developed formed somewhat atypical membranes (gelatinous films), those that did not develop formed no membranes. The membrane formation did not necessarily occur while the eggs were in the hypertonic solution but often considerably later, while the eggs were already beginning to segment. Those eggs which had begun to segment but did not form the atypical membrane stopped developing and went back into the resting stage again. Those which formed membranes went on developing into larvae. The specific difference between the eggs of different females is therefore a difference in the facility with which the

hypertonic solution causes membrane formation. The fatty acids, if properly applied, cause a typical or an atypical membrane formation in practically every sea urchin egg. The hypertonic sea water, however, causes an atypical membrane formation in the eggs of only a small percentage of the females of *purpuratus* and a larger though limited percentage of the eggs of *Arbacia*. As the writer pointed out years ago, the purely osmotic method of artificial parthenogenesis gives better results if the solution is rendered more alkaline.

6. In order to avoid misunderstandings it may be well to point out that in the purely osmotic method of artificial parthenogenesis the causation of development is also due to two factors: a membrane-forming and a corrective factor. The peculiar fact is that one and the same external agency, the hypertonic solution, produces both effects simultaneously. It produces the corrective effect in all cases, if the eggs are left long enough in the solution. It induces membrane formation in a limited number of cases. The eggs develop into larvae only if both effects are produced by the hypertonic solution, otherwise the membrane formation has to be induced by some other agency, for example, the addition of some alkali to the solution or the treatment of the eggs afterward with a fatty acid.

It may be that the membrane formation by the hypertonic solution is always the combined effect of the HO ions and the hypertonic solution, even in neutral hypertonic solutions, where the  $C_{OH} = 10^{-7}N$ . But this conception may be unnecessary.<sup>6</sup>

7. We are now in a position to answer the question from which we started as to which of the two factors of fertilization (the membrane-forming and the corrective one) was responsible for the saving of the life of the egg. The answer must be that it is the combined effect of both factors. Our experiments have shown

<sup>6</sup> In a former paper the writer had suggested that the HO ions contained in the hypertonic solution might furnish the first factor; this conclusion was reached under the influence of the experiments on *purpuratus* where a neutral hypertonic solution as a rule never induces artificial parthenogenesis. In *Arbacia*, however, a neutral hypertonic solution does easily induce the formation of parthenogenetic larvae.

that the corrective effect if once imparted is permanent. If the corrective factor alone were the life-saving factor, eggs treated with a hypertonic solution alone should live indefinitely, or at least much longer than the non-treated unfertilized eggs. This is, however, not the case. In all the experiments the unfertilized eggs of *purpuratus* treated with the hypertonic solution alone died as quickly as those not treated at all, that is, in three days or less, at room temperature. *For these eggs the artificial membrane formation became a life-saving act*; while the artificial membrane formation had just the opposite effect in sea urchin eggs, to which the corrective effect was not imparted. This admits of only one conclusion, namely that the life-saving effect of the act of fertilization is not due to one but to the combined action of both factors of fertilization.

This harmonizes with the observations on the eggs of such forms as the starfish (*Asterina*) in which the artificial membrane formation by a fatty acid suffices to call forth development. Not all the eggs, however, will develop into larvae under the influence of artificial membrane formation alone, but only a small percentage. R. S. Lillie has shown that the percentage of eggs of *Asterias*, which will develop into larvae after artificial membrane formation by heating, can be increased by a subsequent suppression of oxidations in such eggs (which treatment as the writer has shown can also supply the second factor of fertilization). This proves that in the egg of the starfish fertilization is also induced by two factors, but that some of the eggs possess or can produce the second factor normally, while this factor can be produced in the majority of eggs only if they are kept without oxidations for some time. In the sea urchin eggs it is a rare exception if the egg contains the second factor normally, that is, if it can develop into a larva by the mere act of membrane formation. As a rule, the second factor has to be supplied either by a treatment with a hypertonic solution or by a prolonged suppression of oxidations. The mature eggs of the starfish die without exception if they are not fertilized and they die much more rapidly than the unfertilized eggs of the sea urchin; while the mere artificial membrane formation saves at least the life of a small percentage of

the eggs of the starfish. All these facts harmonize with the view that both factors of fertilization are required for the life-saving effect of fertilization. A similar situation exists in the case of the eggs of some annelids.

#### SUMMARY OF RESULTS

1. Since the unfertilized egg dies in a comparatively short time while the act of fertilization saves the life of the egg, it was proposed to find out which of the two factors of fertilization is responsible for this effect, the membrane formation or the corrective effect (produced in artificial parthenogenesis by the hypertonic solution). In former experiments the writer had shown that the artificial membrane formation alone hastens the death of the unfertilized egg while the treatment of such eggs with a hypertonic solution saves the life of the egg. This would make it appear as if the second factor was solely responsible for the life-saving effect of the act of fertilization.

2. The writer had formerly shown that the treatment of the egg with a hypertonic solution may precede the artificial membrane formation. It is shown in this paper that if the unfertilized eggs of *purpuratus* have once been treated with a hypertonic solution which in itself will not induce development they will develop at any time after artificial membrane formation has been induced. If the second factor is once imparted to this egg it keeps it as long as it is alive. The hypertonic solution induces an irreversible change in the egg.

3. It is shown that the treatment with a hypertonic solution alone does not prolong the life of the unfertilized egg. Eggs treated in this way live no longer than unfertilized eggs which have not been treated at all. If in such eggs artificial membrane formation is induced they will live indefinitely. For eggs treated with a hypertonic solution the artificial membrane formation becomes a life-saving act.

4. It follows from this that for the prolongation of the life of the unfertilized egg both factors of artificial parthenogenesis, the alteration of the surface as well as the second factor (usually supplied by the treatment with hypertonic solution) are required.



# FEEDING EXPERIMENTS WITH MICE<sup>1</sup>

RUTH WHEELER

*The Sheffield Laboratory of Physiological Chemistry of Yale University, and the Connecticut Agricultural Experiment Station, New Haven, Connecticut*

## SIX CHARTS

In *Science* for November 24, 1911, Osborne and Mendel reviewed briefly their experience in feeding isolated food substances to albino rats.<sup>2</sup> The results of these experiments are so important and far reaching that it seemed desirable to repeat some of the work on another species. Albino mice were chosen because they are omnivorous, nearly as easy to handle as rats, apparently less susceptible to lung diseases, cost less to feed, being only about one-tenth as heavy as rats of corresponding age, and have an even shorter life span. Miss A. E. C. Lathrop of Granby, Massachusetts, who has had a long experience in breeding mice, says that the average life of a white mouse is a little less than two years. They are sexually mature at two months and fully grown at 150 days or less.

With the exception of experiments of Röhmann,<sup>3</sup> details of which have not yet been published, mice have not been kept alive much longer than a month on food containing a single protein; Lunin<sup>4</sup> fed casein as the only protein, as did Hall<sup>5</sup> also; the latter

<sup>1</sup> This investigation was made possible through the assistance of Drs. Thomas B. Osborne and Lafayette B. Mendel in connection with the Carnegie Institution of Washington. The helpful co-operation of Miss Edna L. Ferry merits special acknowledgement here.

<sup>2</sup> Carnegie Institution of Washington, Publication 156, Parts I and II, 1911. Cf. also: *Science*, N. S., vol. 34, p. 722, 1911; *Journ. Biol. Chem.*, vol. 12, p. 81, 1912; *Zeitschr. f. physiol. Chem.*, Bd. 80, p. 307, 1912; *Journ. Biol. Chem.*, vol. 12, p. 473, 1912; *ibid.*, vol. 13, p. 233, 1912.

<sup>3</sup> See *Biochem. Zeitschr.* Bd. 39, p. 507, 1912.

<sup>4</sup> *Zeitschr. f. physiol. Chem.*, Bd. 5, p. 31, 1881.

<sup>5</sup> *Arch. f. Anat. u. Physiol., Physiol. Abth.*, 1896, p. 142; also p. 49.

kept his animals alive twenty-one days, though they began to lose weight on the ninth day or earlier; Willcock and Hopkins,<sup>6</sup> feeding zein with and without tryptophane, carried their experiments not longer than sixteen days; mice fed on casein with cane sugar and sodium carbonate by Abderhalden and Rona,<sup>7</sup> lived about three weeks; Röhmann was able to get second and third generations on a mixture of proteins<sup>8</sup> and more recently,<sup>3</sup> in the work referred to above, on a single protein—fed with carbohydrates, fats and salts. The details are not yet available. The mice fed by Suzuki, Shimamura and Odake<sup>9</sup> on casein, lecithin, starch, 'oryzanin' from rice, and salts all died within thirty-nine days.

Certain problems of nutrition can be solved only by feeding experiments that extend over a considerable time and in which the food substances are furnished in a purified form. Animals have a surprising power of getting along for a time on a qualitatively inadequate diet. Mice kept upon a ration in which two-thirds of the protein was gelatin and the other third casein did not show a decline in body weight for twenty-one days, although the ultimate decline of every animal fed upon this food showed it to be insufficient; another group made material gains in weight for seventeen days on a diet upon which all ultimately lost weight rapidly and died unless the food was changed. Even more essential for decisive results is the purity of the individual food substances employed. When the composition of the food is known with certainty and can be altered at will, the solution of dietetic problems begins to look more possible and also vastly more interesting. Differences in the nutritive properties of proteins such as the glutenin and gliadin of wheat, for example, may be altogether hidden if either retains a small amount of the other. For the present work it fortunately was possible to use the foods prepared for the feeding trials in the experiments of Osborne and Mendel already cited.

<sup>6</sup> Journ. Physiol., vol. 35, p. 88, 1906.

<sup>7</sup> Zeitschr. f. physiol. Chem., Bd. 42, p. 528, 1904.

<sup>8</sup> Abstracts in Maly's Jahresbericht, Bd. 38, p. 659, 1908.

<sup>9</sup> Biochem. Zeitschr., Bd. 43, p. 89, 1912.

Long continued constancy of body weight was used as the chief criterion of the adequacy of a ration for maintenance, although general appearance and behavior were found to be surprisingly indicative of health or of malnutrition. Mice kept on diets which ultimately proved inadequate were either sluggish and stiff in their movements or extremely restless, constantly running around their cage almost like dancers; and their coats quickly acquired a very noticeable roughness. On the other hand, mice on diets which prolonged feeding showed to be adequate were active without restlessness, had sleek, smooth coats, and in general could not be distinguished from the control animals on a mixed diet of dog biscuit, sunflower seeds, meat and carrots.

On a food paste consisting of a single protein, fat, carbohydrate and the 'protein-free milk' first employed by Osborne and Mendel with rats, mice were kept alive and apparently in excellent health for six months, a quarter of their normal life-time. At the end of that period the experiment had to be terminated owing to my departure from New Haven; but there was no evidence that these mice could not have lived for a longer time without change of diet. Different proteins, including the animal proteins casein, lactalbumin, and gelatin, and vegetable proteins glutenin, gliadin, edestin, and zein were fed for periods of from one to six months.

Because of the importance of mere traces of various ions, especially in animals as small as mice, a suitable salt mixture is difficult to find. As was found by Osborne and Mendel with rats, in every case a steady loss of weight and ultimate death followed the feeding of foods containing the inorganic salts in the form of Röhmann's mixture<sup>10</sup> if a change in the diet was not

<sup>10</sup> Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> .....	10 grams
K <sub>2</sub> HPO <sub>4</sub> .....	37 grams
NaCl.....	20 grams
Na citrate.....	15 grams
Mg citrate.....	8 grams
Fe citrate.....	2 grams
Ca lactate.....	8 grams

From Osborne and Mendel: Carnegie Institution of Washington, Publication 156, Part I, p. 32, 1911.

made. The lower curve in chart 1 shows a typical case. In this, as in the subsequent charts, body weight in grams is plotted on the ordinates, time in days on the abscissae. The abbreviation 'pfm.' is used to indicate 'protein-free milk.' The inorganic ingredients of the dietaries in the successful experiments were furnished in the form of the 'protein-free milk'<sup>11</sup> of Osborne and Mendel, made by removing the fat and proteins from milk and leaving chiefly lactose and salts, except sodium chloride, in

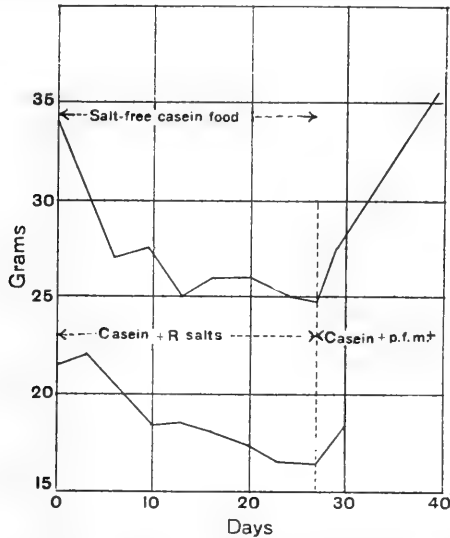


Chart 1 Showing the steady loss of weight of mice on salt-free casein food or on casein food containing Röhmann's salt mixture (R. salts) followed by rapid recovery when casein food containing protein-free milk (casein + pfm.) is given.

the same relative amounts, as in milk itself. In chart 1 the upper line shows the rapid decline of a mouse on salt-free food and the immediate recovery that took place when protein-free milk was employed. This experience, as well as the fact that for months at a time mice thrive on food in which 'protein-free milk' was the only source of salts, indicates that they find in it in available form all the inorganic constituents necessary for their maintenance for long periods.

<sup>11</sup> Ibid, Part II, p. 82.

During the time that the experiments lasted all the proteins used and enumerated above, except gelatin and zein, seemed to be interchangeable in so far as maintenance is concerned. Casein was fed as the sole protein for six months; lactalbumin, glutenin, gliadin, and edestin each for shorter periods varying from one to five months. Half-grown or still smaller mice did not grow on these foods nearly so fast as the control animals grew on a mixed diet (i.e., the foods, while entirely adequate for maintenance, were inadequate for growth); but the animals were still active, sleek, apparently well, with no decline in weight at the end of four, five and six months, and some had made considerable gains in weight—130 per cent in the case of one mouse on casein food. During this time hemoglobin and other blood proteins, elastin, collagen, the keratin of the skin, and hair, and so forth, must have been manufactured from a single protein of widely different composition from these or from its cleavage products. With casein a synthesis of glycocholl must be assumed; if the phosphorus-free edestin is the source of nitrogen, the organism's supply of complex phosphorus compounds, nucleoproteins, phosphatids, and so forth, must be synthesized, with only the phosphorus compounds of the protein-free milk as raw material. Gelatin and zein, however, are not equal to the other proteins used, perhaps because the body is not able to manufacture one or more of the amino-acid groups missing from them. When either of the two was fed as the *only* protein, decline in weight began at once and other evidences of ill health appeared.

All the animals living on gelatin as the sole protein became very restless and acquired a characteristic peculiar appearance; they were emaciated, the rump was flattened, the hind legs drawn up under the body and the coats rough, as though they had fallen into water and partly dried. If one-fourth of the gelatin was replaced by casein, the roughness disappeared and the decline stopped, but no recovery of lost weight took place; that is, for a time there was maintenance, without repair, on a diet in which three-fourths of the protein was in the form of gelatin. Both on this food and on a similar one in which one-third of the gelatin was replaced by casein, the body weight after a

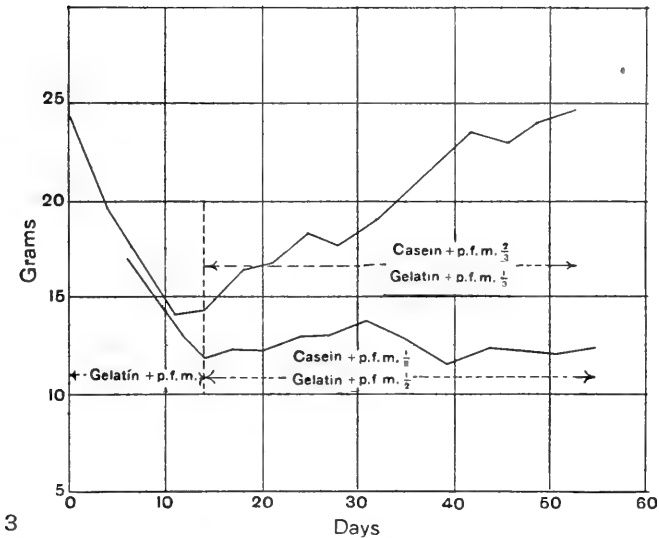
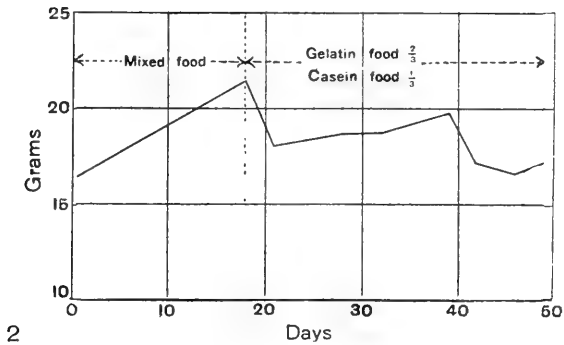


Chart 2 Showing the cessation of growth and slow decline of mice on food in which two-thirds of the protein is gelatin and one-third casein.

Chart 3 Showing the rapid decline of mice on gelatin food and recovery when two-thirds of the gelatin was replaced by casein and merely maintenance when only one-half was replaced.

preliminary decline was maintained from two to three weeks, followed by a very slow decline (chart 2). If equal quantities of the two proteins were used, maintenance was attained whether the animals were in normal condition or reduced one-third of

their proper weight by gelatin feeding (chart 3). One mouse lived thus for fifty days. In no case did recovery of lost weight occur unless at least two-thirds of the gelatin was replaced by casein or some other 'adequate' protein.

With zein as the only protein the decline was likewise continuous but much slower, one-third the body weight being lost in twenty-five days as compared with twelve days for mice fed with gelatin. The appearance and behavior of the animals was quite different; mice fed on the zein foods never exhibited the wet-looking coat or the hunched up position, the excessive activity or the ravenous appetite of the mice fed with gelatin. When zein was replaced by casein, weight was regained fairly rapidly; but the original weight was not quite reached by the single mouse with which realimentation was attempted. A diet in which half the protein was zein and the rest casein was apparently satisfactory for maintenance; two mice, half-grown, thrived on it for a month, one making a substantial gain in weight. The mice fed by Willcock and Hopkins on zein died before the twelfth day; those fed with zein + tryptophane were alive and active on the sixteenth day when the experiment was terminated, but had lost weight. In my experiments mice fed with zein lost on the average one-third of their weight in twenty-five days; while two mice which had an addition of tryptophane equal to 3 per cent of the zein fed lost only about one-fifth of their original weight by the fiftieth day.

There was no evidence of loss of appetite on either gelatin or zein foods until the mice had lost so much weight as to be no longer in normal health. That the character of the protein was responsible for the failure became evident from the recovery that promptly ensued when the diet was changed by substituting casein for the inappropriate protein first fed. The inadequacy of the gelatin or zein appears to be due to the inability of the animal to synthesize one or more of the missing amino-acids; for apparently improvement results from the addition of tryptophane to the zein food. The success of Kauffmann<sup>12</sup> and of Ab-

<sup>12</sup> Pfüger's Archiv. Bd. 109, p. 440, 1905.

derhalden<sup>13</sup> in getting nitrogen equilibrium in dogs by adding to 'incomplete' proteins or their digestion products the amino-acid radicals missing from their food likewise supports such an assumption. The more striking are the experiments described by Osborne (cf. *Science*, N.S., vol. 37, p. 185, 1913) who has also found that the addition of tryptophane to zein prevented decline in weight of rats for a very considerable time. Apparently the animal body cannot manufacture cyclic compounds, nor can it maintain itself on food entirely devoid of tyrosine or tryptophane.

Monotony of diet appears to be of little importance as a detriment to the nutrition of white mice, provided the diet is an otherwise sufficient one. Even after six months' feeding with precisely the same food they ate as liberally and as eagerly as did those kept on mixed foods. Anorexia seemed always to follow rather than to precede a loss of weight and to be the result rather than the cause of the decline. Mice on inadequate diets ate an average of one to one and one-half grams of food per day until they became ill—in appearance at least.

Growth is different from maintenance in its nutritive requirements. The most successful of the foods discussed above, for example, one containing casein + 'protein-free milk,' although satisfactory for adult animals, allowed only a minimal growth in small mice. Osborne and Mendel's rats grew as fast on it as on mixed food; mice, on the other hand, did little more than maintain themselves. Mice four to six weeks old, weighing 8 to 12 grams, seemed able to live on this food indefinitely; two survived six months with no sign of ill health; they were plump, smooth-coated, and normally active without undue restlessness, but they did not grow. Another mouse, for example, gained 4 grams in forty-three days less than one-third as much as the controls on mixed food; it continued to grow slowly however and in 140 days had gained the 8 grams for which the controls required two weeks. This is typical. Some of the smallest ones did not grow at all, were feeble and had a dwarf-like appearance. One gained only 2 grams in fifty-five days; in ten days on a mixed diet it

<sup>13</sup> Discussed in Abderhalden's *Synthese der Zellbausteine in Pflanze und Tier*, p. 61, et seq.



gained 8 grams, lost all its dwarfish look and weakness. The mouse subsequently remained healthy for eighty-two days on the casein food used at first, but gained in all that time less than 3 grams. In recovery from inadequate diets, the body weight never rose above its original value if any of the foods so far described were used, that is, there was only repair, no real growth.

Mice one-half or one-third grown were stunted in a most striking manner by gliadin foods. In three months the body weight of some of them did not vary more than one gram from the mean; in several cases the curve of growth might almost have been drawn from start to finish as a straight line. But through these long periods, the growth impulse was merely held in abeyance; and even after three to four months, suitable food induced a gain more rapid than normal, as the organism apparently tried to make up for the delay. Chart 4 shows the variation during 125 days in the weights of three small mice, two fed on casein and one on gliadin food; and shows also the tremendous gains made immediately after changing to a mixed diet. In two days the mouse fed on gliadin, represented in the chart, when supplied with mixed food, gained 5 grams—50 per cent of its weight! This was the more remarkable as the stunting had continued 100 days, making the mouse at the end of that time nineteen weeks old, six weeks past the age when the control animals on mixed food had practically stopped growing. (The controls weighed 28 grams twelve weeks after birth. Some of them stopped growing then; some reached 30 grams during the next two to three months). The casein-fed mouse whose record is given in the lower curve of chart 4 showed almost as rapid growth after 120 days stunting, when it was more than seven weeks past the age at which anything more than extremely slow growth is ordinarily made, and had indeed passed the age at which any of the controls showed any increase in weight whatever. Nevertheless on a mixed diet it gained 8 grams in the first twelve days, at the end of which time it became pregnant and eventually bore four young which were apparently normal. Evidently the capacity to grow is not necessarily lost because of its suppression during early life, even though the suppression continue all through the time of life during which growth ordinarily occurs.

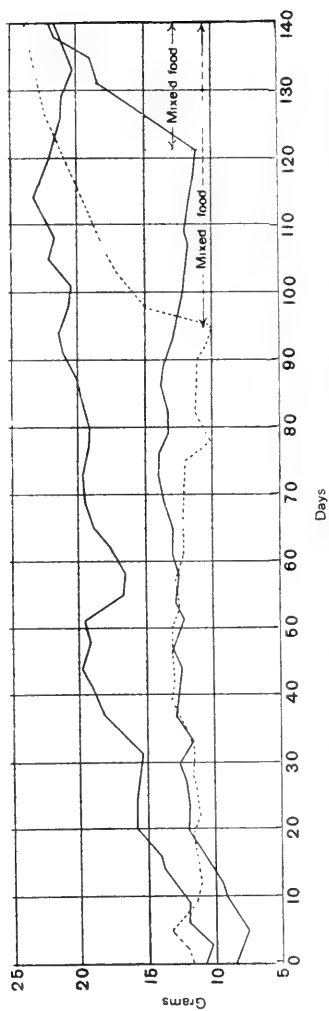


Chart 4 The upper solid line shows maintenance with slight growth over a long period on a diet containing casein + pfm. The lower solid line is the weight curve of a mouse, which was merely maintained on the same diet, but had not lost the capacity to grow on mixed food at an age at which growth normally ceases. The interrupted line shows the dwarfing on a diet containing gliadin + pfm., with subsequent growth on mixed food.

Failure to grow may have a variety of causes which have been discussed by Osborne and Mendel.<sup>14</sup> The inorganic constituents may be at fault. With the Röhmann salt mixture used in their earlier work with rats, Osborne and Mendel secured successful maintenance;<sup>15</sup> but no substantial growth took place until this salt mixture was replaced by their 'protein-free milk,' whereupon the rate of gain was greater than that of the controls on mixed food. Whether the remarkable superiority of the 'protein-free milk' is due to the presence of traces of some essential substance or to greater availability of the combinations in which the ions are held is not yet certain.<sup>16</sup> The mice did not even live on food containing either the Röhmann salt mixture or one artificially made up to resemble as closely as possible the 'protein-free milk.'

Stunting may also be due to inadequacy of the proteins of the food, as was the case, for example, with gliadin. Both rats<sup>17</sup> and mice show remarkable constancy of weight when gliadin is the only protein fed, the body weight often exhibiting scarcely more variation than would be accounted for by the daily food intake.

In the present experiments, the stunting of the mice on the casein diet already described would seem to be due to neither of the above named conditions, but perhaps to an unsuitable proportion of the ingredients of the ration for the species in question. Recovery from the decline caused by feeding with gelatin, zein, or salt-free foods was rapid and complete on the casein food; but the gain always stopped when the original weight was reached or soon thereafter. As has been said, young mice made only a very slow gain on the casein diet; but there was so little difference when lactalbumin, glutenin, or edestin was substituted that it was evident that the trouble was not with the protein. A change in the percentage of protein present made surprisingly little difference; the content of protein alone was not the difficulty.

<sup>14</sup> *Zeitschr. f. physiol. Chem.*, Bd. 80, p. 307, 1912.

<sup>15</sup> See Charts XLVI and XLVII on p. 103 of Publication 156, Part II, Carnegie Institution of Washington, 1911.

<sup>16</sup> Cf. Hopkins: *Journ. Physiol.*, vol. 44, p. 425, 1912; Hopkins and Neville: *Biochem. Journ.*, vol. 7, p. 97, 1913.

<sup>17</sup> See charts on p. 128, Osborne and Mendel, loc. cit.

The growth on milk food of varying protein- and ash-content was significant from several standpoints. Chart 5 shows the increase in body weight on three milk foods whose composition is given in table 1. The numbers on the chart correspond with those in the table. The upper interrupted line is a composite of the growth charts of all the control mice on mixed food; the lower (dotted) line, of only such controls as were related in origin to the mice on the three milk foods represented in the chart.

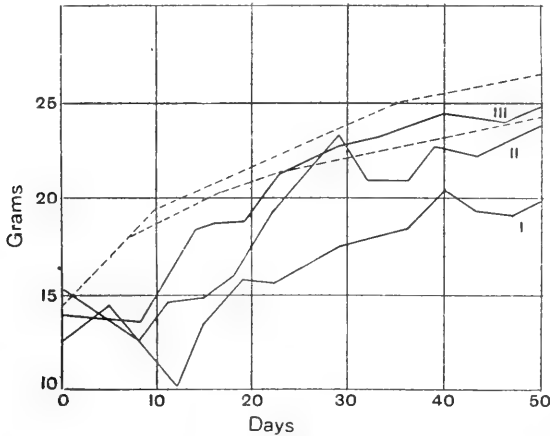


Chart 5 Showing the comparative growth of three mice on milk foods varying in protein and ash content. The dotted lines represent the normal curves of growth.

A comparison of chart and table shows that the rate of growth and the final weight attained increased with the percentage of salts and of protein, until when the protein was 30 per cent and the ash 6.8 per cent<sup>18</sup> both growth and final weight were comparable with those of the controls. The similarity is even greater if only controls of related origin are considered—as in the lower dotted line.<sup>19</sup>

The curves on chart 6 taken from the publications of Osborne and Mendel indicate the growth of rats on mixed food (normal

<sup>18</sup> Milk food III in the table.

<sup>19</sup> The mice represented in the lower dotted line and in line III grew as did the others, but they were evidently of a small variety.

TABLE 1

	I	II	III
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Milk powder.....	60.0	100	50.0
Starch.....	16.7	0	0.0
Lard.....	23.3	0	7.7
Protein-free milk.....	0.0	0	25.0
Casein.....	0.0	0	17.3
	100.0	100	100.0

or

Protein.....	15.4	25.6	30.7
Carbohydrate.....	39.0	37.2	38.6
Fat.....	39.7	27.4	21.4
Ash.....	3.6	6.0	6.8
Water.....	2.3	3.8	1.9

growth) and the milk food represented above in column I respectively. The close approximation of the growth curve of milk-fed animals of the two species to that of the controls ('normal' growth) indicates how satisfactory the milk foods were: milk food I (table 1) to the rats and milk food III to the mice. Rats and mice are quite comparable during the fourth week after birth, since both species at this time begin to take extraneous food in addition to their mother's milk. Using as a starting point the weight reached by the two species respectively, twenty days after birth,<sup>20</sup> rats double their weight in twenty days; mice in about half that time. The milk food used for rats contained 18 per cent protein and 3 per cent ash. On this mice are stunted as they are on the corresponding casein food; but with the protein (in milk-food) increased to 29 per cent and the ash to 6.8 per cent normal growth may take place. The smaller animals, growing nearly twice as fast, apparently need a double amount of bone- and flesh-forming food substances. The same principle has been found to pertain in the natural milk of various

<sup>20</sup> See Charts xxii (p. 87) and xxiii (p. 88) Osborne and Mendel: Publication 156, Part II, Carnegie Institution of Washington, 1911.

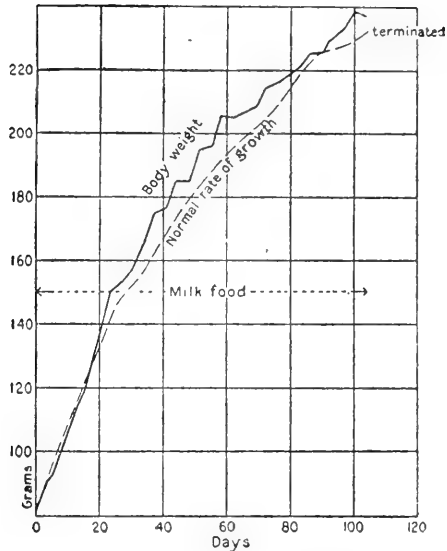


Chart 6 From Osborne and Mendel, Carnegie Institution of Washington, Publication 156, Part II, p. 95, 1911, showing the growth of rats on milk food.

mammals; the more rapid the growth, the greater is the protein- and ash-content of the milk. This is, however, not necessarily the whole secret; for when attempts were made to imitate with isolated food substances the milk food found most satisfactory for mice, the mice did not grow. It is of course possible that this is due to the presence in milk of some special substance that makes it so wonderfully adapted to its purpose; but it is also possible that the physical characteristics of the casein food rather than the absence from it of some essential constituent account for the result.<sup>21</sup> Further work will be necessary to settle this point.

<sup>21</sup> More recent investigation by the author in the Department of Household Science of the University of Illinois indicates that the trouble was due partly to the physical character of the food, partly to too high calorific value.

## SUMMARY

The experiments here reported have demonstrated the availability of mice for nutrition investigations of this sort. White mice have been kept in health for six months on an 'artificial' diet containing a single protein, casein, and for periods of from one to five months on similar foods in which the casein was replaced by lactalbumin, by either of the chief proteins of wheat, or by edestin. Gelatin and zein cannot replace more than one-half the protein in the food of mice; and in the case of gelatin at least, an even smaller proportion than that must be used if there is to be repair as well as maintenance. The nutritive requirements for growth may be quite different from those for maintenance only; and experiments in feeding with milk food indicate that mice require a higher percentage of protein and of ash for growth than do the slower growing rats. The capacity to grow is not necessarily lost during youth; for an increase in weight more rapid than the normal sometimes takes place after long periods of stunting.

The investigation was undertaken at the suggestion of Prof. Lafayette B. Mendel, to whom the author's sincere gratitude is due.





# SEX-DETERMINATION IN ASPLANCHNA AMPHORA<sup>1</sup>

CLAUDE W. MITCHELL

*University of Nebraska*

## INTRODUCTION

While studying the polymorphic rotifer, *Asplanchna amphora*, and while seeking to ascertain the factors which determine its remarkable transitions of form, attention was directed again and again to the value of this species as material for the study of sex-determination in rotifers. It was in the genus *Asplanchna* that male rotifers were first discovered and few other genera produce them in so great numbers. The species in question is hardy, easily reared, and capable of being subjected to a great variety of conditions. Especially in the matter of feeding, its polyphagous habits are well known, and are productive of marked physiological and morphological results. Of special significance is the fact that the polymorphic character of the species itself, mirroring as it does marked underlying differences, it is of utmost use in following any and all effects of controlled conditions. Thus the production or non-production of males does not occur as a fact which stands alone but as the concomitant of morphological changes which may be of much value in determining its cause.

Finding the material thus favorable it was suggested by Dr. J. H. Powers that experiment be undertaken along this line, in the hope that work upon this unstudied species might aid materially the solution of the problem which has hitherto been attempted solely upon a single rotifer, namely, *Hydatina senta*. I wish again to thank Dr. Powers for his valuable suggestions and aid, but above all for the interest shown in each experiment and in the results of the entire work.

<sup>1</sup>Studies from the Zoölogical Laboratory, University of Nebraska, No. 109.

In brief, the results of the work previously done upon the problem of sex-determination in rotifers is as follows: The well known work of Maupas upon *Hydatina senta* led him to the conclusion that a rise in temperature results in an increase of male production. Following Maupas, Nussbaum found the factor of temperature negative, while he attributed to starvation alone the fact of male production. Punnett discarded the factors of both temperature and starvation, finding, as he thought, that high or low male production ran in given lines or strains, which latter fact he attributed to an internal factor, 'zygotic constitution.' Whitney again concludes that sex-determination is controlled by external conditions, male production being favored by the presence of some substance in solution, the nature of which is undetermined, but the absence of which predisposed to the production of parthenogenetic females only. Shull at first also maintained that sex is determined by external conditions—lack of certain chemical matter in solution—though later, finding that different strains of the same species produce varying numbers of males even under identical conditions, he returns to the assumption of some internal factor inherent in lines or races. In progeny of a cross between different lines of high and low male-producers he finds the potentiality of male production increased in some instances and lowered in others. In a later work he returns to the assumption of 'genotypic constitution' as the determining factor, which is in essence a return to the position of Punnett. The relation of such internal factors to the influence of external conditions remains quite unexplained. It seems plain, therefore, that additional work is much needed before final conclusions can be reached.

Before proceeding further it is necessary to justify briefly the use of the phrase 'sex-determination' in its application to the phenomena we are studying. Shull and writers in allied fields have maintained that the phenomena in question are not really matters of sex-determination as such, but represent transitions from parthenogenetic reproduction on the one hand to sexual reproduction on the other. Viewed broadly this contention at first seems natural; but under analysis we fail to agree with it.

In typical parthenogenetic reproduction in rotifers it is plain that all the individuals are females<sup>2</sup> and all the eggs which develop into females are, in a true if somewhat special sense of the word, female eggs. They must certainly contain the full anlage of one type of female constitution. The mere fact that they do not require fertilization for their development can not rob these eggs of their character as female gametes any more than it can rob the resulting parthenogenetic adults of their character as females. In short in any series of purely parthenogenetic generations the entire race is female. Now whenever this typical parthenogenetic reproduction is so changed that certain eggs become male producers or males, it seems plain to us that sex has been determined—namely that an exclusive female production has become in part a male production. In point of fact, viewing the phenomena more closely, we may see exactly what takes place: the egg or reproductive cell of certain young females becomes modified (either in the course of one or two generations) so as to determine—without fertilization—the production of a male. These eggs therefore have become male eggs and whatever factor has determined this, their character, is a factor of sex-determination. It is true that these same eggs, in the event of fertilization, are again redetermined as female eggs, becoming resting eggs, which always hatch as typical females. It can not be too clearly understood that the factor we are seeking is something which modifies an egg, determining its inherent tendency towards the development of a male or a female. It matters not in the least whether we conceive the determining factor as acting directly upon the egg in its earlier or later growth period or whether its influence may have been indirect, having been cumulative through one or more generations preceding its development. In any case we have phenomena which it seems can appropriately be designated as those of sex-determination.<sup>3</sup>

<sup>2</sup> It is curious that in the discussion of sex-determination this fact seems usually forgotten or quite neglected, despite the fact that the same writers in all other connections habitually designate parthenogenetic individuals as female.

<sup>3</sup> In advocating the view opposite to the one which we are here defending Shull has claimed that the source of confusion in the case of rotifers lies in the fact that there is no outward distinction between the parthenogenetic female and the

Certain general conclusions previously arrived at by the author, which bear upon the question of sex-determination may be briefly stated before proceeding to the actual experiments. The rotifer in question is a tri-morphic if not a poly-morphic species. The form which emerges from the resting egg is preponderatingly parthenogenetic, while the later, larger, and more complex types are copious male producers. This smaller, saccate type may also be reached by degeneration from the others, the humped and campanulate types. It is plainly upon a lower physiological level.

The study of the species has also disclosed that a marked metabolic rhythm runs through long series of generations and manifests itself in a rhythm of reproduction, even comprising periods of marked depression as well as acceleration. As stated in a preceding article this rhythm is not the result of external conditions. Depressions and accelerations in the reproductive rate manifest themselves strongly when external conditions are as uniform as possible. Nevertheless, it should be stated that this inherent rhythm although relatively independent of external conditions is not absolutely independent of them. A rise in temperature or a fresh nutritive stimulus may check a tendency towards depression or raise still higher the wave of acceleration.

As it has been shown there exists a definite relation between the amount of male production and the type of individual, and as it appears that there is also a correlation between the type of individual and physiological factors such as we have mentioned,

so-called 'sexual female,' whereas these two classes of individuals are sharply distinguished in certain other groups. In contradistinction to this claim we would raise the question whether the exact opposite is not nearer the truth, namely, whether the obvious morphological differences which sometimes accompany these diverse reproductive habits have not led to the over-accentuation of their inherent differences. Sex-determination there would be in any case. But the nature, degree, or importance of the phenomenon in this special instance is bound up with the whole question of the relation between parthenogenetic and sexual reproduction. Do not many recent advances point to the fact that the differences between the two have in the past been over-estimated? Work which is nearing completion upon certain problems in the genus *Asplanchna* point with surprising emphasis to the fundamental unity between parthenogenetic and sexual reproduction in this species.

physiological rhythm, high nutrition, and so forth, it was conceived that a definite relation must also exist between the extremes of this series as well, namely, between what we shall call the physiological potential and amount of male production. Having found that the type of rotifer, even regardless of rhythm, could be controlled through the factor of nutrition alone, it seemed that this must be directly or indirectly a sex determining factor. The experiments which follow were in large part directed towards ascertaining the truth of these suppositions, that male production and consequently sex-determination is a matter of physiological potential and under the more or less direct control of nutrition. It was hoped that the degree of such relation might be rendered fairly definite. Search was also made, however, for any other factors, major or minor, negative or positive, which may play a rôle in sex-determination.

Throughout all experiments the same technique was used in rearing the *Asplanchna* as was described in a preceding article,<sup>4</sup> consequently details need not be given here. The food supply consisted of a mixed diet of *Paramecia*, *Hydatina*, and *Brachionus* by which the humped type as well as the saccate type could be continually reared.

#### MASS CULTURE FROM SACCATES

During the winter of 1910-1911, a number of pedigree series were reared which remained in the saccate form throughout many generations and showed but few instances of male production. As offshoots from these series thirteen mass cultures were started, from time to time, to determine the influence of such conditions upon the form, type, as well as male production. The histories of these mass cultures are given in table 1.<sup>5</sup> Examination of the table will show that in seven of these cultures the transition to the humped type occurred and that in four of this same number males were also formed.

<sup>4</sup> C. W. Mitchell: Jour. Exp. Zool., vol. 15, pp. 91-130.

<sup>5</sup> Table 2 of preceding paper.

Although in these mass cultures, conditions could not, of course, be definitely controlled, yet all ordinary factors, food, fluid medium, temperature, mass stimulus, and metabolic products were made as uniform as possible and the fact that but four of the seven cultures produced males indicates the probability that no marked factor of sex-determination was to be found in these conditions. Two other conclusions may also be drawn from these early mass cultures, namely, that the potentiality of male production was present even in the all but exclusive parthenogenetic

TABLE 1

*Mass cultures derived from individuals of Series A and D<sup>1</sup>*

NO. OF INITIAL GEN.	SERIES	TIME OF ISOLATION	TIME OF BIRTH OF FIRST HUMPED INDIVIDUALS		REMARKS
			1910	1910	
9	A	Nov. 3	Nov. 5		Born as humped ind.; produced humped young
9	A	Nov. 3	Nov. 5		Born as humped ind.; produced humped young; males
5	D	Oct. 25	Oct. 28		Parent turned cannibal; produced humped young; males
5	D	Oct. 27	Oct. 30		One humped ind. only
7	D	Oct. 26	Oct. 30		Many humped ind.; males; discarded Nov. 10, 1910
17	D	Nov. 23	Jan. 2, 1911		Saccate and humped ind.; carried for four weeks
17	D	Nov. 23	none		All saccate; discarded Jan. 12, 1911
3	D	Oct. 18	none		All saccate; discarded Nov. 3, 1910
5	D	Oct. 31	none		All saccate; discarded Nov. 4, 1910
5	D	Oct. 23	none		All saccate; discarded Nov. 4, 1910
5	D	Oct. 24	Nov. 4, 1910		Produced humped young; males appeared
15	D	Nov. 20	none		All saccate; discarded Dec. 26, 1910
17	D	Nov. 23	none		All saccate; discarded Jan. 12, 1911

<sup>1</sup> Table 2 in article in *Jour. Exp. Zool.*, vol. 15, no. 1, 1913

lines, and second, the conclusion, previously mentioned, that male production is partially though not completely correlated with the appearance of the humped type. In none of the six saccate cultures were males produced.

In the spring of 1911 a definite method was discovered by which the humped type might be produced and reared at will. Besides isolation cultures, sixteen mass cultures of this type were started. By means of these cultures such experiments as our facilities allowed were made to test the influence of temperature change. Although the experiments, owing to limited facilities, were not performed with the precision desired, yet the results of ample observation agree fully with the results of Shull, Nussbaum, and Punnett. Temperature, within fairly wide limits, is essentially a negative factor in regard to sex-determination.

#### LOW FEEDING AND STARVATION

Fifty mass cultures of the humped type, drawn from the same pedigree series as the preceding, were treated as follows: The ordinary culture medium was filtered and to the filtrate a minimum quantity of food was added. This formed the fluid medium of each culture and compelled low feeding, to practical starvation, of the individuals. The chief results were similar to those described in the previous paper, namely, degenerative changes resulting in transitional individuals, then the saccate type. In only two out of the fifty cultures were males produced during the two to three weeks which they were maintained. The results thus, of general starvation in mass cultures, resemble those of some of Nussbaum's experiments on *Hydatina senta*, which likewise resulted in parthenogenetic female production. An added interest attaches to the results of starvation on *A. amphora*, in that the regressive change from the larger humped type to the smaller saccate type, discloses the tendency toward the lowered physiological potential already mentioned in connection with this form.

## WELL FED MASS CULTURES OF THE HUMPED TYPE

To twenty mass cultures derived as were the preceding, food—chiefly *Paramecia*—was supplied in sufficient quantity to ensure abundance for each individual. The humped type was maintained in these cultures and in every one of the twenty males were produced at some time during the three weeks they were continued. Yet male production was by no means simultaneous or uniform in amount; in but one culture did the number of males at any one time become noticeably great, this toward the end of the period. It is difficult and perhaps impossible to interpret these minor, though striking differences. They could not be referred to variations in conditions surrounding the cultures; but within a mass culture cannibalism or many other unobservable incidents may take place which complicate results. It is plain however, that the results of this experiment, indicate a greater tendency toward male production in well fed mass cultures than in those poorly fed or starved. This result stands in evident contradiction to statements of workers upon *Hydatina senta*, although a careful reading of Nussbaum's experiments suggest the possibility of a like interpretation.

## INFLUENCE OF CULTURE MEDIA UPON MALE PRODUCTION

To test the possible effect upon male production, of metabolic products or other substances in solution, the entire culture medium was filtered from the mass culture mentioned above in which an unusual number of males had appeared. This filtrate was added unsparingly to a mass culture of the humped type in which as yet no males had been produced. The results were wholly negative and indicated, in so far as one experiment is of value, the improbability of the culture medium containing the sex-determining factor. We will merely add that extensive observation in regard to various media and especially in regard to older mass cultures seem to negative the probability of any direct influence of dissolved matter upon male production. It has been repeatedly observed that male production in this rotifer is frequent and copious in the most diverse fluid-media and even in the heaviest compost filtrate.



## HIGH NUTRITION FOLLOWED BY BRIEF STARVATION

Fifteen new mass cultures were started from the general stock as above, some of them begun from humped individuals while others were started from saccates which were then so fed as immediately to bring about the transition. All the cultures were full-fed and the following three-fold test showed in general the success of the feeding: first, the humped type as soon as produced was maintained; second, the digestive tracts of all individuals examined contained abundance of food; while, third, uncaptured food remained free in the cultures. As soon as these nutritional conditions were established each culture was starved at least once for a period of twenty-four hours. The results from two of these cultures, four and nine, may best be ignored for they had produced males before starvation. Nothing in their conduct contradicts the results of the other cultures in the experiment although the results with them were more complex. A detailed description of the results is given in table 2.

In eight of the remaining thirteen cultures copious male production followed closely (within twenty-four hours or less) upon the starvation period, while five cultures produced no males, one of them reverting to the saccate form as a result of the starvation. One culture (11) was subjected to repeated periods of starvation; no second production of males followed, but instead a retrogression to the saccate type.

This immediate male production, following in eight out of fifteen cases of combined high feeding and starvation, is at once suggestive as confirmation of our previous hypothesis as to the cause of male production. Moreover, it is easily possible to interpret the five cases of failure. They were all cultures in which the transition from saccate to humped form had but recently taken place; this was also true in a portion of the eight positive cultures. But, it is natural to assume that the food stimulus which had brought about the transition by so raising the physiological level in the different cultures that the humped type was produced, had not acted long enough or with sufficient force to make male production possible in the five, while it had in the

case of the eight. But the probability of this interpretation must be tested by further and more definite experimentation.

Before proceeding to the consideration of isolation experiments, however, it is well to note that the results of all the preceding

TABLE 2

*Mass cultures under high nutrition subjected to twenty-four-hour periods of starvation*

H, humped; S, saccate; Cam, campanulate; R. E., resting egg; Pro., male producer; Iso., isolated.

NO. OF EXP. CULTURE	NAME OF SERIES	NO. OF GEN. IN SERIES	NO. OF IND. IN FAMILY SERIES	DATE OF ISOLATION	TYPE	DATE OF BIRTH OF HUMPED TYPE	DATE OF STARVATION	DATE OF BIRTH OF MALE	REMARKS
1	F	20	4	1911 Mar. 2	H	1911	1911 Mar. 8	1911 Mar. 10	60% M. R. E.
2	F	21	5	Mar. 3	H		Mar. 10	Mar. 11	50% M.
3	F	23	2	Mar. 5	H		Mar. 10	Mar. 12	Many
4	F	26	6	Mar. 10	S	Mar. 14		Mar. 16	Fed on Moina
5	D	55	2	Mar. 2	S	Mar. 16	Mar. 18		No males
6	E	13	3	Mar. 5	S	Mar. 8	Mar. 10	Mar. 13	50% M.
7	D	58	3	Mar. 7	S	Mar. 9	Mar. 13	Mar. 14	Few
8	F	34	9	Mar. 19	S	Apr. 7	Apr. 10		No males
9	G	15	1	Mar. 21	S	Mar. 30		Apr. 1	M. and Pro. iso.
10	F	34	6	Mar. 21	S	Apr. 7	Apr. 8 Apr. 11	Apr. 9	M.-S. No males
11	D	69	6	Apr. 1	S	Apr. 4	Apr. 7 Apr. 9 Apr. 11	Apr. 8	Starved became S. 4-13
12	D	69	7	Apr. 1	S	Apr. 4	Apr. 6 Apr. 12	Apr. 8	M.+R.E. Became Cam.
13	F	37	3	Apr. 3	S	Apr. 6	Apr. 8		No males
14	D	91	7	May 14	S	May 20	May 24	May 28	M.;R.F.
15	D	95	5	May 20	S	May 26	May 28		S

mass cultures point to the following general conclusions: First, all lines, if not all individuals of the species, possess the inherent potentiality of male production, but whether this potentiality shall lead to actual male production or remain latent depends upon external conditions. Second, successful high feeding of the humped type results in an increase of male production. Third, continuous low feeding or starvation results in the parthenogenetic production of females only. Fourth, sudden starvation of cultures possessing a low metabolic level also results in pure parthenogenetic production of females and may even cause a reversion from the more sexual or humped form of the *Asplanchna* to the saccate or more purely asexual form. Fifth, slight starvation of the well fed cultures may result in male production.

To test the correctness of these tentative conclusions from mass cultures, we turn to the task of definitely controlled experiments with individual or isolation cultures.

#### MALE PRODUCTION OF INDIVIDUALS UNDER HIGH NUTRITION

From cultures of Series D (1910-11), were isolated fifty newly born humped individuals, each of which was placed in an individual culture abundantly supplied with *Paramecia*. Twelve of the fifty, or 24 per cent became male producers (table 3). This is about an average percentage, no more at least, than is shown in the usual pedigree series of humped individuals.

#### MALE PRODUCTION OF INDIVIDUALS STARVED IMMEDIATELY AT BIRTH

During a period of six weeks, thirty-eight individuals were isolated, within one hour after birth, from the general pedigree series. The period of time during which the isolations were made insured the elimination of accidental external factors. Each individual was examined under the microscope to be sure that no food had been ingested and that no resultant development of ovarian cells had taken place. All were placed in filtered culture medium. To four of these cultures food was added to constitute special controls, although besides these there were the

general pedigree series which served as general controls. To the remaining thirty-four no food was given for a period of twenty-four hours. They were then again examined microscopically and developing ova noted. Food was now added to these starvation cultures and in all but four cases it was ingested. With four individuals death followed starvation. In the others growth was resumed and male or female young were produced. Eighty per

TABLE 3  
*Isolation cultures under normal food supply*

NO. OF CULTURE	NO. OF GEN. IN SERIES	DATE OF ISOLATION	TYPE OF YOUNG PRODUCED	NO. OF CULTURE	NO. OF GEN. IN SERIES	DATE OF ISOLATION	TYPE OF YOUNG PRODUCED	
		1911				1911		
1	67	Mar. 27	F	26	72	Apr. 12		
2	67	Mar. 27	F	27	72	Apr. 12	F	
3	67	Mar. 28		28	72	Apr. 13	F	
4	67	Mar. 28		29	72	Apr. 13		
5	67	Mar. 28		30	72	Apr. 13		
6	67	Mar. 29	F	31	72	Apr. 13		
7	67	Mar. 29	F	32	72	Apr. 13		
8	67	Mar. 29	F	33	72	Apr. 14	F	
9	67	Mar. 29	F	34	72	Apr. 14	F	
10	67	Mar. 29	F	35	72	Apr. 14	F	
11	67	Mar. 30	F	36	72	Apr. 14	F	
12	68	Mar. 30	F	37	72	Apr. 14		
13	66	Mar. 26	F	38	72	Apr. 15	F	
14	66	Mar. 26	F	39	72	Apr. 18	F	
15	66	Mar. 26	F	40	73	Apr. 15		
16	66	Mar. 26	F	41	73	Apr. 15		
17	66	Mar. 27	F	42	73	Apr. 15	F	
18	66	Mar. 27	F	43	73	Apr. 15	F	
19	66	Mar. 27	F	44	73	Apr. 15	F	
20	66	Mar. 28	F	45	73	Apr. 15	F	
21	66	Mar. 28	F	46	73	Apr. 15	F	
22	66	Mar. 28	F	47	73	Apr. 15	F	
23	72	Apr. 12	F	48	73	Apr. 15	F	
24	72	Apr. 12	F	48	73	Apr. 15	F	
25	72	Apr. 12	F	50	73	Apr. 15	F	
Average per cent.....							76	24

cent of these starved mothers became male producers (table 4). This percentage is seen to be nearly three times that in the preceding experiment. Of the four special controls one only produced males, which is close to the normal average, 21 per cent, in the general well fed humped series which were the additional controls. The result of starvation in these isolation cultures thus bears out to a considerable extent the conclusions suggested by the mass cultures. By repetition of such experiments it would seem that evidence might be accumulated to establish the general fact that a check in nutrition, or sudden starvation tends to produce an unusual proportion of males.

In the course of our experiments, however, we preferred to check this conclusion in the attempt to solve the more delicate problems offered for instance, by the individual cases in which our control cultures produced males without apparent preceding starvation and again, by the lesser portion of the starved cultures which did not show male production. In short we hoped if possible to achieve a complete explanation of sex-determination. It seemed probable that this must be sought not only in food and starvation but also in the matter of rhythm which, as we have pointed out, involves profound physiological and morphological differences as well as differences in rate of reproduction.

To do this required that individuals chosen for experiment should be isolated from pedigree lines at known stages in their physiological rhythm, that is when reproduction is at its maximum and minimum.

#### EFFECT OF LOW FEEDING AND STARVATION UPON INDIVIDUALS CHOSEN FROM SERIES DURING PERIODS OF DEPRESSION

Thus to demonstrate the scarcity of males produced by individuals chosen at periods of rhythmic depression and bred under unfavorable nutritive conditions, which tend toward low potential, twenty-five humped individuals were chosen from families during known periods of depression. These were isolated in cultures containing no food, but after twenty-four hours food was added in small quantities. The young were in every case females.

TABLE 4

*Starvation at period of birth of young females*

NO. OF CULTURE	NAME OF SERIES	NO. OF GEN. IN SERIES	NO. OF IND. IN FAMILY SERIES	DATE OF ISOLATION	PERIOD OF STARVATION	DATE WHEN FOOD WAS GIVEN	TYPE OF OFFSPRING	REMARKS
				1911	hours	1911		
1	F	24	7	Mar. 9		Mar. 9	F	Control
2	F	24	8	Mar. 9	10	Mar. 10	M	
3	F	24	9	Mar. 9	10	Mar. 10	M	
4	F	24	10	Mar. 9	10	Mar. 10	M	
5	F	24	11	Mar. 9	10	Mar. 10	M	
6	F	24	12	Mar. 9	10	Mar. 10	M	
7	F	24	13	Mar. 10	10	Mar. 11	died	
8	F	24	14	Mar. 10	10	Mar. 11	F	
9	D2	60	6	Mar. 12	10	Mar. 13	M	
10	F	28	5	Mar. 15	8	Mar. 16	M	
11	F	28	6	Mar. 16	8	Mar. 16	M	
12	F	28	7	Mar. 16	8	Mar. 17	M	
13	F	28	8	Mar. 17		Mar. 17	F	
14	F	32	2	Mar. 25		Mar. 25	M	
15	F	32	3	Mar. 25	10	Mar. 26	M	
16	D	67	4	Mar. 18	7	Mar. 28	M	
17	D	67	5	Mar. 18	7	Mar. 28	M	
18	D	67	6	Mar. 18	7	Mar. 28	F	
19	D	67	7	Mar. 29		Mar. 29	F	
20	D	72	{ from mass	Apr. 10	6-8	Apr. 12	M	
21	D	72	1	Apr. 10	6-8	Apr. 11	M	
22	D	72	2	Apr. 10	6-8	Apr. 11	M	
23	D	72	3	Apr. 10	6-8	Apr. 11	M	
24	D	72	4	Apr. 10	6-8	Apr. 11	died	
25	D	72	5	Apr. 10	6-8	Apr. 11	F	
26	D	72	6	Apr. 10	6-8	Apr. 11	F	
27	D	72	7	Apr. 10	6-8	Apr. 11	M+R.E	Male added to fert.
28	D	72	8	Apr. 10	6-8	Apr. 11	M+R.E	Male added to fert.
29	D	72	9	Apr. 10	6-8	Apr. 11	died	
30	D	72	10	Apr. 10	6-8	Apr. 11	M	
31	D	72	11	Apr. 10	6-8	Apr. 11	M+R.E	Male added to fert.
32	D	72	12	Apr. 10	6-8	Apr. 11	M+R.E	Male added to fert.
33	D	72	13	Apr. 10	6-8	Apr. 11	F	Males added * to fert.
34	D	72	14	Apr. 10	6-8	Apr. 11	F	
35	D	75	7	Apr. 16	36	Apr. 18	died	
36	D	75	8	Apr. 16	36	Apr. 18	M	
37	D	77	7	Apr. 21	no food		M	
38	D	77	8	Apr. 21	no food		M	

80 per cent male producers  
20 per cent female producers

Total, 24 male producers  
6 female producers  
4 controls  
4 died

Individual young from each of twenty cultures were again isolated in like cultures and allowed to reproduce. These were also parthenogenetic females. A third generation was reared in nine of the latter cultures under the same conditions with a corresponding result of no male production. The more detailed history is given in table 5. As expected from previous experiments, both with mass and individual cultures, there was accompanying this non-male production a reversion to the saccate type which was all but universal.

This experiment was made with a double purpose, that of checking our results with those obtained from the mass culture experiments as well as checking the starvation experiments of Punnett, Whitney, and Shull on the rotifer *H. senta*. Our results agree fully with those of the two former experimenters but are somewhat at variance with those of the latter. We shall return to this apparent disagreement in the final discussion.

#### STARVATION OF INDIVIDUALS CHOSEN FROM LINES AT PERIODS OF HIGH POTENTIAL

In order to experiment with material of high potential and homogenous character, forty individuals were derived from four parents. Two of these parents were chosen from Series E and two from Series D, both series being at the time in question in the full humped type and in a crescendo of reproduction. Of the four individuals, two from each series were isolated in cultures of low nutrition while the other two corresponding individuals were isolated in cultures of high nutrition. These four individuals thus situated produced the forty young above mentioned; each of these was isolated at birth. Of these young thus isolated one or more from each parent were used as controls and placed under nutritive conditions identical to those of the well-fed parents. All the others, when isolated shortly after birth, were subjected to eight to ten hours complete starvation, followed by uniform and abundant food. The detailed results are given in table 6.

The results confirm in high degree previous expectations. Individuals from poorly nourished parents themselves subjected to starvation followed by high feeding gave no male producers. In-

TABLE 5

*Starvation at birth of individuals produced by low potential females*

NO. OF CULTURES IN EXP.	GEN. IN SERIES D	GEN. IN EXP.	DATE OF ISOLATION	PERIOD OF STARVATION	DATE OF BIRTH OF FIRST YOUNG	NO. OF YOUNG	TYPE
1	72	1	1911 Apr. 9	hours 24	1911 Apr. 13	3	H
		2	Apr. 13	24	Apr. 16	2	I
		3	Apr. 16	24		1	S
2	72	1	Apr. 10	24	Apr. 13	3	H
		2	Apr. 13	24		-	S
3	72	1	Apr. 10	24	Apr. 13	3	H
		2	Apr. 13	24	Apr. 16	2	I
		3	Apr. 16	24	Apr. 20	2	S
4	72	1	Apr. 10	24	Apr. 12	4	H
		2	Apr. 15	24	Apr. 18	1	I
		3	Apr. 18	24			S
5	72	1	Apr. 10	24	Apr. 14	1	H
		2	Apr. 14	24	Apr. 17	3	I
		3	Apr. 17	24		2	S
6	72	1	Apr. 11	24	Apr. 13	3	H
		2	Apr. 13	24	Apr. 16	1	S
		3	Apr. 16	24		1	S
7	72	1	Apr. 11	24	Apr. 13	1	H
		2	Apr. 13	24			S
8	72	1	Apr. 11	24	Apr. 14	3	H
		2	Apr. 14	24		2	I
9	72	1	Apr. 11	24	Apr. 14	6	S
		10	73	1	Apr. 12	24	Apr. 13
10	73	2	Apr. 13	24	Apr. 15	4	I
		3	Apr. 15	24			S
		11	73	1	Apr. 12	24	Apr. 13
12	73	2	Apr. 13	24	Apr. 16		I
		1	Apr. 12	24	Apr. 13	3	H
13	73	2	Apr. 13	24	Apr. 15	2	S
		1	Apr. 12	24	Apr. 13	6	H
		2	Apr. 13	24	Apr. 16	4	S-I
14	73	3	Apr. 16	24		2	S
		1	Apr. 12	24	Apr. 13	3	H
		2	Apr. 13	24	Apr. 16	2	I
15	73	3	Apr. 16	24		1	S
		1	Apr. 12	24	Apr. 14	3	H
		2	Apr. 15	24		1	S
16	73	1	Apr. 13	24	Apr. 16	2	H
		2	Apr. 16	24	Apr. 18	1	S
		3	Apr. 18	24			S



TABLE 5—Continued

NO. OF CULTURES IN EXP.	GEN. IN SERIES D	GEN. IN EXP.	DATE OF ISOLATION	PERIOD OF STARVATION	DATE OF BIRTH OF FIRST YOUNG	NO. OF YOUNG	TYPE
			1911	hours	1911		
17	73	1	Apr. 13	24	Apr. 16	6	H
		2	Apr. 16	24	Apr. 18	2	I
18	73	1	Apr. 13	24	Apr. 15	3	H
		2	Apr. 15	24		4	S
19	73	1	Apr. 13	24	Apr. 16	2	H
		2	Apr. 16	24	Apr. 18	1	S
20	73	1	Apr. 13	24	Apr. 16	5	S
21	73	1	Apr. 14	24	Apr. 16	3	S
22	73	1	Apr. 14	24	Apr. 16	3	S
23	73	1	Apr. 14	24	Apr. 18	3	H
		2	Apr. 18	24	Apr. 20	5	I
		3	Apr. 20	24			S
24	73	1	Apr. 15	24	Apr. 18	4	S
25	73	1	Apr. 15	24	Apr. 19	3	H
		2	Apr. 19	24	Apr. 21	2	I

TABLE 6

*Experiment with individuals of families derived from Series E and D during known high potential*

NO. OF EXP.	NAME OF SERIES	GEN. IN SERIES	DATE OF ISOLATION	DATE OF BIRTH OF THE OFFSPRING, SURROUNDING CONDITIONS, AND TYPE PRODUCED											
				1st	2d	3d	4th	5th	6th	7th	8th	9th	10th	11th	12th
1	E	18	Mar. 13, 1911	16	16	17	17	17	18	19	19	19	19		
				well fed	Starved for eight to ten hours										
2	D	74	Apr. 13, 1911	13	M	M	F	M	M	M	M	F	F		
				well fed	well fed						Starved for eight to ten hours				
3	E	18	Mar. 14, 1911	F concl. <sup>1</sup>	F	F	F	M	F	M	M	M	F	M	D
				well fed	Starved for eight to ten hours										
4	D	74	Apr. 13, 1911	14	F	F	F	F	F	D	F				
				starved	well fed						Starved for eight to ten hours				
				F concl.	F	F	F	F	F	F	F	F			

<sup>1</sup> Concl., Control culture.

dividuals likewise from poorly nourished parents themselves subjected to high nutrition without starvation also gave negative results. Individuals from well-nourished parents themselves subjected to high nutrition without starvation give but 11 per cent of male producers. Individuals from well nourished parents, subjected to starvation at birth, followed by a period of high nutrition gave seventy per cent of male producers. Although the numbers employed in this experiment are insufficient to fully warrant general conclusions, yet they form valuable confirmation of the preceding results. The first and most fundamental condition for male-production is plainly a high-potential grand-parent which will transmit its high potential under good food conditions to its progeny. It can be obtained from a generation in the crescendo of the metabolic rhythm. The second though hardly less important factor is a slight nutritive interruption or period of starvation in the generation of potential male-producers itself. This conclusion suggested the attempt to determine still more definitely the period at which starvation is effective. Such experiments would of course serve at the same time as further test of the conclusions reached.

#### EFFECT OF STARVATION AFTER THE INITIAL GROWTH PERIOD OF THE FIRST EGG

Thirty young individuals of the humped type were chosen from pedigree lines in high crescendo and were isolated in a culture containing food in such quantity that a normal supply could be obtained by each individual. These young were allowed to remain in this culture while the first egg began to appear growing at the apex of the ovary. Each individual, about the time the connection of the egg with the ovary was broken, was isolated in a typical starvation culture where it was kept without food for a period of eight to ten hours. Food was then given. As table 7 shows, there resulted but 30 per cent of male producers, only a moderate increase over the per cent in the normal well fed series of humped individuals.

TABLE 7

*Starvation of high potential females five hours after birth*

NO. OF CULTURE	DURATION OF STARVATION PERIOD	TYPE OF YOUNG PRODUCED		NO. OF CULTURE	DURATION OF STARVATION PERIOD	TYPE OF YOUNG PRODUCED	
	<i>hours</i>				<i>hours</i>		
1	8-10	F		16	8-10	F	
2	8-10	F		17	8-10		M
3	8-10		M	18	8-10		M
4	8-10	F		19	8-10	F	
5	8-10	F		20	8-10		M
6	8-10		M	21	8-10	F	
7	8-10	F		22	8-10	F	
8	8-10		M	23	8-10	F	
9	8-10	F		24	8-10		M
10	8-10	F		25	8-10	F	
11	8-10	F		26	8-10	F	
12	8-10	F		27	8-10	F	
13	8-10	F		28	8-10	F	
14	8-10		M	29	8-10	F	
15	8-10	F		30	8-10		M
Average per cent .....						70	30

## STARVATION AT PERIOD OF BIRTH

This experiment is not only in contrast to the preceding experiment but is essentially a repetition of a portion of it. Nine individuals of high potential were again chosen from the same series as had been used in the former experiments and at a period of maximum reproduction. A number of the earlier individuals in the progeny of these nine were again isolated in cultures under similar food conditions. These constituted controls. The remainder of the young born to the same parents were subjected at birth to the above mentioned period of starvation. The individual results are given in table 8. Of the thirty-nine full fed young constituting the controls born of the nine parents but six became male producers; while of the remaining sixty-eight subjected to starvation at birth and then fed, fifty-one became male producers. Thus, the number of male producers in a highly nourished uniform stock is raised in this experiment by the brief period of starvation, from 15 per cent to 76 per cent. And if

TABLE 8  
*Starvation at birth of high potential females from Series D*

NO. OF CULTURES	DATE OF ISOLATION OF PARENT	INDIVIDUALS IN ORDER OF THEIR BIRTH										Total							
		Well Fed					No. of F	No. of M	Starved					No. of F	No. of M				
Apr. 1911																			
1	16	F	F	F	F	F	F	F	7	0	M	M	M	M	M	M	0	6	13
2	16	F	F	M	F	F	M	4	2	M	F	M	F	M	M	2	4	12	
3	16	F	F	M	F	F	4	1	F	M	M	M	M	F	F	3	5	13	
4	17	F	F	F	F	5	0	M	F	F	M	M	F	M	M	3	6	14	
5	17	F	M	F	F	3	1	F	M	M	M	M	F	M	M	2	6	12	
6	17	F	F	F	F	4	0	M	M	M	M	M	F	M	F	2	7	13	
7	17	M	F	F	2	1	F	M	M	M	F	F	M	M	3	5	11		
8	18	F	F	F	3	0	F	M	F	M	M	M	M	2	6	11			
9	18	F	M	1	1	M	M	M	M	M	0	6	8						
Total.....		33					6	Total.....					17	51	107				
Per cent.....		85					15	Per cent.....					25	75					

we contrast the results of starvation immediately following birth as in this experiment with the results of starvation after the initial ovarian development we get the contrast of 76 per cent to 30 per cent.

These results at least confirm, if not absolutely prove, the conclusions before reached, that male production follows upon the summation of favorable external and internal conditions, plus a sudden interruption by a nutritive check. The results of the last two experiments also show that this nutritive check is highly effective only when applied during a brief period of the young female's existence.

There still remains a residuum of unexplained results, both negative and positive: why do 25 per cent of the individuals most favorably treated for male production still remain female producers? And, on the other hand, if starvation is a vital course of male production, why do 15 per cent of highly nourished individuals become male producers without this apparent stimulus? We can only answer these questions theoretically. But to one who has noted minutely the phenomenon of variation in this species and the unusual amount of variation which involves struc-

ture, constitutional vigor, and reaction to stimuli, the residuum of apparently negative results is readily interpreted. Thus, that certain high bred females should become male producers without enforced starvation may follow naturally from mere accidents and irregularities in feeding which the most uniform of control conditions can not eliminate. And, on the other hand, that in starvation experiments, a certain percentage still develop as female producers, is readily explicable upon the assumption of inherent variations in constitutional vitality. These individuals are simply not of as high potential as are the others. General experience shows that sister individuals in the most normal families often differ in the rate of growth and in reproductive power.

To the foregoing results which comprise all the experiments performed when working directly upon the problem of sex-determination, we may add brief analysis of certain mass cultures conducted subsequently upon the same species when working upon certain problems of heredity. The reason for turning aside and again repeating mass culture experiments lay in the fact that we deemed ourselves in possession of stocks of the rotifer in still higher potential of development. They were of the humped form but at periods when this easily gave rise to the third, still larger or campanulate type. Each of the mass cultures was indeed derived from humped stock descending from resting eggs produced by the giant campanulates and which had been fertilized by males so derived. A considerable number of mass cultures were instituted and reared from the above stock, and a number of them were treated according to the methods described for producing maximum male production. Every one of the attempts with this highly developed stock was successful; swarms of male producers, males, and individuals with resting eggs resulted. So great were these epidemics of male production and resulting resting eggs that the cultures were all but swept out of existence. Careful records of male production were also kept of all other mass cultures at this time to note whether male epidemics occurred under other conditions; but none such were encountered. Likewise in all mass cultures which produced few males, at least some one of the favoring factors was absent,—

either the majority of individuals were of the saccate type, or the slow rate of reproduction indicated that the individuals, though humped, were not of a high physiological potential, or the general food supply had been inadequate.

So extreme was the male production in the mass cultures indicated above that a few of the smaller ones were examined, individual by individual, with the microscope. The percentage of male producers is given in table 9. It will be seen that in one case the number of male producers simultaneously present reached the surprising maximum of 92 per cent of the entire culture.

TABLE 9  
*Starvation of mass cultures of humped individuals*

NO. OF GEN.	DATE OF BIRTH OF INDIVIDUAL	PERIOD OF STARVATION	DATE OF ANALYSIS	NO. OF F PROD.	PER-CENTAGE OF F PROD.	NO. OF M PROD.	PER-CENTAGE OF M PROD.	REMARKS
	1912	1912	1912					
21	Jan. 15	Jan. 17	Jan. 19	6	39.0	14	70.0	
23	Jan. 18	Jan. 19	Jan. 21	1	7.7	12	92.3	
11	Feb. 23	Feb. 28	Mar. 1	1	11.0	8	89.0	4 old F and 7 young discarded
82	Apr. 28	Apr. 30	May 1	3	18.0	13	82.0	Old F discarded

#### GENERAL DISCUSSION OF LITERATURE

In thus reaching the conclusions that male production—hence sex-determination—in *Asplanchna* is a phenomenon all but wholly under nutritive control, it naturally becomes incumbent to consider briefly the discrepancy between this conclusion and the results which have been reached by others.

There is of course, first of all, the possibility that nutrition is a more important factor of sex control in *Asplanchna* than even in other genera of rotifers. But it seems more probable that its rôle is the same in all with the exception of the fact that the enormous nutritional irregularities natural to *Asplanchna* enable one to produce results which in degree of definiteness and contrast may well exceed those producible in *Hydatina*. We ascribe, thus, much of our success to the choice of a species favorable for experimental work.

At the same time we would lay much emphasis upon the necessity of refined feeding technique with whatever species work is done. Few if any rotifers feed exclusively upon one type of food, and it probably does not suffice, in experimenting with them, to employ a single food organism even though such nutrition be capable of maintaining growth and reproduction.

We would next point out that in one way or another, all previous workers—Nussbaum, Punnett, Whitney, Shull—have found that certain strains or races of *Hydatina* were more predisposed to male production than others. Nussbaum did not himself thus interpret his results; but it has been pointed out none the less that the cultures with which he worked certainly differed in this respect. It has been further shown by Whitney that these 'strains' which thus differ in their male producing capacity may be more or less altered in this fundamental respect by external conditions. No one, however, seems to have definitely associated the conditions which thus modify the male producing capacity of a strain directly, as a cause, with male production. Now, with *Asplanchna amphora* we have pointed out that these male and non-male producing strains not only exist but are characterized by marked morphological differences and we have further pointed out that these strains are also produced by nutritive change. Is it not probable that a like causal relation obtains between the less marked though different strains of *Hydatina senta*?

Considering more definitely the results of individual investigators, we turn first to those of Nussbaum. After negating the hypothesis of temperature as a sex-determinant, he arrived, through a long series of experiments, at very definitely formulated ideas that starvation was the positive factor inducing male production. These general conclusions are so important and definite that they may best be stated in his own words:

Bei *Hydatina senta* bestimmt während einer gewissen Entwicklungsphase die Ernährung das Geschlecht des ganzen Geleges eines jeden jungfräulichen Weibchen. Wird das auskriechende Weibchen bis zur Reifung seines ersten Eies gut ernährt, so legt es nur weibliche Eier, wird es bis zur Geschlechtsreife mangelhaft ernährt, so legt es nur männliche Eier. Vor und nach dieser Periode hat die Ernährung auf das Geschlecht keinen Einfluss.

It will be seen that these conclusions coincide with our own, though in part only. The general emphasis upon nutrition is the same. But Nussbaum places a much simpler construction upon the facts than they will bear and in place of his conception of simple starvation as the cause for male production we find ourselves obliged to substitute the supposition of sporadic or interrupted nutrition. Indeed our experiments show conclusively that of the two aspects of this latter factor, the first or high feeding is more important than the second or interruption by starvation. The experiments upon which Nussbaum based his conclusions consisted mainly of numerous mass cultures which were subjected to quantitative variations in food supply. Many cultures were carried simultaneously but definite controls were not made. Shull has pointed out that Nussbaum's results are sometimes contradictory, similar mass cultures producing or not producing males. We believe however, that a closer study of the conditions which Nussbaum has recorded for his experiments will sometimes show that they were not uniform, and that, in the light of our results, the contradictions disappear. It is unfortunately also true that Nussbaum has not recorded the conditions of some of his important experiments with sufficient fullness so that their real significance can now be determined.

In brief résumé, our analysis of the sixty-four experiments which were really addressed by him to sex-determination, is as follows—in some cases basing our judgment of the nutritive condition upon Nussbaum's statement of the number of individuals present and the number of eggs produced: Fourteen mass cultures, well fed, then starved, produced males. Six mass cultures, well fed, apparently not starved, produced males. Five cultures, continuously starved, produced no males. One culture, periodically starved, produced no males. Twelve large mass cultures, apparently well fed and without starvation, produced no males. Thirteen small mass cultures, apparently well fed and not starved, produced no males. Thirteen mass cultures, conditions and nutritive supply not given or inferable from Nussbaum's statements, produced males.



It is plain that Nussbaum's varying results are in general closely parallel to our own variable results with our first general mass cultures, obtained before a successful feeding technique and a successful analysis by means of isolation cultures had fully indicated the probable solution. In so far as the exact conditions of Nussbaum's experiments are given they harmonize fully with our own results, while Nussbaum's main conclusions show the strong impression made upon his mind by the copious male production which was, by him, at least frequently observed to follow starvation.

In the work of Punnett which follows upon that of Nussbaum, four pedigree series of *Hydatina* were carried through twenty-two to seventy-two generations. These series yielded varying numbers of male producers, from which fact, Punnett concludes that they belong to 'strains' possessing differing inherent capacity for male production. He admits that these different 'zygotic constitutions' may perhaps be modified by external conditions, though he himself did not determine the cause of such modification. In one instance he did record the production of a purely parthenogenetic female-producing ('thelytokous') strain from a slightly male-producing ('arrenotokous') strain. In one experiment the effort was made to produce male production by starvation of young females, after hatching, from a line which had produced no males. The result was wholly negative. It is plain that these results of Punnett offer no difficulties to our interpretation. His lines showing no male production and male production may well have been parallel to our lines of saccate and humped *Asplanchna* respectively. They may have been merely upon different physiological levels. That his starvation experiment failed was, in the light of our experiments inevitable, in that it was begun with individuals undoubtedly upon too low a level to be capable of male production.

Uniform feeding was used throughout Punnett's experiments with the exception of a single instance of a substitution of an all but fatal diet in the case of a strain already of low potential. The one instance where a strain dropped from low male produc-

tion to no male production is also interpretable as due to a lowering of vitality, resulting, possibly, from too uniform feeding.

In Whitney's first work<sup>6</sup> on *Hydatina*, we find marked advance in the definite proof that the 'strains' of Punnett may be derived from one and the same pure race and may freely give rise to each other. Definite external causes, however, were not assigned for the transitions. Starvation experiments were tried without apparently modifying male production. But it must again be pointed out that Whitney did not consider the nutritive condition either of the race or of the individual from which the starved animals were derived.

In later articles Whitney develops first the rather theoretical hypothesis of some chemical substance dissolved in the culture medium as the cause of male production, while in a later publication<sup>7</sup> he demonstrates the negative influence of certain culture media as inhibitory of male production, agreeing thus essentially with Shull. We return to this matter later. But for the present and from our stand point the conduct of Whitney's chief series as recorded in his second article<sup>8</sup> is of special interest. Two series were reared for one hundred generations fed upon a uniform diet of the flagellate, *Polytoma*, for which was then substituted the flagellate, *Chlamydomonas*. Throughout the entire one hundred generations fed upon the uniform diet no males appeared. But immediately—within a few generations after the food change—copious male production occurred. Still Whitney does not see in this striking sequence even the probability that change in nutrition induces male production. He evidently regards the influence of nutrition as a discarded hypothesis although in his latest work as well, several instances occur where results strongly suggest the influence of this factor. It is worth noting that in our cultures of *A. amphora*, preceding the work for the present paper, there occurred an instance practically duplicating Whitney's experiment. A pedigree series uniformly fed upon *Paramecia* for fifty-seven generations had shown not a single male producer. Then upon sudden substitution of *Euglena* for

<sup>6</sup> Jour. Exp. Zoöl., vol. 5, p. 1-26, 1907-1908.

<sup>7</sup> Jour. Exp. Zoöl., vol. 12, no. 3, pp. 227-62.

<sup>8</sup> Science, N. S., vol. 32, pp. 345-49.

food there followed in the fifty-ninth generation the transition to the humped type and also to male production. In our work at this time, however, the duplication of Whitney's experiment was followed by a series of experiments which completely eliminated the possibility, for *Asplanchna*, of the truth of Whitney's assumption, namely, that dissolved substances in the culture medium determine the sudden advent of male production. The fact of nutritive change remains the one residual causal factor.

The work of Shull upon the general topic of the life cycle and the sexual phase of *H. senta* is so extensive that a detailed review of it here is impossible. Certain of his essential conclusions however require mention and comment. In several of his earlier articles he develops at length the hypothesis that substances dissolved in culture medium influence male production; yet the influence ascribed to these substances is, curiously enough, the reverse of that which Whitney ascribes to his hypothetical matter in solution. He deems that various dissolved substances act as sex inhibitors, predisposing to exclusive parthenogenetic female production.

Shull<sup>9</sup> in his later work again finds much evidence of lines or races possessing somewhat definite capacities for male production.<sup>10</sup> He has also carried out interesting experiments in the matter of crossing these diverse lines, the results of such crosses leading sometimes to an increase and sometimes to a decrease in the amount of male production. But no farther facts as to the cause of male production were discovered. Shull noted a frequent if not general decrease in the amount of male production throughout his long continued series. He thinks this gradual approach toward pure parthenogenesis to be "independent of both genotypic constitution and the immediate external environment." In light of our experiment it is natural to raise the question whether this gradual cessation of male production may not have been the consequence of the unchanged and unmixed diet employed? Experiments with more varied feeding of *Hydatina* could hardly fail to be of interest.

<sup>9</sup> Jour. Exp. Zool., vol. 12, no. 2, 1912.

<sup>10</sup> He attributes these diverse races to fundamental differences in 'genotypic constitution.'

In one instance only we wish to refer more minutely to the results of Shull's experiments. In one of his earlier articles<sup>11</sup> he presents the results of the "Influence of food culture on the percentage of male producers." His immediate conclusion as to results is somewhat favorable to the positive action of starvation as increasing male production. But the increase is not great and is later ascribed to other causes. Without commenting upon this general conclusion, we wish to call attention to the interesting nature of the individual results in this starvation line. The first striking fact shown by his table is an apparent rhythmic variation. Almost without exception we find one or two very small families of, say, from three to twelve individuals followed by a succession of from four to six families with from two to three times this number. This variation in the size of family obviously demonstrates that, however food was supplied, effective starvation was not uniform. The individuals producing the larger families must have ingested more food than those producing the minimum number of offspring. In noticeable correlation with this change in size of family, we find the fact of increase and decrease in the number of male producers. The smaller families scarcely produce males at all, whereas the larger families frequently contain 50 per cent or more of male producers. It seems very natural to interpret these results as due to the fact that the degree of starvation which produced the smaller families precluded, as we always found it to do in our experiments, the possibility of male production; while the higher nutrition, productive of the larger family made such production possible. A more minute examination of Shull's table shows the results to be in even closer accord with our own. Thus it is not the first large family that usually shows the high rate of male production spoken of; indeed in some instances the first large family following upon one or more small ones shows not a single male producer. This coincides strikingly with the results of our experiments which show that it is not the first generation of high potential individuals which produce males. They rarely do so, but them-

<sup>11</sup> Jour. Exp. Zool., vol. 8, pp. 311-54.

selves become the mothers of many young, which, through the slightest check in nutrition, become male producers. We think that any one with this idea clearly in mind will find Shull's table in close accord with our hypothesis. Without wishing to depreciate the value of Shull's work we may here point out the sharpness of the difference between our viewpoint and his. He interprets the table of which we have spoken in terms of five-day periods and is plainly seeking for some factor which may exert a general influence upon male production of the whole series, regardless of rhythm or special known nutritive condition. Our results lead us to find the cause of male production in the rhythm of the series and in definite factors working upon single individuals of every generation.

In conclusion we wish to point out, further, that in the entire range of experiment upon *Hydatina senta* no investigator, with possible exception of Nussbaum, has developed a definite method by which the rate of male production can be increased at will or even definitely controlled at any point other than by its reduction to zero. Second, attention may be called to the general fact that all investigators either used uniform feeding or at least laid little stress upon qualitative food change. Third, we may well point out the probability of the correctness of the negative or inhibiting factors in regard to male production as demonstrated by Shull and Whitney. There may very probably be many such factors. If male production depends primarily upon a high state of effective nutrition it would seem natural that any inhibitory or deleterious substance should limit or prevent its occurrence.

In thus rehabilitating the all but discarded factor of nutrition as a sex-determinant, and as the effective sex-determinant, in *Asplanchna amphora*, we do not wish to be understood as passing adverse judgment upon the claims that other and diverse modes occur elsewhere in the animal kingdom. But as far as rotifers are concerned certain experiments conducted in our laboratories upon other species, together with sufficient correlated observations in nature, lead us to the probable though tentative conclusion that qualitative and quantitative changes in nutrition will be found the universal sex-controlling factors in this group.

## SUMMARY

1. Continued low nutrition of the rotifer, *Asplanchna amphora*, reduces male production to zero.

2. Well-fed individuals, of the humped type, tend to give birth to about 20 per cent of male producers.

3. Dissolved metabolic products or other substances are not significant factors in male production.

4. Starvation of low potential females (produced by poorly nourished mothers) results in non-male production.

5. Starvation of high potential young females (produced by well-nourished mothers) during the first five hours after birth, or the growth period, results in copious male production.

6. Starvation of high potential young females during the second five hours after birth increases little, if at all, the amount of male production.

7. Maximum male production is determined by three factors, physiological rhythm, high nutrition, and starvation during the growth period.

8. The first of these influences is one which extends through a number of generations; the second works upon the generation preceding the male producer; the third acts directly upon the male producing generation itself.

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# FURTHER STUDIES ON PHYSIOLOGICAL STATES AND RHEOTAXIS IN ISOPODA

W. C. ALLEE

*The University of Illinois*

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## INTRODUCTION

This is the second of a proposed series of experimental studies upon the relation between physiological states and rheotaxis in the isopod *Asellus communis* Say. In an earlier paper (Allee '12) I showed that the species *A. communis* is composed of pond and stream mores (Shelford '11 a). These mores are easily distinguished, since when tested in a circular current the normal stream isopods give a high percentage, while those from ponds give a low percentage of positive rheotactic responses. Experiments have shown that conditions that are known to depress the rate of metabolism decrease the percentage of positive responses given by stream isopods. The depressing

conditions used experimentally were: low oxygen, chloretone, potassium cyanide, low temperature, sudden extreme increase of temperature, increased carbon dioxide, and starvation. On the other hand, atmospheric or complete saturation of oxygen, caffeine, and an increase of temperature if not too extreme, caused the pond isopods to give a higher percentage of positive responses. These last conditions are known to increase the metabolic rate of the organism.

All this first work was done on groups of isopods selected at random from the general stock. Usually from five to eight were tested at the same time and the averaged results from ten successive trials were taken as representing the condition of the group under experimentation. The present series of experiments deals with the individual isopod rather than the group. In order to give time for more intensive study these experiments deal with individuals of the stream mores only. They were conducted to throw light on the following questions:

1. What is the relation between the efficiency of movement in the current and the sign of response in rheotactic reactions?
2. Is there any relation between the reaction to a uniform shock stimulus and the sign of the rheotactic response, or the efficiency of that response?
3. How great a variation is there in the rheotactic reaction of individual isopods kept in uniform conditions?
4. What is the effect of the depressing agents, potassium cyanide and low oxygen, upon the rheotactic and shock reactions of the individual isopods?
5. What is the effect of the molting period upon rheotaxis?

These experiments are being prosecuted from the ecological point of view. The term 'ecology,' as I understand it, means that portion of general physiology which has for its problem the relation between the organism and its complete environment (cf. Semper '81, pp. 25-35; Shelford '12 c, pp. 333-34) I fully agree with Shelford ('12 c, p. 365) that the ecologist must specialize upon some aspect of the subject, because it is unusually large and its concepts unusually complex. Since the data as collected in field observations are so complex and since there

are so few who are prepared to make field observations and to fill in logically the inevitable gaps in such data, it seems the more important that ecological views be subjected to the most rigorous experimental tests before they are accepted.

Fortunately there is a large mass of data that has been collected in the fields of general physiology and general psychology, called 'animal behavior,' upon which the ecologist may draw. But unfortunately for the ecologist, most of this experimental work has been done either from the standpoint of the physiologist, where a minimum of emphasis is placed upon the natural environment and a maximum upon the method of the reaction (Loeb '05; Jennings '06 and Mast '11); or from the standpoint of the comparative psychologist (Holmes '11). It is true that even in much of this work the idea of response to environment is present, sometimes in a marked degree, as with Dawson ('11) and more rarely it is the dominant thought (Shelford and Allee '13).

#### STOCK

The isopods were all *Asellus communis* of a stock collected in County Line Creek (Shelford '11 a, maps) near Glencoe, Illinois. They belong to the same general stock which furnished material for almost all the earlier experiments on stream isopods. After being kept in an aquarium supplied with Chicago city tap water, at the University of Chicago for a year the isopods were transferred to the University of Illinois and placed in aerated<sup>1</sup> university tap water. The chemical analyses of the two waters are shown in table 1.

The change in water did not greatly affect the rheotactic reaction of the isopods. One lot received October 24, 1912, which had been two days in transit in small containers, gave an average of 62 per cent positive for 150 trials when first received. Four days later the positive response had increased to

<sup>1</sup> The aeration was accomplished either by allowing the water to flow slowly through a series of pans (Marsh '10) or by allowing air from the university compressed air system to bubble through small openings in a rubber tube laid around the bottom of the aquarium.

TABLE 1

*A comparison of chemical analyses of Chicago and University of Illinois tap water. Analyses of solids in parts per million and gases in cubic centimeter per liter*

	CHICAGO TAP <sup>1</sup>	U. OF I. TAP <sup>2</sup>
Potassium, K.....	6.0 <sup>3</sup>	2.6
Sodium, Na.....	42.1 <sup>3</sup>	29.0
Ammonium, NH <sub>4</sub> .....	0.04	2.3
Magnesium, Mg.....	11.3	34.9
Calcium, Ca.....	34.6	70.1
Iron, Fe.....	0.15	1.0
Aluminum, Al.....	0.00	1.3
Silica, Si.....	3.3 <sup>3</sup>	18.9
Nitrate, NO <sub>3</sub> .....	1.7	0.7
Chlorine, Cl.....	12.0	3.5
Lead, Pb.....	0.01	0.00
Sulphuric acid, SO <sub>3</sub> .....	0.04	2.3
Oxygen, O.....	10.46	0.12 <sup>4</sup>
Free carbon dioxide.....	2.5	18.0
Half bound carbon dioxide.....	32.5	101.12

<sup>1</sup> Shelford and Allee '13, table 4, p. 217.

<sup>2</sup> Bartow '06, p. 34.

<sup>3</sup> Bartow.

<sup>4</sup> After allowing air to bubble through the water, the dissolved oxygen was raised to air saturation (5.5 to 7 cc. per liter). The free carbon dioxide was removed and the half bound carbon dioxide was reduced to 88.58 cc. per liter.

71 per cent of 150 trials, and seven days later, 87 per cent of 150 trials were positive. There was little mortality—practically none after those injured in shipping had died. This indicates that the change in the chemical content of the water either did not affect the rheotactic response or that the isopods quickly became acclimated.

The animals under experimentation were placed in glass dishes which held about 1.5 liters of water. These were placed in running tap water to insure more even temperature. The stock was kept in a glass-sided room and all tests were performed in a part of this room curtained to exclude direct light. The majority of the tests were made from 8 to 12 A.M. Records were taken of the daily reactions of twenty individuals covering a period of from three to seventy-five days. This period did not include the breeding season.

## RESPONSE TO CONCUSSION COMPARED WITH THE SIGN AND EFFICIENCY OF THE RHEOTACTIC REACTION

## METHODS

In testing for the sign of the rheotactic reaction in a circular current the methods described in the preceding paper ('12, p. 276) were largely used. Forceps wrapped with soft cloth were substituted for the rounded glass rod in producing the current since they are less apt to cause injuries. The isopods were stirred loose from the bottom in order to diminish the interference of positive thigmotaxis with the reaction to the water current. Not more than three animals were tested at one time and records were kept of their individual reactions.

*Method for determining efficiency in a circular current*

During the earlier work the sign of the response was not distinguished from the vigor of the reaction in determining the positiveness of the animals under experimentation; but it is evident that an isopod might head upstream at each trial and yet be unable to make headway against the current. Its physiological condition then would be obviously different from that of an isopod that could make rapid progress against the current although in percentage of positive responses their reactions might be identical.

In order to test the relation between the sign and the efficiency of movement, the following arbitrary standard of efficiency in the current was adopted. This represents numerically the distance covered by an isopod in a minute's reaction period:

- 0 no reaction
- 1 slight movement
- 2 any response between 1 and 3
- 3 progress one-third around the pan, positive (approximately 27 cm.) or two-thirds, negative (54 cm.)
- 4 progress two-thirds around the pan, positive or one and one-third negative
- 5 progress once around the pan, positive or twice around, negative
- 6 any distance over 5

In indefinite reactions the efficiency was estimated as closely as possible from the varying course taken by the isopod.

The basis upon which the relation between positive and negative reactions was worked out is shown in table 2. In this work one isopod was used at a time. One person held the watch and recorded observations while another with the aid of a reading glass counted the number of movements of the fourth thoracic leg during a minute's reaction period and afterward measured the distance covered in that time. Distance covered per leg movement with the isopod going with the current was found to be approximately double that given when the isopod was reacting positively.

In testing for efficiency of response in a water current the rate of the current becomes of much greater importance than it is in finding the sign of the rheotactic reaction. Therefore even

TABLE 2

*Showing the relation between the efficiency of movement with and against the current. Temperature 19 to 20°C. Oxygen 5.5 to 6.5 cc. per liter*

POSITIVE				NEGATIVE				
No. trials	No. leg movements	No. centimeters covered	Ave. no. centimeters covered per leg movement	ISOPOD NUMBER	No. trials	No. leg movements	No. centimeters covered	Ave. no centimeters covered per leg movement
6	357	269	0.75	VII	10	367	455	1.24
8	607	255.5	0.42		8	560	593	1.06
3	146	69	0.47		6	547	429	0.78
7	462	269	0.58		10	749	657	0.88
			0.53	{ Ave. cm. per leg movement }				0.95
9	642	175	0.27	I				
10	907	254	0.27	VI				
10	868	219	0.24	II	1	35	11	0.31
6	348	116	0.33	XI				
10	775	397	0.51	VIII				
7	507	232	0.45	XV	10	879	577	0.65
					7	548	338	0.61
76	5676	2023.5	0.35	{ Totals and averages }	52	3685	3060	0.81

greater care was exercised to produce a uniform rate of current in the successive trials than during the preceding experiments.

*Reaction to concussion*

In testing rheotaxis in a circular current there is no means of measuring the stimulus acting at any given time; consequently one is not sure that the isopod is reacting to equivalent stimuli in successive tests. This is more especially the case since the circular current has a spiral rather than a direct course. It

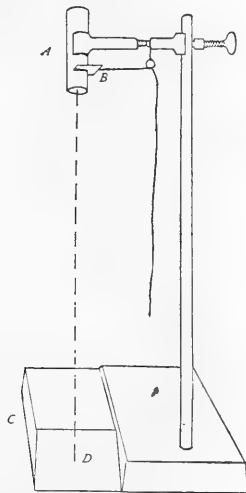


Fig. 1 Concussion apparatus.

seemed possible that an adaptation of the concussion method employed by Banta ('10, p. 453) might therefore furnish a more exact method for determining the differing physiological states.

Banta dropped a steel or lead ball to a pine board upon which the isopod to be tested was placed in a wax bottomed Stender dish. He does not speak of precautions to prevent the ball from rebounding, and presumably none were taken. Of course the vigor and number of the rebounds would vary partially as the distance through which the ball was dropped; but when this rebound is present the animals may and probably do react to the succession of smaller shocks after being aroused by the

initial shock. This might happen although no reaction was given to the initial stimulus.

In order to eliminate these rebounds, the apparatus was devised as shown in figure 1. A short cylinder *A* was fastened directly above the slanted portion of the steel block *C*. The steel ball to be dropped rested upon a cardboard *B* which worked in a slot in the cylinder. This was arranged 50 cm. above the point of concussion *D* on the block *C*. The block *C* is made of forged steel 10 cm. wide, 2.5 cm. high, and 20 cm. in greatest length. The upper surface is 15 cm. long. The sloping part measured 5.6 x 10 cm. and was machined to give a true surface. The slope is such that the falling ball rebounds at an angle and thus strikes the block but once. A steel ball weighing 11.927 grams was found to furnish the most satisfactory stimulus when dropped 50 cm. At the moment of striking the block, this had a momentum of 3734 C. G. S. units.

The isopod to be tested was confined in a petri dish 3 cm. in diameter, placed on the level surface 5 cm. from the point of impact. The isopod was allowed to stand five minutes to recover from the shock of handling. Then the responses were taken to twenty successive concussions. A reading glass was used in order to make out slight movements. After each individual trial the isopod was allowed to come to rest if a response had occurred. In all cases the reactions were taken for the first twenty trials and the lack of a reaction was considered to be quite as significant as a definite movement. Whenever the animal showed a tendency to crawl without the action of an external stimulus, it was allowed to come to rest before further trials were made. With practice the response to shock could be readily distinguished from movement due to other causes.

Numbers with the following values (cf. Banta '10, p. 453) were used to record the strength of the reactions:

- 0 no reaction
- 1 movement of antennae or slight movement of other appendages
- 2 extended movement of the antennae or strong enough movement of the legs to result in a slight stirring of the body



- 3 decided movement of the anterior end of the body or crawling one or two steps
- 4 more extended crawling covering about a centimeter
- 5 crawling over a centimeter
- 6 a definite jump followed by rapid running

The use of these numbers in recording the reactions is illustrated in table 3.

Banta records a mixed type of response. In number four of his classes of reaction he states that the isopod moved antennae or other appendages or bent the anterior end of the body and followed this by crawling. Reactions of this kind were not given under the conditions of my experiment and may have been due to the repeated rebounds acting as a cumulative stimulus. Concussion occurring while the animal was crawling usually caused a cessation of action. Often when the isopod was quiet the only reaction given was a settling of the body.

Table 3 shows some tests for one isopod made on successive days. The test recorded in column 7 was made immediately after that in column 6 but with a ball weighing only two grams and hence it developed a momentum of only 626 C. G. S. units. This appears to be near the threshold of stimulation when the isopod is as free as possible from thigmotactic interference.

The tests recorded in columns 5 and 9 immediately followed those given in columns 4 and 8 respectively. The difference was that in the first series of trials the isopods were resting more or less closely in the angle at the edge of the dish, while in the second they had been stimulated until they came to rest free from the edge. In column 5 there is some evidence that part of the increased response was due to this recent mechanical stimulation. By comparing these reactions it becomes apparent that the position of the isopod with reference to the angles of the container may affect their response to a shock stimulus. The position of the isopod in the containers used cannot be readily controlled by the experimenter without increased mechanical stimulation. Suitable dishes with hemispherical inner surfaces were not obtainable when these experiments were performed. Since the error from this cause is the same for normal

TABLE 3

Reactions of isopod no. II to shock stimulus of 3734 C. G. S. units<sup>1</sup> on successive days

	1	2	3	4	5	6	7 <sup>2</sup>	8	9
Oxygen in cubic centimeters per liter.....	7.0	7.0	6.5	6.5	6.3	6.3	7.0	7.0	7.0
Temperature.....	15	16	17	20	20	16	16	16	16
Position in Petri dish.....	free	free	free	loosely in angle	free	free	free	closely in angle	free
Reactions.....	3	4	2	1	2	2	0	1	1
	0	1	2	1	1	1	0	0	4
	1	1	2	1	1	0	0	1	4
	0	2	2	1	0	1	0	1	1
	1	0	6	1	3	2	0	1	5
	0	1	2	1	3	3	0	1	0
	0	1	1	1	1	1	1	1	3
	0	1	2	1	5	1	0	1	0
	0	2	2	1	1	4	0	1	1
	0	1	2	1	2	1	0	1	1
	4	3	1	1	0	2	0	1	1
	0	1	2	1	1	2	0	1	0
	1	3	2	1	3	3	0	1	2
	1	2	1	1	2	1	0	0	1
	1	2	1	1	2	2	0	1	0
	2	1	1	1	1	1	0	1	2
	3	1	1	1	1	1	5	0	1
4	1	1	2	1	1	0	1	4	
1	0	1	1	1	1	0	0	2	
1	0	0	0	0	1	1	0	4	
Sum.....	23	28	33	20	32	35	1	17	37
Number responses other than zero..	12	17	19	19	18	19	1	17	17
Average vigor									
$A = \frac{\text{sum}}{\text{no. of trials}}$	1.15	1.4	1.65	1.0	1.6	1.75	0.5	0.85	1.85
$B = \frac{\text{sum}}{\text{no. movements}}$	1.9	1.6	1.73	1.05	1.77	1.84	1.0	1.0	2.18
Per cent positive rheotaxis.....	90	80	90	100		80		90	
Efficiency.....	3.4	3.4	3.5	3.3		2.8		2.6	

<sup>1</sup> Except column 7.

<sup>2</sup> 2 gram ball falling 50 cm. gives a momentum of 626 C. G. S. units.

and depressed animals and since all were tested under uniform conditions, this source of error is probably not vital as far as the larger variations in physiological condition are concerned.

Two average vigors of response are given in table 3. The first (A) is based on the principle that an animal may respond to a given stimulus by remaining quiescent, when its reaction must be expressed numerically as zero; that this reaction is as significant as when the most violent movement occurs and should be included in a scheme of averages. The second (B) follows the reasoning that only movements should be counted as reactions. This system was followed by Banta in his work with concussion. The fallacy of the reasoning may be seen by comparing the reactions listed in columns 4, 7 and 8. According to the second system of averaging all these would be considered as equivalent while a glance at the responses in detail shows that this is not the case. Consequently the first method has been taken in this work to summarize the reactions into one significant figure.

#### INTERRELATIONS BETWEEN RESPONSE TO UNIFORM CONCUSSIONS AND THE SIGN AND EFFICIENCY OF RHEOTAXIS

As the percentage of positive rheotactic responses varied from day to day, did the efficiency of movement and the reaction to shock vary in like or opposite manner? If all are the direct expression of the physiological state of the animal the variations should be similar and a decrease in positiveness should be accompanied by a decrease in efficiency and in the vigor of the concussion reaction.

The relation or non-relation between these factors is shown in charts 1-6 (pp. 275-282). These show that in the main the larger variations in efficiency and positiveness are in agreement although this differs somewhat with individuals, notably with isopod no. XIV, chart 6. The larger concussion variations show little agreement with the other factors although there are some cases of notable disagreement which will be discussed later (p. 273).

The minor daily variations of the number of positive reactions and the efficiency of the rheotactic response run more closely parallel with each other than either does with the variations in the reactions to shock. The shock variation and the variation in positiveness oppose each other almost as often as they agree. The reaction to shock and efficiency are a trifle more uniform in their variation but not enough so to warrant any claim of agreement. It is probably significant that in all cases the agreement is more pronounced when the vigor of reactions decreases than under other conditions.

There is another way of determining the relation between these different factors: one may compare the concussion responses and efficiency accompanying a state of high positiveness with those found with a low percentage of positive responses. The results of such a comparison are shown in table 4.

In this table the efficiency and shock reactions which were given by animals showing a positive response of 80 per cent or over are compared with those accompanying 40 per cent or less of positive rheotactic reactions. The table shows that in every case the high percentage of positiveness is accompanied by a high degree of efficiency in the current. In the main a low efficiency accompanied a low positive reaction; but this was not always true, notably in isopods nos. VII, XII and XIX. These isopods gave more negative responses to the current than positive and showed as high efficiency when negative as when they were positive. However, the average shows almost as marked a difference in the efficiency as in the positiveness.

Again, there is no relationship between the reaction to shock and the positiveness of the isopods. It is evident from the table that while low vigor of reaction to shock may accompany a similar status of the rheotactic factors such is not necessarily true. The case of isopod no. I is significant. This animal gave much more vigorous shock reactions when giving a low positive and low vigor response than under the reverse conditions (see also p. 272). The average of all the responses is practically the same regardless of whether given with high or low rheotactic positiveness.

TABLE 4

Showing the efficiency and response to shock accompanying high and low positive-ness. The responses are averages of all the reactions given by the individual under the conditions of the table. The general averages are obtained by multiplying the number of instances by the average response and dividing by the total number of instances.

RHEOTAXIS 80 PER CENT, OR ABOVE POSITIVE						ISOPOD NUMBER	RHEOTAXIS 40 PER CENT, OR BELOW POSITIVE					
No. tests of rheotaxis	Ave. positiveness	No. tests of efficiency	Ave. efficiency	No. tests of concussion	Ave. response to concussion		No. tests of rheotaxis	Ave. positiveness	No. tests of efficiency	Ave. efficiency	No. concussion tests	Ave. concussion reaction
38	91	36	2.8	24	0.86	I	12	25	12	1.7	12	1.40
33	93	33	2.9	22	1.60	II	12	12	12	1.4	12	1.36
19	94	17	2.5	4	1.40	III	5	0	5	0.0	5	1.04
36	94	35	2.8	20	1.12	IV	2	35	2	3.2		
2	90	1	2.1			V	12	26	12	1.8	2	0.45
35	93	35	2.7	4	1.38	VI	5	8	5	1.7	4	0.50
11	90	11	2.7	8	1.40	VII	26	19	26	2.9	11	1.30
29	96	29	2.8	11	1.40	VIII	1	40	1	2.7		
21	91	21	2.9	8	0.96	IX	6	17	6	3.6		
6	81	6	2.8			X	1	0	1	0.0		
32	90	32	2.9	14	1.13	XI	9	23	9	1.5	5	0.79
8	84	8	2.5	5	0.56	XII	16	24	16	2.5	3	1.13
10	86	10	2.7	1	0.40	XIII	5	28	5	1.7	2	0.63
32	93	32	2.6	21	0.99	XIV						
10	82	10	2.4	5	0.66	XV	12	22	12	2.2	8	0.74
9	91	9	2.7	8	0.70	XVI	3	0	3	1.5	3	0.20
						XVII	3	37	3	2.6	2	1.45
7	84	7	2.6	5	1.31	XVIII	3	27	3	2.2	1	1.10
1	90	1	2.7	1	1.50	XIX	10	20	10	2.9	8	1.62
4	87	4	2.5	3	1.12	XX	3	14	3	2.3	3	0.72
363	91	357	2.7	165	1.03	{ Total and averages }	134	21	134	2.2	81	1.04

Table 5 shows a similar state of affairs when the responses accompanying high and low efficiency are compared. An efficiency of 2.8 or more is regarded as high and one of 1.8 or less as low. As before isopods nos. VII, XII, XV and XIX, which showed a large number of negative responses, indicate that high efficiency and a high degree of positiveness do not necessarily go together. On the other hand, the individual items and averages indicate that in the main they do. In stream isopods, how-

ever, low efficiency is always accompanied by low positiveness. Again the averages show almost as striking differences as when the positiveness of the response was taken as a basis of comparison in table 4.

The accompanying reactions to concussion indicate that a high vigor of shock response may occur with either a high or low rate of efficiency and vice versa. Again the averages of concussion responses are practically identical and show no apparent relation to efficiency.

TABLE 5

*Showing the rheotactic and shock reactions accompanying high and low efficiency. The data were obtained as in table 7*

EFFICIENCY 2.8 OR ABOVE						ISOPOD NUMBER	EFFICIENCY 1.8 OR LESS					
No. tests of rheotaxis and efficiency	Ave. per cent positiveness	Ave. per cent negativeness	Ave. efficiency	No. tests of concussion	Ave. concussion response		No. tests of rheotaxis and efficiency	Ave. per cent positiveness	Ave. per cent negativeness	Ave. efficiency	No. tests of concussion	Ave. concussion response
18	89	2	3.1	11	0.90	I	9	28	8	1.5	9	1.43
18	89	6	3.1	12	1.64	II	10	18	3	1.6	10	1.16
5	92	4	2.9	1	1.20	III	6	8	2	0.3	5	1.04
33	80	13	3.1	15	1.15	IV						
						V	9	21	11	1.5	2	1.00
19	91	7	3.2	7	1.50	VI	3	23	0	0.9	3	0.95
25	36	56	3.2	7	1.22	VII	2	5	5	1.0	2	1.00
14	96	1	3.0	4	1.54	VIII						
21	81	8	3.1	6	1.08	IX						
4	83	13	3.0			X						
18	86	8	3.1	10	1.39	XI	3	7	0	0.7	3	0.00
10	53	43	2.9	5	0.84	XII						
6	80	0	3.1			XIII	3	23	3	0.9		
14	89	5	3.1	7	1.00	XIV						
12	51	41	3.0	6	1.20	XV						
4	90	3	3.0	3	0.57	XVI	2	0	5	1.3	2	0.25
2	40	50	2.8	1	1.45	XVII <sup>1</sup>						
3	74	26	2.8	2	0.70	XVIII						
15	39	48	3.0	13	1.40	XIX						
3	60	37	3.1	2	1.07	XX	1	40	40	1.5	1	0.95
234	74	15	3.1	112	1.22	{Totals and averages}	46	17	6	1.2	30	1.26

<sup>1</sup> Tested only during molting period.

Table 6 shows the relation between the negative and indefinite rheotactic responses with the rate of efficiency and with shock reactions. Part I gives the data taken when the negative responses were 60 per cent or more of the total reaction. Under these conditions the percentage of positive responses is low but both the efficiency rate and the concussion responses are high. The second part of the table shows the responses when over 60 per cent of the total reactions were indefinite. Here again the positive reactions are necessarily few. But it will be noted that the rate of efficiency is low, much lower than when low positiveness was taken as the basis of comparison. The concussion response is almost the same as was shown to accompany high and low positiveness and high and low efficiency.

TABLE 6

*Showing the responses accompanying high negative and high indefinite rheotactic response*

RHEOTAXIS 60 PER CENT OR ABOVE NEGATIVE						ISOPOD NUMBER	RHEOTAXIS 60 PER CENT OR ABOVE INDEFINITE						
No. tests of rheotaxis and efficiency	Ave. per cent negativity	Ave. per cent positiveness	Ave. efficiency	No. tests of concussion	Ave. concussion response		No. tests of rheotaxis and efficiency	Ave. per cent indefiniteness	Ave. per cent positiveness	Ave. efficiency	No. tests of concussion	Ave. concussion response	Cause of indefinite reaction
1	80	10				I	8	77	14	1.85	8	1.57	KCN
2	60	25	3.0	2	1.20	II	11	87	11	1.20	11	0.85	KCN
						IV							
						V	8	75	14	1.60	2	1.00	inj.
						VI	3	97	0	1.00	3	0.50	KCN
18	77	18	2.9	5	1.36	VII	6	78	15	1.90			low O <sub>2</sub>
1	60	40	2.7			VIII							
1	70	20	2.2	1	1.45	XI	1	60	30	2.80			molt
11	71	17	2.6	2	0.90	XII	3	60	33	2.10	1	0.30	unknown
4	80	18	3.0	2	0.65	XV							
						XVI	3	97	0	1.50	2	0.20	low O <sub>2</sub>
1	60	30	2.1	1	1.45	XVII							
1	70	30	2.3			XVIII	1	60	30	2.40	1	1.10	unknown
6	78	20	3.0	6	1.66	XIX							
2	95	5	2.6	2	0.60	XX							
50	70	17	2.6	22	1.3	{Totals and averages}	44	74	13	1.6	28	0.97	

The responses accompanying low negativeness and low indefiniteness may be seen in the first parts of table 4. Of necessity where either response is above 60 per cent of the total number of reactions the other two must be low. From table 6 it is evident that a high percentage of negative reactions may accompany a high rate of efficiency. But this condition was found only fifty times in the course of these experiments while the opposite response of high positiveness and high efficiency occurred 357 times as shown in table 4. For these 357 times the positive responses averaged 91 per cent, and the negative ones 4 per cent of the total response. From this it follows that under natural conditions the negative response does not vary with the efficiency or vigor of movement as does the positive response. The relationship may be stated as follows:

The rate of positiveness is usually high when the efficiency is high and is always low when the efficiency is low. Normally the negative response is low in the stream mores. It is always low when the efficiency is very low and is usually low when the efficiency is high. However it is never high unless the efficiency is fairly high and in this alone it agrees with the positive reaction.

On the other hand the indefinite response is rarely given with high efficiency and is usually given when the rate of efficiency is low. It is not high in stream isopods under normal conditions but always occurs when the metabolic rate of the isopod has been experimentally decreased.

From the data presented so far the response to shock has apparently no direct relation to any of the other factors. When the positiveness and efficiency of the rheotactic reactions that accompany concussion responses with a vigor of 1.2 or more are compared with those accompanying 0.8 or less, the following average results were obtained: 160 trials with the shock reaction 1.2 or more averaged 1.52. The average positive response was 71 per cent and the average efficiency, 2.6. Ninety-four trials with the shock response 0.8 or less averaged 0.46. The accompanying rheotactic reactions averaged 59 per cent with an average efficiency of 2.1. Eight of the twenty isopods gave as strong or



stronger positive rheotactic reaction with a low as with a high vigor of shock reaction. This gives some indication of relationship between the shock and rheotactic reactions but the evidence is too slight to warrant the conclusion that such a relationship exists, particularly in the face of the conflicting evidence given in other instances when this possible relationship has been considered.<sup>2</sup>

#### DAILY VARIATIONS IN THE RHEOTACTIC REACTION

In my earlier work on rheotaxis with groups of isopods ('12, p. 340) irregularities were observed in the reactions of different members of a group kept under constant experimental conditions. It was also found (p. 277) that the reaction of a group of eight highly positive isopods varied from day to day. From these observations the question arose as to the difference in the rheotactic response of the individual isopods and the degree of daily variation of this response when kept under similar experimental conditions. In other words, to what extent is the internal mechanism of different isopods from the same habitat unlike and how great a variation of this internal mechanism takes place automatically from day to day?

In testing for these points isopods selected at random were isolated in glass jars in a liter of water which was changed daily. University of Illinois tap water saturated with oxygen at

<sup>2</sup>Experiments on a different phase of this subject performed since the above was written, incidentally confirm these results. 150 isopods were tested for from one to twenty-five days with the following results:

NO. TRIALS	RHEOTACTIC RESPONSE				EFFICIENCY	NO. CONCUSSION TRIALS	AVE. VIGOR OF CONCUSSION RESPONSE
	+	-	$\alpha$	0.			
306	96	2	2		2.85	149	1.23
140	16	49	23	12	2.06	76	1.14
69	11	81	8		2.65	37	0.95
22	12	5	75	8	1.6	14	1.03

These later concussion tests were made with the isopods in a hemispherical container and show but little more correlation with the rheotactic factors than did the response in petri dishes.

atmospheric pressure was used throughout. The vessels were placed in running water to keep the temperature more uniform. During the period of experimentation which lasted seventy-five days the temperature ranged from 15 to 22°. These variations in temperature usually took place slowly and according to previous experiments ('12, pp. 316, 326) probably did not affect the rheotactic response and certainly cannot be held to account for all the variations.

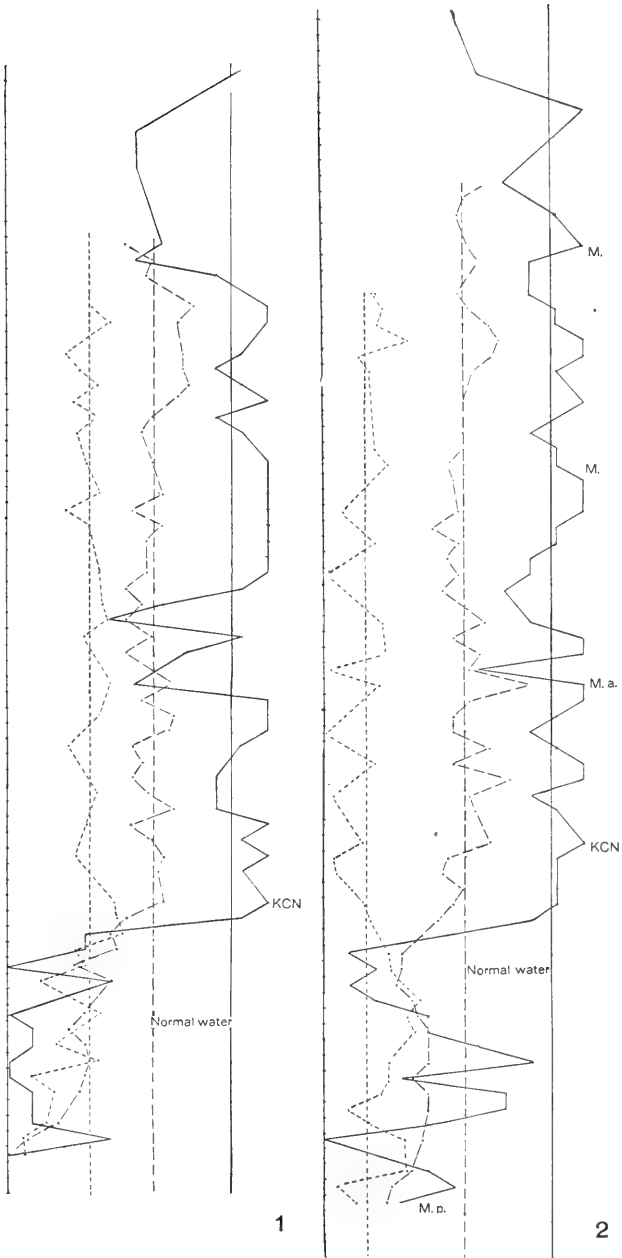
The oxygen varied from 5.5 to 7.0 cc. per liter but this does not affect the rheotactic reaction (Allee '12, p. 281). The amount of carbon dioxide present was practically constant. Food was furnished by placing a few green leaves of *Elodea* with each isopod; these were renewed often. The records of these tests are shown in charts 1 to 6 pp. 275-282.

Molting was found to be responsible for part of the variation, but there was some variation other than that caused by the molting period in the response of the most uniformly positive isopods. Thus isopod no. I, chart 1, gave a normal positive response of 88 per cent of the total number of trials and yet repeated the response of the preceding day only 14 times in 60 trials. Isopod no. II, chart 2, with an average positive response of 86 per cent repeated the preceding reaction 16 times in 53 trials. Isopod no. VIII, chart 4, with an average of 90 per cent positive reac-

Chart 1 The rheotactic and concussion responses of isopod no. I.

Chart 2 The rheotactic and concussion responses of isopod no. II.

In the graphs the abscissae represent time; the ordinates give the response. In all instances unless otherwise mentioned the intervals marked on the base line indicate days. The solid line gives the positive rheotactic responses of the isopods expressed in per cent of the total number of trials. The slightly broken line shows the efficiency of the rheotactic movement, while the dotted line gives the concussion reactions. In each case the straight line shows the average response given by the individual under consideration under normal conditions. In the plotting the scale for the percentage of positive responses is one-twentieth of that for either of the other responses. The letters on the charts have the following meanings: *M.a.*, molted anterior part; *M.p.*, molted posterior part; *M.*, molted whole covering; *KCN*, put in potassium cyanide; *Normal water*, changed from potassium cyanide to the water normally used; *Low oxygen*, put in water having an oxygen content of 0.5 to 1.0 cc. per liter; *High oxygen*, returned to the water normally used ( $O_2$  5.5 to 7.0 cc. per liter).



tions gave identical responses 11 times in 33 trials. These are typical of the responses of the highly positive isopods.

Some of the isopods show greater variation, typically represented by no. VII. This isopod had as strong tendency to give negative as positive reactions (table 7). The positive response varied greatly from day to day often without a corresponding variation in the efficiency of the reaction, although this latter item varied more than in the more uniformly positive isopods cited above.

Attention should be called to the facts illustrated in table 7. This table shows that those stream isopods which naturally give a low percentage of positive responses give a higher percentage of negative than of indefinite reactions. That is, if a stream isopod does not go positive to the current under normal conditions it has a distinct tendency to go more negative than indefinite. Exactly the reverse is true of pond isopods.<sup>3</sup>

Another state of affairs is illustrated by the reactions of isopod no. V, table 7. This individual was injured in handling on the sixth day of observation. Trials were continued until its death 28 days later. In this case, however, although the positive response shows the decided cut that would be expected, the indefinite, not the negative response increased. Under these conditions the efficiency was also markedly decreased, bearing out the experimental results of a high degree of indefiniteness accompanying low efficiency in stream isopods, however the low efficiency be induced.

The cases of the isopods (nos. VII, XII, XV and XIX) that gave an unusually high negative response for the stream mores have been discussed at some length (p. 272) and will be summed up later (p. 280). In general the table shows that there is a relation between the positiveness of the rheotactic reaction and the rate of efficiency and there is slight evidence of a relationship between these two rheotactic factors and the concussion response.

<sup>3</sup> Of 827 trials of normal pond isopods ('12, table 6, p. 290) 29 per cent of the reactions were positive, 30 per cent were negative and 41 per cent were indefinite. This shows the high percentage of indefiniteness in the typical rheotactic reaction of pond isopods.

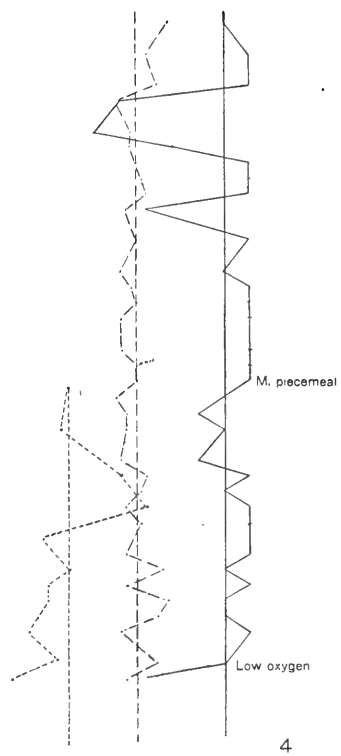
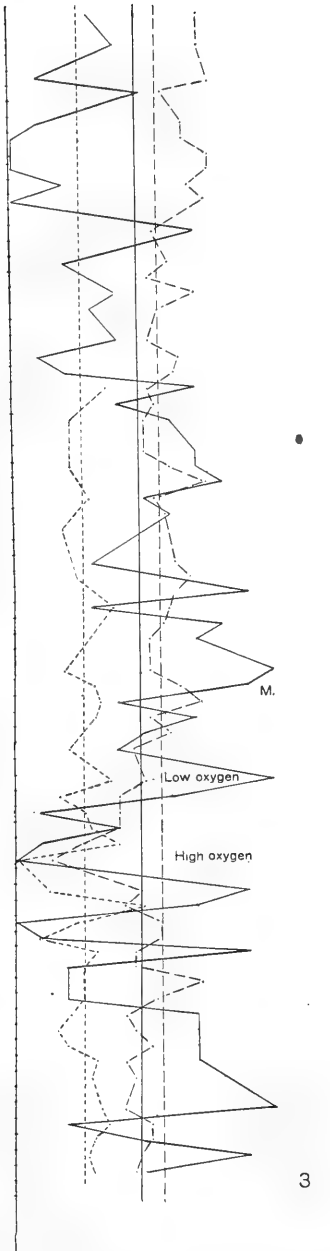


Chart 3 The rheotactic and concussion responses of isopod no. VII. For details of charting, see chart 1, p. 274.

Chart 4 The rheotactic and concussion responses of isopod no. VIII. For methods of plotting, see chart 1, p. 274.

TABLE 7

Showing the average reactions of the individual isopods under identical conditions. The data were compiled from the successive reactions of isopods before they were subjected to changes in the environment. Thus isopod no. I was tested 39 times under such conditions making a total of 390 individual trials. Of these 88 per cent were positive, 3 per cent negative and 9 per cent indefinite. The efficiency was recorded 36 times and averaged 2.83. The average vigor of 19 concussion trials (380 individual tests) was 0.86. The final averages were obtained by multiplying the significant figures by the number of trials of which they are averages and dividing by the total number of trials

ISOPOD NUMBER	NO. TRIALS RHEOTAXIS	AVE. PER CENT POSITIVE	AVE. PER CENT NEGATIVE	AVE. PER CENT INDEFINITE	NO. TRIALS EFFICIENCY	AVE. EFFI- CIENCY	NO. CONCUS- SION TRIALS	AVE. CONCUS- SION RE- SPONSE
I	39	88	3	9	36	2.83	19	0.86
II	32	86	10	4	28	2.83	23	1.61
III	23	87	3	10	20	2.45	6	1.39
IV	52	82	11	7	49	2.86	18	1.26
V	21	44	14	42	19	1.82	5	0.80
VI	35	87	6	7	35	2.86	7	1.50
VII	56	49	41	10	56	2.85	35	1.35
VIII	34	90	6	4	34	2.80	10	1.50
IX	39	72	12	16	39	3.00	14	1.03
X	7	83	7	10				
XI	32	76	17	7	32	2.80	10	1.39
XII	33	48	41	11	33	2.50	10	0.83
XIII	26	67	7	26	26	2.50	6	0.12
XIV	38	88	6	6	38	2.60	26	0.97
XV	42	56	32	12	42	2.57	32	0.68
XVI	6	92	3	5	6	2.80	5	0.96
XVIII	16	62	27	11	16	2.46	9	1.16
XIX	23	44	44	12	23	2.86	20	1.30
XX	9	59	35	6	9	2.40	7	1.00
Totals and averages. . .	563	71	18	11	548	2.74	362	1.07

## THE QUESTION OF A DAILY RHYTHM

One other problem in the variation in response remains to be mentioned. That is the question of a daily rhythm in the rheotactic reaction such as Bohn found to exist in *Convoluta* (Bohn '03, '03 a). A large number of tests have been made throughout the entire period of experimentation covering the time from

7 A.M. to midnight without any evidence of such a variation. Thus the tests upon which this paper is based were made largely from 8 A.M. to 12 noon. Those recorded in the preceding publication ('12) were made largely in the afternoon and evening up to midnight, although some tests were made in the early morning. This leaves the interval from midnight till 7 A.M. untested.

In order to find if there was evidence of a daily rhythm three uniformly positive isopods (nos. I, II and VI) were tested every hour for twenty-four hours. The results are shown in chart 7, p. 283. The reaction of isopod no. I is not given since it does not differ essentially from that of no. II. There is no evidence of a daily rhythm. The decrease in the positiveness of the reaction of no. VI towards the end of the period is due to fatigue as is shown by the fact that it was three days before the isopod recovered its normal positiveness. Since there was no evidence here of a daily periodicity of reaction and since the earlier data all point against the existence of such a daily rhythm, no further tests were made along this line.

#### EFFECT OF LOW OXYGEN<sup>4</sup> AND POTASSIUM CYANIDE ON THE REACTIONS OF INDIVIDUAL ISOPODS

It has been shown that both potassium cyanide and low oxygen decrease the positiveness of stream isopods ('12, pp. 298 and 314). Experiments were run to determine the effect of these conditions upon the reactions of individuals, especially as regards the efficiency of the rheotactic response and the vigor of the concussion reaction. Typical effects of potassium cyanide may be seen in charts 1 and 2 (p. 275) of low oxygen in charts 3, 4 and 5, pp. 277-281.

Both the positiveness and the vigor of the reaction to current were diminished, though from one to five days might elapse before the reaction was greatly affected. The length of this period differed with different isopods. On the other hand, the response to concussion at first increased (charts 1, 2, 3 and 5)

<sup>4</sup> Potassium cyanide,  $\frac{N}{100000}$  solution. Low oxygen ranged between 0.50 and 1 cc. per liter.

but later, as the animals became more affected, this response also decreased to zero some time before death occurred.

As the positive rheotactic movement decreases either the negative or indefinite reactions must increase. Under normal conditions it has been shown that in stream isopods the negative rather than the indefinite reaction tends to be given in cases of low positive response. But little evidence was forthcoming concerning the relative metabolic condition of the animal giving highly positive or highly negative responses.

Under experimental conditions when the positive response is cut by depressing agents some evidence is available and is given in table 8. The high positive response may give way directly to a state of high indefiniteness as in the first, sixth, seventh and ninth instances cited in the table. But when the negative response is increased it stands intermediate between the two and when it is given under these conditions the efficiency has always decreased. Later with further decrease in efficiency the reaction becomes largely indefinite. The tenth item shows that this

TABLE 8

*Showing the relative effect on the sign of the rheotactic reaction when the metabolic rate is known to have been reduced enough to cause an intermediate rate of efficiency*

ISOPOD NO.	CAUSE OF DEPRESSION	EFFECT ON POSITIVENESS	EFFECT ON NEGATIVENESS	EFFECT ON INDEFINITENESS	CONDITION OF RATE OF EFFICIENCY
I	KCN	decreased	unaffected	increased	intermediate <sup>1</sup>
II	molting <sup>2</sup>	decreased	increased	increased later	intermediate
	KCN	decreased	increased	increased later	intermediate
VI	fatigue	decreased	increased	increased	intermediate
	KCN	decreased	increased	increased	intermediate
VII	low O <sub>2</sub>	decreased	unaffected	increased	intermediate
VIII	low O <sub>2</sub>	decreased	unaffected	increased	intermediate
IX	low O <sub>2</sub>	decreased	increased	unaffected	intermediate
XI	low O <sub>2</sub>	decreased	unaffected	increased	intermediate
	after				
	low O <sub>2</sub>	increasing	increased	decreasing	intermediate
XVI	low O <sub>2</sub>	decreased	increased	unaffected	intermediate

<sup>1</sup> By 'intermediate' is meant a rate between that given when the isopod is highly positive and when it is almost completely indefinite.

<sup>2</sup> In this instance molting resulted in death.



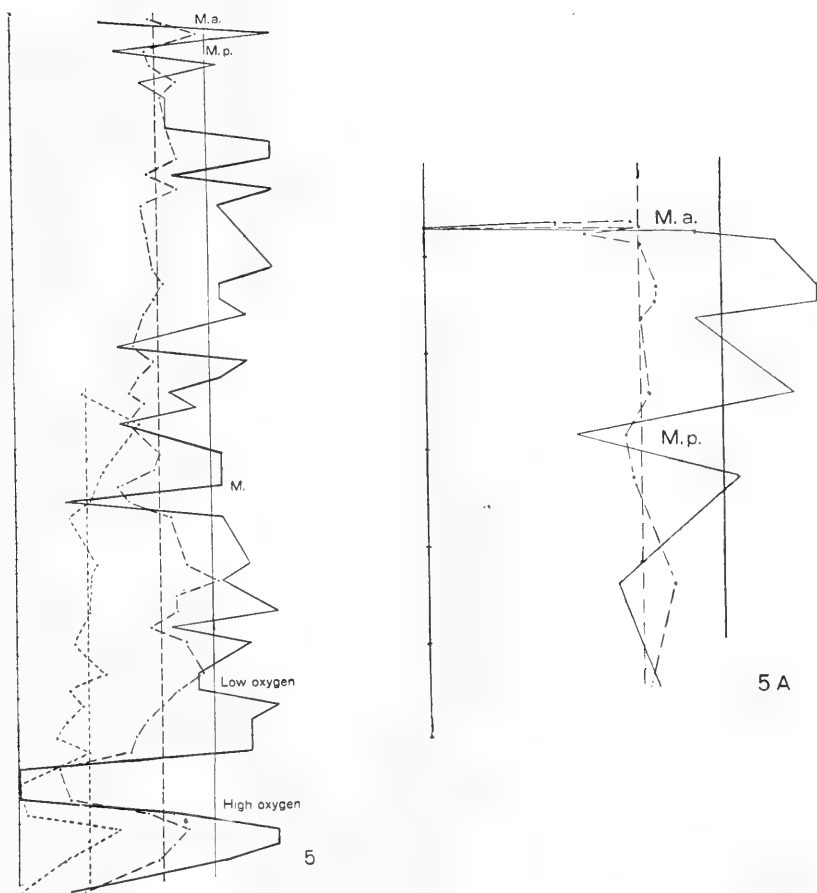


Chart 5 The rheotactic and concussion responses of isopod no. XI. For methods of charting, see chart 1, p. 275.

Chart 5 A The effect of molting on the positive response and efficiency in isopod no. XI. For detailed account, see p. 285. The system of charting is the same as that given in chart 1, p. 275. The spaces on the base line represent days.

holds whether the isopod is coming out from or going under the effect of the depressing agent.

This same state of affairs is shown in the tables of the preceding paper<sup>5</sup> which deal with the effects of depressing agents. The data presented in these tables may be illustrated by the

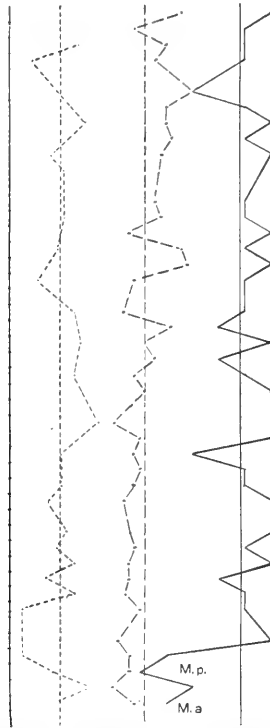


Chart 6 The rheotactic and concussion responses of isopod no. XIV. For methods of charting, see chart 1, p. 275.

following responses taken from table 15 which shows the effect of high carbon dioxide and low oxygen upon rheotaxis in stream isopods. The results are in percentages of 80 trials:

1. Eighty-nine per cent positive; 7 per cent negative; 4 per cent indefinite.

<sup>5</sup> Allee '12, tables 11, p. 299; 15, p. 310; 16, p. 312; 17, p. 315; 18, p. 315; 19, p. 316; and 20, p. 318.

2. Two days later 65 per cent positive; 25 per cent negative; 10 per cent indefinite.

3. Three days later 33 per cent positive; 23 per cent negative; 44 per cent indefinite.

That is, in the early stages of the effects of these depressants the isopod tends to show increased negative responses and later when the depression is more complete the greater number of responses are indefinite.

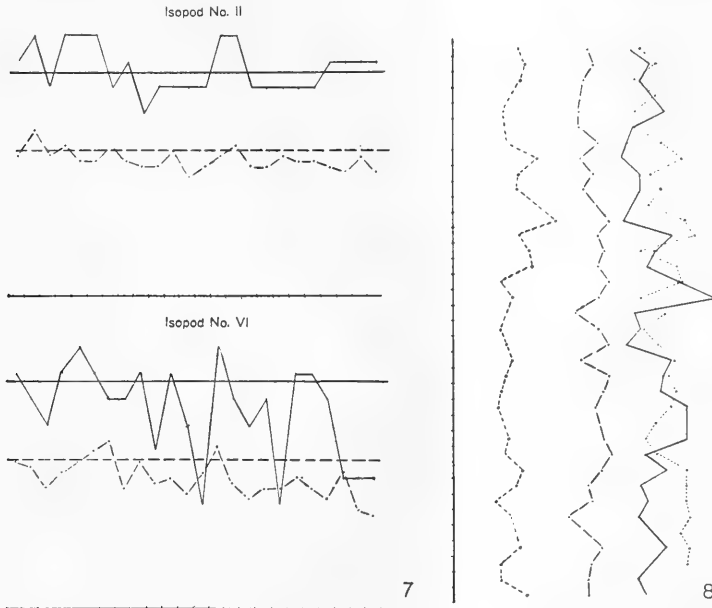


Chart 7 Showing the positive rheotactic response and efficiency of isopod nos. II and VI for twenty-four consecutive hours. The dots along the base line represent hours. For details of charting, see chart 1, p. 275.

Chart 8 A comparison of the positive reactions obtained from group tests with the average daily positiveness of three or more individual isopods. The group reactions are shown by the dotted line; the averaged individual responses are shown as chart 1, p. 275. For discussion, see p. 292.

#### RHEOTAXIS DURING THE MOLTING PERIOD

Wege ('11) gives the following account of the molting process in *Asellus aquaticus*:

Sometime before molting, usually twelve to twenty-four hours, the hypodermis begins to draw back from the body covering, whereby it

plainly becomes folded in many places, a process which may be due to growth. At the same time a new chitinous covering is excreted by the hypodermis. When this is strong enough the old covering splits around the body between the fourth and fifth thoracic segments. Then the hinder part, consisting of the covering of the thoracic segments and their appendages, is shed. Later the process is repeated for the forward part, head, four thoracic segments and their appendages. Twelve to twenty-four hours may intervene between the shedding of the hinder and forward parts. This process usually but not always takes place at night.

Wege did not find, as did Ost ('06) for *Porcellio*, any cases of autotomy, although he observed about a hundred animals.

The discarded covering is very thin and of a milky white color and consists mostly of chitin. The molting process goes on through life. The time interval between molts is usually from eight to thirty days, although over sixty days may elapse. The variation depends upon the age of the animal, temperature of the medium and the time of year. The interval increases with age and with winter, irrespective of the temperature. Wege's observations were carried on from October to the last of December in an evenly heated room, yet the interval increased for adults. The effect of temperature was still more marked for the intervals were much longer when the animals were kept in an unheated cellar.

This description of Wege's is more detailed than that of Zuelzer ('07). Her account agrees in essential features with that just given except that she observed that one to two days might elapse between the molting of the anterior and posterior parts.

In my observations on the molting process in *Asellus communis* I have no data to offer regarding the cause of variation in the interval between molts. In the main the process seems to resemble closely that described by Wege for *A. aquaticus*. However there are a few important exceptions. Thus in *Asellus communis* either the anterior or posterior part may shed its covering first and as long as four days may elapse between the molting of the halves. While this is the typical process yet the molting may occur by segments or even parts of a segment may break off and the molt extend over several days. In one instance the isopod molted all but the abdominal covering, which

was molted piecemeal and was not entirely finished until nine days later. In another instance the antennae and head shed their covering although the general molt did not occur until seven days later.

The general effect of the molting period upon rheotaxis is shown in table 9; it is best illustrated by the following account taken from notes made at the time of the first observed molting of isopod no. XI (see also charts 5 and 5 a). This isopod was a male, 12 mm. long, which averaged normally 86 per cent positive reactions. The molt began just after the first test of eleven individual trials which resulted in a response of 35 per cent positive, 35 per cent negative and 30 per cent indefinite, with an efficiency of 2.5.

The covering broke between the fourth and fifth thoracic segments and the forward part was worked off over the head by a series of undulating motions of the body and by movements of the legs, both the posterior ones and those immediately concerned. It took seventy seconds to complete the process. One of the antennae that had been dragging before the molt occurred was dropped off with the exuvia. Immediately after the molt the fore legs were noticeably smaller than usual. During the process the isopod stayed in one place and disregarded all currents. A current set up immediately after the molt was completed was also disregarded. After about two minutes in the same place the isopod moved across the pan and stopped in an angle 17 cm. away. It rested there quietly for seventeen minutes when it was again tested ten times for rheotactic reaction. No movement occurred. At thirty minutes after molting ten trials showed 30 per cent positive, 60 per cent indefinite and 10 per cent no response, with an efficiency of 2.4.

At this time the isopod was much more sensitive to touch stimuli than usual. In the response just given it twice ran into another isopod with its antennae and jumped back over a centimeter each time, although usually there would have been almost no reaction.

Three hours after molting the response was 70 per cent positive, 10 per cent indefinite and 20 per cent no reaction. The

TABLE 9

Showing the rheotactic and concussion responses at the time of molting. The interval between molts expressed in days is given in the second column, the interval preceding the first observed molt is given as over the appropriate number of days; thus, in number I the first molt occurred after fourteen days of observation and the interval is listed as being over fourteen days.\* The starred numbers indicate that these figures are repeated in the preceding or succeeding line of the table due to the short time interval between the anterior and posterior molts

ISOPOD NUMBER	INTERVAL BETWEEN MOLTS	PART MOLTED	PERCENTAGE OF POSITIVE RESPONSES			EFFICIENCY			VIGOR OF SHOCK REACTION			SEX	LENGTH IN MILLI-METERS
			Day before molt	Day of molt	Day after molt	Day before molt	Day of molt	Day after molt	Day before molt	Day of molt	Day after molt		
I	over												
	14	anterior	90		100	2.6		2.8					
	15	all	90	90	100	2.7	2.4	2.5	1.0	1.25			
	13	posterior	100	60*	100*	3.0	2.8*	4.0*	1.2	0.15*	1.1*		
	1	anterior	60*	100*	100	2.8*	4.0*	2.8	0.15*	1.1*			
	28 <sup>1</sup>	posterior	50	30		1.3	1.2		0.25	0.6		♀	7
II	over												
	70											♂	10
III	over												
	14	posterior	90	90*	80*	2.6	2.8*	2.9*					
	$\frac{1}{2}$	anterior	90*	80*	90	2.8*	2.9*	2.6					
	25 <sup>2</sup>											♀	7
IV	over												
	28	posterior	90	30* <sup>3</sup>	90*	2.8	3.0*	2.9*		2.0*	1.2*		
	1	anterior	30*	90*	20*	3.0*	2.9*	2.8	2.0*	1.2*			
	29	anterior <sup>4</sup>	90	70	90	2.8	2.0	2.6	1.0	1.7		♂	9
V	over												
	27	posterior	40	40*	40*	1.9	1.8*	2.0*					
	1	anterior	40*	40*	60	1.8*	2.0*	2.0		0.4	1.0	♂	10
VI	over												
	42	all	90	100	40 <sup>5</sup>	2.5	3.9	2.8				♀	11
VII	over												
	44	all	90	40	70	3.2	3.6	2.6	1.55	1.65	1.55	♂	11
VIII	over												
	24 <sup>6</sup>											♂	10
IX	over												
	1	anterior	70	0	60	3.9	4.0	2.6					
	27	posterior	90	90	30	3.3	2.9	3.9				♀	11
X	over												
	1	anterior	80	90	80	2.5	2.8	2.8					
	3	posterior	90	0 <sup>8</sup>		2.6	0.0					♀	9

TABLE 9—Continued

ISOPOD NUMBER	INTERVAL BETWEEN MOLTS	PART MOLTED	PERCENTAGE OF POSITIVE RESPONSES			EFFICIENCY			VIGOR OF SHOCK REACTION			SEX	LENGTH IN MILLIMETERS	
			Day before molt	Day of molt	Day after molt	Day before molt	Day of molt	Day after molt	Day before molt	Day of molt	Day after molt			
XI	2	anterior		0	100*		0.0	3.0*						
		posterior	100*	40	80	3.0*	2.6	2.7						
XII	31	all	80	20	80	2.0	2.2	3.0	1.45	1.45	1.05	♀	12	
		over										♀	4	
XIII	40 <sup>9</sup>	over												
		1	anterior		70*	50*		3.2*	2.1*					
XIV	1 <sup>10</sup>	posterior	70*	50*	30*	3.2*	2.1*	1.8				♂	13	
		over												
XV	44	posterior	60	50	70*	2.3	2.3	2.0*						
		2	anterior	70*	60 <sup>11</sup>		2.0*	2.4					♂	10
XVI	over	1	posterior	20	90	90	2.4	2.7	2.9		1.2			
		3	anterior	90	10	40	2.9	3.8	2.1		2.4			
XVII	21	all	100	70	80	2.0	2.4	2.9						
		7	all	70	60	70	2.0	2.5	2.5	0.25	0.9			
XVIII	17	middle <sup>12</sup>	60	30	50	2.1	2.0	2.9	1.35	0.3	1.6	♀	6	
		1	anterior		40*	40*		2.8*	2.9*		1.51			
XIX	1	posterior	40*	40*	30	2.8*	2.9*	2.1		1.45		♂	9	
		over												
XX	8	posterior	80	20		2.7	2.0							
		2	anterior		80	50		2.2	2.5		0.75	1.2		5
XXI	over	11	posterior	40	20*	60*	2.9	3.1*	3.1*	1.3	1.85*	1.65*		
		1	anterior	20*	60*	90	3.1*	3.1*	2.9	1.85*	1.65*	1.15	♀	10
XXII	over	3	all	70	80	90	2.6	2.9	2.1	1.4	1.2		♂	8

<sup>1</sup> Trying to molt for eleven days, but was weakened by exposure to potassium cyanide. Died from molting.

<sup>2</sup> Died from molting trouble.

<sup>3</sup> Molted within six hours before the trial was made.

<sup>4</sup> Molted posterior part piecemeal and did not finish until nine days later.

<sup>5</sup> Put in potassium cyanide which probably accounts for the depression.

<sup>6</sup> Molted piecemeal.

<sup>7</sup> Did not molt again in the forty-four days it was watched.

<sup>8</sup> Died from molting.

<sup>9</sup> No molt seen in forty days although piecemeal molting may have occurred.

<sup>10</sup> No more molts in thirty-one days before death.

<sup>11</sup> Escaped from petri dish during interval before concussion tests.

<sup>12</sup> Molted piecemeal.

efficiency was 2.1. Two hours later the response was 90 per cent positive, 10 per cent no reaction, efficiency 2.8. This last trial was characterized by steady slow movements and quick orienting, the latter seldom taking over five seconds.

In this case the molt of the posterior end occurred two days later, after 9 P.M. At nine the response was 40 per cent positive, 40 per cent negative, 20 per cent indefinite, efficiency 2.6. The next morning the response was 80 per cent positive, 20 per cent negative, efficiency 2.7. On the morning after the next molt, thirty-one days later, this isopod would start positive and then quickly turn negative as if the current pressure against the more sensitive covering were painful.

Isopods stand higher from the bottom when nearing molting, which is probably due to the increasing stiffness of their legs. This makes resistance to the current more difficult. At this time the posterior legs appear harder to move and may become tangled, thus throwing the isopod as it tries to crawl. Immediately after molting they are thrown from their feet more easily than during other parts of the cycle.

The more gradual molts mentioned above may also affect the rheotactic reaction. Thus in one instance an isopod (no. IV) molted the fourth thoracic segment and legs while its response was being tested. This occurred about thirty to thirty-six hours after the anterior molt and was accompanied by a cut of 20 per cent in the positive reaction. Another tendency is indicated by the molt in isopod no. V (table 9). This animal had been injured in handling and before molting gave a high percentage of indefinite responses but afterward the percentage of positive reactions was suddenly and markedly increased.

As is seen in the account for the molting observed in isopod no. XI, the effect of the molting lasted for about five hours after the actual ecydysis took place. If the period extended as long beforehand it would make the time during which rheotaxis was affected by the molting process extend over a period of about ten hours. It is conceivable that the general morning tests such as were usually made might not hit this period at all. Sometimes this is the case, as is illustrated in the molting of no. VII (table



9, chart 4) but usually there was a more or less decided decrease in the percentage of positiveness (table 9 and charts). When this occurred there was nearly always a decrease in the efficiency of the reaction and an increase in the negative response.

Sometimes there was an increase in the vigor of the concussion response at this period but this is too infrequent and the data on this point too meager to warrant a definite statement on this relationship.

#### SUMMARY OF RESULTS

I. Relation between efficiency and the sign of rheotactic response of stream isopods:

1. In the daily variations to the current the efficiency of movement tended to vary with the percentage of positive reactions (p. 268).

2. The larger variations of the positive reactions are usually accompanied by similar variations in efficiency (charts 1 to 8).

3. Low efficiency is accompanied by a low percentage of positive and negative and by a high percentage of indefinite rheotactic reactions (p. 270).

4. High efficiency is always accompanied by a low percentage of indefinite responses; usually by a low percentage of negative and a high percentage of positive reactions (p. 270).

5. In exceptional individuals high efficiency may be accompanied by either low or high percentage of positive responses (p. 272).

6. Under the influence of potassium cyanide and low oxygen both the positive responses and the rate of efficiency is decreased. The rheotactic reactions may go directly from a preponderance of positive to one of indefinite reactions, or there may be an interval of increased negative response. In the latter case the increased negative response always accompanies an intermediate rate of efficiency (p. 280).

7. At the time of molting, sensitiveness to currents of water is reduced to zero, but recovery is rapid. The rheotactic reactions may be affected for a ten-hour interval at each molt. During this time both the number of positive reactions and the

efficiency is decreased while the number of negative reactions is often increased (p. 285).

II. Relation between vigor of response to concussion and rheotaxis:

1. In the daily variations there is no apparent relationship between the vigor of the response to shock and either the sign or the efficiency of the rheotactic reaction (p. 268).

2. The greater variations show no more relation than do the smaller ones (charts 1-6, pp. 275-282).

3. The average vigor of shock reaction which accompanies low and high efficiency; low and high percentage of positive reactions; and low and high percentage of indefinite reactions are practically identical. The vigor of concussion response that accompanies a high percentage of negative reactions is slightly higher than the others (pp. 269-273).

4. Under the influence of potassium cyanide and low oxygen the vigor of the shock reaction often increases at first as the percentage of positive responses and rate of efficiency decrease, but later decreases and becomes zero before death occurs (p. 279).

5. There is some evidence of an increased sensitiveness to shock stimuli during the molting period (p. 289).

III. More variations occur in the percentage of positive responses than can be accounted for by variations of known external or internal factors (p. 274).

IV. Potassium cyanide and low oxygen act at different rates in different isopods but in all there is finally a depression reaction from which the isopods may recover if placed in normal conditions (p. 279).

V. The length of the molting cycle varies greatly in different individuals and in successive cycles of the same individual and causes certain variations in the rheotactic reaction (p. 285).

#### DISCUSSION

With these results in mind it becomes pertinent to inquire into the relation between the reactions of individual stream and pond isopods. Nothing can be said of the limits of individual variation among pond mores because no work as yet has been done

from this viewpoint, but regarding the efficiency of movement of pond isopods there is some data. By comparing the graphs of thirty-five individual trials<sup>6</sup> of stream with fifty of pond isopods it appears that the relative vigor of the pond is about half that of the stream mores, while on the average the positive rheotactic response is about three-eighths as strong. Here again there is apparent agreement between the degree of positiveness and the rate of efficiency.

The response to concussion failed to furnish an exact method for testing the metabolic state. This was first suggested in the responses listed in table 3. Further evidence is found in charts 1 to 6, which show that the variations of the vigor of the reaction to shock oppose those of positiveness and efficiency almost as often as they agree; also by the fact (tables 4, 5 and 6) that practically the same vigor of concussion is given with both high and low positiveness, high and low indefiniteness and high and low efficiency. It seems probable that the vigor of the concussion reaction varies inversely as the thigmotactic response rather than directly as the rheotactic reaction.

In the early periods of depression the vigor of the concussion response often increases. This action corresponds with that usually given during early stages of the action of depressing agents. The cause of the increased reaction is now generally regarded as being due to the decrease of the normal inhibitory power of the nervous system (Sherrington '06, p. 106). Later with increased action of the depressing agent this decrease in the action of the inhibiting nerves gives way to depression of the entire nervous system. This period corresponds to the time when weakened shock responses were given in the later stages of depression.

In the previous experiments ('12, p. 285) it was shown that stream isopods cannot permanently maintain themselves in a stream where they cannot withstand the current during the weakened responses of the breeding season. From the present experiments it appears that the molting period offers for brief

<sup>6</sup> These graphs are those from which figures 1 and 2 (pp. 278, 291) of the preceding paper were taken.

but frequently recurring periods, equally weakened responses, and hence is of equal importance in the ecology of the species. The minor variations in positiveness are probably of little ecological importance. The isopods that sometimes showed a high percentage of negativeness gave on the average (table 7) as many positive as negative reactions. This means that for these isopods the sign of the rheotactic reaction is of practically no importance in maintaining the position in the stream.

Following the experiments upon the rheotactic reactions of individual isopods the question arises as to the relation of averaged results obtained by this method with those obtained by group tests as performed in preceding experiments ('12). The two types of results may be seen plotted in chart 8 (p. 283). The group responses are taken from table 2, page 280 (Allee '12). The chart shows that the results given by the two methods are essentially the same.

Four of the twenty isopods tested showed a tendency to give a large number of negative reactions under normal conditions. This shows why five stream isopods taken at random give a positive response of from 70 to 80 per cent. The chances are that four of the five will give a positive response of from 80 to 100 per cent averaging some place in the upper eighties. The other will give a response of about 50 per cent positive which will cause the average reaction to be between 70 and 80 per cent positive.

By substituting the plan of individual for group tests the amount of individual variation becomes emphasized. In the highly positive isopods this consists of 20 per cent variation both in the degree of positiveness and in the efficiency of the response. In other and more exceptional cases the range is still greater. It also shows that in some stream isopods a high negative response may accompany a high rate of efficiency although this condition is too infrequent to greatly affect average results.

In the preceding paper ('12, pp. 338-39) it was shown that the degree of positiveness depends upon the rate of metabolism and can be controlled by certain factors known to affect animal metabolism. In the present series of experiments it has been

shown (table 8, p. 280) that under experimental conditions positive isopods may come to give negative reactions when the efficiency or vigor of response is intermediate between that which usually accompanies high positiveness and high indefiniteness.

Then if we use the degree of positiveness as an index of the physiological state of the stream isopods we shall have the following results:

RHEOTAXIS	EFFICIENCY	PERIOD WHEN GIVEN
High +, low - and $\alpha$	high	usually accompanies optimum conditions
Lowered +, heightened - and low $\alpha$	high	sometimes accompanies apparently optimum conditions
Lowered +, heightened - and low $\alpha$	intermediate	near beginning or close of periods of depression
Low + and -, high $\alpha$	low	at periods of strong depression
Low +, -, and $\alpha$ high 0	zero	at times of extreme depression
Minor variations of any of the above		occur when the conditions are apparently uniform

That is, the work with individuals has shown that in the physiological states that accompany the highest efficiency there is something as yet unanalyzed that sometimes causes negative responses where positive reactions are usually given. Also that the varying physiological states that produce the minor variations are as yet inexplicable in their entirety (cf. Pearl '02, with *Planaria* and Jennings '02, with *Stentor*) although each class may be in part explained by the influence of the molting period. One other factor that may affect the minor variation is the fact that the isopods tend to continue in the direction in which they are started regardless of the response they will ultimately make ('12, p. 276), so some of the variations may be due to this error in the method. It is probable that factors enter into the control of the positive rheotactic response other than the rate of metabolism of the organism. Thus during the molting period there is an interval when the mechanical difficulties are such that movement is limited regardless of the metabolic condition of the isopod. Yet on the average the data at hand strongly indicate that the degree of positiveness and the efficiency of *Asellus* com-

munis in a water current depend primarily upon the rate of metabolism.

It is possible to measure the rate of metabolism by the survival time in a standard strength of potassium cyanide (Child '13, p. 155). Since the purpose of these experiments was to study the extent of variations in individual isopods this procedure was obviously impossible. Now that something has been learned concerning the amount of individual variation, the cyanide method can be used to advantage.

I am indebted to Dr. V. E. Shelford and Dr. C. M. Child for criticism and suggestions during the progress of this work; to Dr. Shelford also for reading the manuscript; to M. H. Allee for assistance with certain of the experiments; to B. W. Benedict for making the concussion apparatus without charge; and to Dr. C. F. Hottes for furnishing laboratory facilities for the prosecution of the work.

April, 1913.

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THE EFFECT OF CASTRATION, SPAYING OR SEMI-  
SPAYING ON THE WEIGHT OF THE CENTRAL  
NERVOUS SYSTEM AND OF THE HYPOPHYSIS OF  
THE ALBINO RAT; ALSO THE EFFECT OF SEMI-  
SPAYING ON THE REMAINING OVARY

SHINKISHI HATAI

*The Wistar Institute of Anatomy and Biology*

The object of the present paper is to report on the condition of several of the internal organs of the albino rat after castration, spaying or semi-spaying. These series of spayed and semi-spayed animals were prepared by Dr. Stotsenburg and have been used by him for studying the growth of the body in weight as affected by the respective operations (Stotsenburg '13). The castration of the animals here used was also performed by Dr. Stotsenburg but in this series the growth of the body was not followed, the effect of this operation having been previously determined (Stotsenburg '09).

I wish to acknowledge the courtesy of Dr. Stotsenburg in allowing me to make the postmortem examinations in all of these interesting cases.

All the necessary details concerning the operations and the care of the animals have been already given by Stotsenburg ('09, '13) but two points should be mentioned here as these are important for the interpretation of the results: (1) in all instances the operation was performed on young rats about a month old and (2) in the cases of castration the testes with epididymis were removed.

Regarding the method of examination of the various organs, as well as the significance of the various formulas used in determining the calculated values from the observed values, the reader is referred to a paper published recently by myself (Hatai, '13).

The total number of litters used by Stotsenburg for the spayed series was 17. These comprised 38 spayed and 35 control rats. In the case of semi-spayed there were 12 litters comprising 25 semi-spayed and 23 control rats.

A few of these litters however lost either some of the operated animals or of the controls before my examination, also some of the organs were used for histological preparations, so that the number of litters, as well as number of individuals represented in the present tables is less than that given by Stotsenburg in his paper.

On the other hand it is important to state in this connection that all of the animals from which complete data were obtained were also used in making the present tables. For determining the modification in the operated groups, the following method was adopted:

First the percentage deviation of the controls from the calculated values for any character as given by the formulas, was determined and then the corresponding percentage deviation of the operated animals. The algebraic difference of the two values thus obtained gives the difference between the operated and controls. In the tables here given the body length was always taken as the basis for comparison.

The values obtained, are given in the accompanying tables and designated as "Percentage by which the operated animals differ from the controls." This method is necessary since the observed body length which is taken as the basis for the weight of the organs is only rarely identical in both operated and controls, and thus a direct comparison of the observed values is rarely justified.

#### CASTRATION

This experiment is based on 11 litters comprising 25 castrated and 12 control rats. The series was divided into four groups each containing 3 litters—except one group, containing 2 litters. In every case the operated and control rats which are compared belong to the same litter. This precaution minimizes the variability of the characters under consideration, since the

variability is greater when individuals of different litters are compared (Jackson '13). This method was followed also in the other two series, that is, the spayed and the semi-spayed. The results of the observations are given in table 1.

#### BODY WEIGHT

Stotsenburg ('09) has shown that in the albino rat castration does not modify the growth of the body in weight. This conclusion is fully supported by the present series. We notice in table 1 that the observed body weight of the castrates is nearly identical with that of the controls, the average body weight of castrates being 122.1 grams contrasted with 123.5 grams for the controls at the same age.

We notice further from the table that not only the growth of the body in weight remains unmodified, but the relation between body length and body weight characteristic for this series is unaffected by castration.

The difference in body weight between the castrated and control rats when body length is taken as the basis for the computation is 3.63 per cent in favor of the controls. This difference can certainly not be regarded as significant when we also consider the normal fluctuations in this character.

In those species which respond to castration by an overgrowth several investigators have noted a definite effect on the development of the skeleton. Poncet ('97) noted that the bones, particularly the femur, the tibia and fibula, of the castrated rabbits were longer. Fichera ('05) and Lannois, and Roy ('02) noted an elongation of the leg bones in castrated guinea pigs, in oxen, in capons and in some other animals. Numerous other confirmatory observations can be found in the literature (see Vincent '12 and Marshall '10).

Since however in the rats not only the growth of the body as a whole remains unaltered, but a perfect normality is shown in the relation between length and weight of the body, it seems improbable that alterations in the length of the leg bones should occur. Observations on this point however are now being made.

TABLE 1  
Showing the weight data for the castrated rats compared with those for the controls. *Castrated Series (11 litters)*

ALBINO RATS	BODY LENGTH	BODY WEIGHT	TAIL LENGTH	WEIGHT OF		PER CENT WATER		HYPOPHYSIS	AGE days	NO.
				Brain	Spinal Cord	Brain	Spinal Cord			
Group I. {	Control	153	131	1.646	0.412	78.11	71.52	0.0037	267	4
	Operated	150	97.8	1.644	0.424	77.87	71.30	0.0061	267	7
Group II. {	Control	158	132	1.742	0.458	78.11	71.40	0.0046	257	3
	Operated	163	124.1	1.800	0.488	78.01	71.11	0.0086	257	7
Group III. {	Control	170	137	1.799	0.488	78.12	71.40	0.0055	264	3
	Operated	170	121.4	1.750	0.508	77.85	70.96	0.0086	264	7
Group IV. {	Control	182	157	1.861	0.550	77.52	70.97	0.0061	284	2
	Operated	174	145.2	1.792	0.504	77.85	71.23	0.0092	284	4
<i>Controls</i>										
Observed (average)	166	123.5	139	1.762	0.477	77.97	71.32	0.0050	268	12
Calculated from formulas	166	106.7	137	1.699	0.433			0.0047		
Percentage deviation from calculation = A	0	15.14	1.45	3.70	10.16			6.38		
<i>Operated</i>										
Observed average	164	122.1	144	1.747	0.481	77.90	71.15	0.0081	268	25
Calculated from formulas	164	102.8	135	1.690	0.425			0.0045		
Percentage deviation from calculation = B	0	18.77	6.65	3.37	13.17			80.00		
Percentage by which the operated animals differ from the controls. i. e. B - A										
	0	3.63	5.20	-0.33	3.01	-0.07	-0.17	73.62		

Weight in gms.: length in mm.

## TAIL LENGTH

The tail of the castrated rats is slightly longer than that of the controls; the difference is somewhat over 5 per cent and occurs in three out of the four groups (see table 1).

Other experiments now in progress in our laboratory also indicate the same relation. Consequently the difference found in the present case should not be disregarded on account of its small magnitude. The following conclusion is therefore made tentatively: that the castrated rats, when compared with their controls tend to have slightly longer tails.

## BRAIN WEIGHT

The difference in brain between castrated and controls is 0.33 per cent in favor of the latter (table 1). Although this difference is so slight, nevertheless by reason of its persistent occurrence it cannot be neglected. It has been found previously (Donaldson and Hatai '11) that castration in the albino rat reduces the weight of the brain and the weight of the cord also somewhat below those weights in the normal control rats. The deficiency in the castrates amounted (as an average of all groups) to 3 per cent in the brain and 5.3 per cent in the spinal cord. The deficiency in the present experiment is far less, nevertheless its constancy in direction, as well as the similar tendency among both the spayed and the semi-spayed rats (see tables 2 and 3) force us to conclude, though with considerable reserve, that castration tends to reduce the relative weight of the central nervous system. The difference in this deficiency among different strains suggests that the reduction may vary considerably according to the general condition of the rats under experiment.

## WEIGHT OF SPINAL CORD

Contrary to the case of the brain, the weight of the spinal cord of the castrates is heavier than that of the control rats. The difference amounts to 3.01 per cent. Thus the present series gives in the cord results contrary not only to the brain but also contrary to our previous findings in which we noted a deficiency

in the cord of the castrates which amounted to 5.3 per cent (an average of all groups—Donaldson and Hatai '11). For the lack of agreement in this character between the two series no adequate explanation can yet be given.

#### PERCENTAGE OF WATER IN THE BRAIN AND IN THE SPINAL CORD

Previous work (Donaldson and Hatai '11) has shown that castration does not modify the percentage of water either in the brain or in the spinal cord. The present series supports this conclusion as will be seen from table 1.

The difference between the castrated and control rats gives 0.07 per cent in the case of the brain and 0.17 per cent in the cord, both in favor of the control. The difference is however too small to be significant and thus justifies our previous statement.

#### WEIGHT OF HYPOPHYSIS

The hypophysis shows a most striking difference in weight between the castrated and control rats. The difference amounts to nearly 74 per cent in favor of the castrated rats. The difference is shown not only in the final average but also in all four groups and in each litter. This enlargement of the hypophysis following castration is certainly the clearest alteration so far found.

So far as I know there is only one investigator who used the albino rat for studying the effect of castration on the weight of the hypophysis. This is Barnabo ('08) who gives three series of experiments which were conducted in the following manner:

Series 1. Bilateral section of vas deferens. This series comprises three rats.

Series 2. Section of vas deferens on one side and castration on the other. This series comprises two rats.

Series 3. Bilateral castration. This series comprises three rats.

The operation was performed on sexually mature rats (112 grams in average body weight) and the period between operation and death was on the average seventy-six days. The average weight of the hypophysis was 0.015 grams corresponding to the

final body weight of 151 grams. (The hypophysis was not examined in two rats.) Barnabo concludes that the weight of the hypophysis is not altered as the result of operation. No data given regarding the control rats, and it is merely stated "Per altro controllo mi sono poi servito di animali normali."

It is true that Barnabo's experiment (except series 3) is different from that of my own, not only in the method of operation, but by reason of the fact that Barnabo operated on the sexually mature animals, while the rats examined by me were operated on while still very young (about twenty-five to thirty days of age). Thus the two experiments may not be strictly comparable, nevertheless according to our formula (Hatai '13) which is based on large numbers of normal rats, the weight of the hypophysis given by Barnabo is almost 150 per cent heavier than it ought to be for the body weight given. It seems highly probable that Barnabo did not realize the existence of the striking sex difference in the weight of the hypophysis, and thus the operated rats may have been compared with the normal female albino rats, in which case but slight differences might appear as the weight of the hypophysis in the female is more than twice that in the male (Hatai '13).

There are several investigators who also have noted in other animals an enlargement of the hypophysis as the result of castration. For instance Fichera ('05) noted it in guinea pigs, rabbits and several other animals, and Kon ('08) in man. The clinical observations on the subject are very numerous, but these will not be discussed at the present moment.

On the other hand, Marrassini and Luciani ('11) also using guinea pigs, rabbits and some other animals, recently obtained contrary results, that is they found that castration does not produce any noticeable enlargement of the hypophysis in the forms studied by them. Marrassini and Luciani's experiments were carried on in a very satisfactory manner, since their operated animals were contrasted with well chosen controls, and in addition the number of animals used was much greater than in most of the other researches. The probable cause of the conflict in these results will be discussed later.

## SPAYING EXPERIMENTS

The present series contains 15 litters comprising 27 spayed and 27 control rats. The series is divided into five groups each containing three litters, according to the method described. The results of the observations are shown in table 2.

*Body weight.* At the time of maximum body weight, that is, at 271 days in age, Stotsenburg ('13) records an average body weight of 226 grams for spayed rats in this series and 176 grams for the corresponding controls. This gives a difference of 28.4 per cent in favor of the spayed animals.

At the time of killing however, some of the rats had lost weight through lung infection or had died, and consequently the body weights given by Stotsenburg and those given by the present writer are not the same.

Nevertheless we see clearly that the spayed rats made a better growth not only in length, but in entire body weight as can be shown by the following differences.

At the same age, the body length of the spayed is 2.8 per cent longer, and when body length is equated, the body weight of the spayed is 3.07 per cent heavier than that of the controls.

We further notice that at the time of examination, the body weight of the spayed was on the average 158.9 grams against 141 grams of the control. This gives a difference of 12.7 per cent in favor of the spayed against 28.3 per cent, at the time of the maximum weight. This means that during the interval the spayed rats lost more than the controls. This loss was mainly in fat.

*Tail length.* Unlike castration, spaying does not modify the length of the tail. The difference of 0.61 per cent or one millimeter in absolute measurement in favor of the spayed, seems to be too slight to insist on.

*Brain weight.* Similarly, as in the case of the castrates, the spayed rats show a slight reduction in brain weight ( $-0.39$  per cent) when compared with that of the controls. To determine how far we can rely on this difference, additional data are necessary.

*Spinal cord weight.* Like the brain, the spinal cord of the spayed shows the figure below ( $-1.57$  per cent) that of the controls.



TABLE 2  
 Showing the weight data for the spayed compared with those for the control rats. Spayed Series (15 litters).

ALBINO RATS	BODY LENGTH	BODY WEIGHT	TAIL LENGTH	WEIGHT OF		PER CENT WATER		HYPOPHYSIS	AGE days	NO.
				Brain	Spinal Cord	Brain	Spinal Cord			
Group I. { Control.....	159	99.5	149	1.664	0.460	78.29	71.51	0.0062	273	5
{ Operated.....	166	130.9	145	1.698	0.479	77.89	71.67	0.0088	273	4
Group II. { Control.....	164	115.4	145	1.766	0.472	78.59	71.51	0.0077	250	5
{ Operated.....	165	109.6	152	1.728	0.470	77.99	71.00	0.0089	250	6
Group III. { Control.....	176	138.5	154	1.807	0.500	78.19	71.47	0.0081	229	6
{ Operated.....	179	151.7	160	1.860	0.516	78.39	71.55	0.0098	229	7
Group IV. { Control.....	185	165.1	156	1.862	0.556	78.28	71.51	0.0104	287	6
{ Operated.....	191	183.6	163	1.862	0.557	78.20	71.44	0.0118	287	6
Group V. { Control.....	196	186.7	167	1.877	0.625	77.44	69.39	0.0135	305	5
{ Operated.....	203	218.9	180	1.915	0.659	77.66	69.84	0.0131	305	4
<i>Controls</i>										
Observed (average).....	176	141.0	154	1.795	0.523	78.16	71.08	0.0092	269	27
Calculated from formulas.....	176	136.8	153	1.740	0.496			0.0090		
Percentage deviation from calculation = A.....	0	3.07	0.65	3.16	5.44			2.22		
<i>Operated</i>										
Observed (average).....	181	158.9	160	1.813	0.536	78.03	71.10	0.0105	269	27
Calculated from formulas.....	181	149.7	158	1.764	0.516			0.0099		
Percentage deviation from calculation = B.....	0	6.14	1.26	2.77	3.87			6.06		
Percentage by which the operated animals differ from the controls. i. e., B - A.....	0	3.07	0.61	-0.39	-1.57	-0.13	0.02	3.84		

Weight in gms.; length in mm.

*Percentage of water in the brain and spinal cord.* The difference between the spayed and controls with respect to water content is negligible, and thus it is concluded that spaying does not modify the water content of these structures.

*Weight of hypophysis.* The weight of the hypophysis in the spayed is slightly greater than that of the controls; the difference amounts to 3.84 per cent. This amount of difference however will easily fall within the experimental error on account of the minuteness of the gland as a whole.

Furthermore, when this difference, 3.84 per cent or 0.0004 grams in absolute weights compared with the difference shown by the castrated rats (73.62 per cent) we feel still safer in concluding that the difference is most likely due to slight experimental errors or incidental variation and that consequently spaying does not modify the weight of the hypophysis.

The present results on the spayed rats are contrary so far as the hypophysis is concerned, to the results obtained, for instance, by Fichera ('05) in guinea pigs and rabbits, and Kon ('08) in women. These investigators noticed a striking enlargement of the hypophysis as the result of spaying. On the other hand Marrassini and Luciani ('11) find no hypertrophy following spaying in guinea pigs and rabbits. Thus we see that discordant results are again presented by these authors. The observations of Marrassini and Luciani appear to have been the more carefully made.

In this connection the following general comment on the subject of the removal of sex glands may be made.

The question of the enlargement of the hypophysis following the removal of either of the sex glands is difficult to solve, not because the operation is complicated, but because the hypophysis is such a minute organ and its normal variability is great.

This difficulty is further increased by the fact that its variation in weight tends to be greater when different litters, or still more, when different strains of the same race, are compared. These difficulties however may be reduced to a minimum by taking both the control and the operated animals from the same

litter and thus making comparisons within the litter. This is certainly the safer method in determining any alterations following an operation. When this method is not available, a considerable number of animals both for control and for operation must be examined in order to determine the extent of variation shown by the two groups.

A search through the literature reveals the fact that such a method as that mentioned above has not been used by most investigators, and thus the conclusions have been drawn mainly from a few operated animals contrasted with even a less number of controls, which in turn did not certainly belong to either the litter or the strain of the operated animals themselves. These facts throw doubt on the correctness of the results thus obtained and indeed the recent work of Marrassini and Luciani ('11) who worked with great care and who, contrary to the previous investigators, find no enlargement of the hypophysis in either guinea pigs or rabbits, accentuate these doubts more strongly.

#### SEMI-SPAYED RATS

The present series contains 12 litters comprising 21 semi-spayed and 20 controls. The series is divided into four groups, each containing three litters. The result of the observations is given in table 3.

*Body weight.* In this series the weight of the body at 280 days in age is given by Stotsenburg ('13) as 140 grams for semi-spayed and 145 grams for controls. At the time of killing (326 days) however, some of the rats had gained in weight, particularly the semi-spayed. As is shown in the table above, a substantial increase in body length and weight of body was made by the semi-spayed over that of the controls after 280 days of age. The difference in body weight, when body length is taken as the basis for the comparison, between the semi-spayed and the control, is insignificant amounting to only  $-2.65$  per cent.

From this it is concluded that semi-spaying does not modify the normal relation existing between body weight and body length, and consequently a slight overgrowth in body weight shown at the time of examination was not due to an accumulation of fat.

TABLE 3  
Showing the weight data for the semi-spayed compared with those for the controls. Semi-spayed Series (12 litters)

ALBINO RATS	BODY LENGTH	BODY WEIGHT	TAIL LENGTH	WEIGHT OF		PER CENT WATER		HYPOPHYSIS	OVARIES	AGE days	NO.
				Brain	Spinal Cord	Brain	Spinal Cord				
Group I.	Control.....	105.0	148	1.703	0.479	77.92	70.67	0.0078		286	3
	Operated.....	121.6	152	1.736	0.498	77.94	70.59	0.0082		286	4
Group II.	Control.....	144.6	151	1.762	0.516	78.06	71.13	0.0109		347	4
	Operated.....	134.9	147	1.766	0.505	78.03	71.17	0.0099		347	5
Group III.	Control.....	159.3	166	1.785	0.562	78.25	70.87	0.0117	0.0520 (double)	343	7
	Operated.....	161.9	171	1.770	0.580	77.86	70.28	0.0110	0.0421 (single)	343	6
Group IV.	Control.....	180.0	170	1.776	0.598	77.90	70.18	0.0154	0.0394 (double)	326	6
	Operated.....	199.2	177	1.819	0.623	77.77	69.93	0.0140	0.0386 (single)	326	6
<i>Control</i>											
Observed (average).....	179	147.5	159	1.757	0.539	78.03	70.71	0.0115	0.0457 (double)	326	20
Calculated from formulas.....	179	144.4	156	1.754	0.508			0.0096	0.0464 (double)		
Percentage deviation from calculation = A.....	0	2.14	1.92	0.17	6.10			19.79	-1.50		
<i>Operated</i>											
Observed (average).....	183	154.4	162	1.773	0.550	77.90	70.49	0.0108	0.0404 (single)	326	21
Calculated from formulas.....	183	155.2	160	1.773	0.524			0.0104	0.0471 (double)		
Percentage deviation from calculation = B.....	0	-0.51	1.25	0	4.98			3.84	-14.13		
Percentage by which the operated animals differ from the controls. i.e. B - A....	0	-2.65	-0.67	-0.17	-1.12	-0.13	-0.22	-15.95	-12.73		

Weight in gms.; length in mm.

*Tail length.* Tail length with respect to body length is not modified by semi-spaying. The difference is negligible, amounting to 0.67 per cent in favor of the controls.

*Brain weight.* Here again the brain weight tends to be small in the semi-spayed to about the same degree as has been noted in the case of the spayed rats; the difference is 0.17 per cent in favor of the controls.

*Spinal cord weight.* The weight of the spinal cord of the semi-spayed is a little over 1 per cent below that of the controls. Here also the difference is very small, nevertheless there appears a constant tendency to slight reduction in the operated rats when compared with the unoperated.

*Percentage of water in the brain and in the cord.* It is clear from the table that the water content in the semi-spayed is practically identical with that of the controls; the difference between the two being 0.13 per cent in the case of the brain and 0.22 per cent in the case of the spinal cord in favor of the controls.

Consequently we conclude that the water content in the central nervous system is not modified by semi-spaying.

*Weight of hypophysis.* The weight of the hypophysis in the operated rats is practically normal to body length, while that of the control rats is considerably heavier than the body length calls for. Furthermore, the normality of all other characters in the semi-spayed rats indicates that the difference of nearly 16 per cent shown by the hypophysis in favor of the control rats is not due to the diminution of this organ in the semispayed, but is rather due to the abnormal size of it in the control rats. Without being able to explain this high value in the controls, I conclude nevertheless that the weight of the hypophysis in the operated rats is not modified.

*Weight of ovaries.* We note here a beautiful instance of compensatory growth. The weight of the ovary of one side in the semi-spayed is almost twice the weight of the normal single ovary; the average departure from the double weight being only 7 mgms. or -14 per cent. Exactly what structures are responsible for such a compensatory growth of the ovary still requires to be investigated.

This observation is not new but the facts noted in this case furnish one more instance in a particularly clear form.

Touching the enlargement of the ovary we note, for instance, that Bond ('06) has shown in rabbits that a removal of one ovary is followed by compensatory growth of the remaining ovary. Bond however asserts that this compensatory growth takes place only when the animals are allowed to become pregnant or at least to have sexual intercourse.

Carmichael and Marshall ('08) tested the question of compensatory growth by using rabbits also. The main conclusion reached by them is that the compensatory growth of the ovary occurs independently of both sexual intercourse and pregnancy; a conclusion contrary to that reached by Bond. This agrees however with the results obtained by the present writer on the albino rat.

By removing portions of the remaining ovary, Carmichael and Marshall showed further that the power of compensatory growth possessed by the remaining ovary is relatively greater the larger the amount of ovarian tissue which has been removed.

It is interesting to note that their method of determining the amount of hypertrophy was to compare the weight of the remaining ovary with that of the other which has been removed two to five months earlier. The rabbits were fully grown at the time of operation. Our study of the albino rats (Hatai '13) shows that the ovary grows in weight even after the rats have become mature, and consequently the amount of compensatory growth can only be determined closely by knowing the amount of normal growth during the period under consideration.

Our results on the albino rats however indicate that the conclusion reached by Carmichael and Marshall was essentially correct, as the amount of normal growth of ovarian tissue during the several intervening months is probably not large in the adult rabbits.

*Remarks on the weight of the hypophysis.* It is to be noted that in the albino rats the hypophysis presents a sexual difference which is very marked (Hatai '13) as the hypophysis of the female is more than twice as heavy as that of the male. In the Norway rats the sex difference is very slight and probably still less, if present

at all, in the case of guinea pigs and rabbits, so far as we are able to determine from existing data furnished by the several investigators.

What this sex difference in the albino rats actually means, I cannot say.

One point is clear from the preceding, namely, that the hypophysis reacts differently according to the sex after the removal of the sex glands. Thus we noticed that spaying in the female does not produce the enlargement of the hypophysis, but on the other hand, overgrowth and obesity invariably follow this operation.

Castration produces in the male a striking enlargement of the hypophysis while overgrowth and obesity are entirely absent. This peculiar relation between the response of the hypophysis on the one hand and overgrowth and obesity on the other, is worthy of further consideration.

Clinical as well as experimental evidence (Cushing '09, and for a general account on the subject, see Vincent '12) shows with a high degree of probability that 'hypo-secretion' of the pituitary gland produces abnormal accumulation of fat possibly due to lowered oxidation. Thus if a compensatory growth of the hypophysis does not follow, as is the case after spaying, the product of the unaltered gland must be employed for two purposes; one, to replace the ovarian hormone and two, for the normal uses, whatever these may be. Spaying thus appears to overtax the normal secretion coming from the unmodified gland and consequently to produce phenomena similar to those following hypo-secretion. On the other hand, the compensatory hypertrophy of the hypophysis in the male albino rat after castration appears to prevent the phenomena of hypo-secretion, and consequently the body changes, overgrowth and obesity, are prevented. The value of this view must be tested. Nevertheless it seems to offer a reasonable explanation for the true growth and the accumulation of fat following spaying on the one hand, and the absence of these reactions after castration, on the other.

The result of semi-spaying in the albino rats gives still further evidence in support of the view just presented.

Semi-spaying produces neither an enlargement of the hypophysis, nor an abnormal fat deposition in the body. This is explained by the fact that the remaining ovary enlarges to twice its normal size, thus we may conclude that the normal secretory function is performed adequately by the tissues of the enlarged ovary, and consequently the changes which would follow hyposecretion of the hypophysis are prevented.

#### SUMMARY

The conclusions here given apply to the domesticated albino rat (*Mus norvegicus albinus*) and in the absence of direct evidence should not yet be extended even to the wild Norway.

1. *Body weight.* The growth of the body in weight is not modified by castration or by semi-spaying. Spaying however increases body growth, some of the increase in weight being due to fat.

2. The normal relation between body weight and body length is not modified in the castrated or the semi-spayed. In the case of the spayed, it is modified by the presence of fat; that is, the animal is heavy for its body length.

3. *Tail length.* The tail tends to be slightly longer in castrates, but no alteration occurs in the other two series.

4. *Brain weight.* In all series the difference between the operated and non-operated is almost negligible, nevertheless in all the operated groups it constantly falls below that in the non-operated.

5. *Spinal cord weight.* In the castrated series the weight is higher than in the controls, but follows the brain in the other two series; the spayed and the semi-spayed.

6. *Percentage of water.* The water content of the nervous system is not altered by any of the operations.

7. *Hypophysis.* A striking and constant increase in the weight of the hypophysis is shown after castration, but practically no change after either spaying or semi-spaying.

8. *Ovaries.* In the semi-spayed series the compensatory growth of the remaining ovary is almost perfect and the single ovary has therefore nearly twice its normal weight.



9. *Relations of sex glands to hypophysis.* After removal of the sex glands, it appears that when compensatory growth of the hypophysis occurs, then there is no overgrowth or obesity. On the other hand, both these effects follow when the enlargement does not occur, as in the spayed rats.

In the semi-spayed, neither enlargement of the hypophysis nor overgrowth and obesity occur because the enlargement of the remaining ovary enables it to furnish the normal amount of ovarian secretion.

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# THE EFFECTS OF ATMOSPHERIC TEMPERATURE UPON THE BODY TEMPERATURE OF MICE<sup>1</sup>

FRANCIS B. SUMNER

THREE FIGURES

## 1. INTRODUCTION

In the course of some investigations, the results of which have already been published in part,<sup>2</sup> certain effects of temperature upon the length of the appendages of mice were found to reappear in a succeeding generation, even though the latter was not subjected to the experimental conditions. The author was well aware that this phenomenon would be explained by many on the assumption that the germ-cells had been directly influenced by the temperature conditions to which the organism as a whole had been subjected. To persons of this way of thinking the parallel modifications of parent and offspring would thus be adequately accounted for.

In reply, it would be hardly sufficient to point out that mammals in general are homothermous, and that the body temperature of a mouse may therefore be regarded as invariable, whatever the atmospheric temperature may happen to be. It has long been known that even in the mammalia the atmospheric temperature is not entirely without its effect upon that of the body. The nor-

<sup>1</sup> The investigations herein considered, together with others to be reported upon later, were conducted at Woods Hole, Mass., during the winter of 1910-1911, with the aid of two successive grants from the Bache Fund of the National Academy of Sciences. Although these studies were completed nearly two years ago, the writer has been prevented by other duties from preparing the results for publication. There have now been added, in a supplementary note, the results of certain other experiments performed since the paper was first prepared for publication.

<sup>2</sup> *Journal of Experimental Zoölogy*, August, 1909; *American Naturalist*, January, 1910; *Archiv für Entwicklungsmechanik der Organismen*, June, 1910; *American Naturalist*, February, 1911.

mal temperature of man in the tropics is believed by the majority of investigators to be somewhat higher than it is in temperate zones, though the difference is probably considerably less than 1°C. Hibernating mammals, and most others while in very immature stages, are known to lack the power of temperature control almost entirely.

For mammals in general, the available information regarding the influence of atmospheric temperature upon that of the body is rather meagre. More than eighty years ago, W. F. Edwards<sup>3</sup> exposed various mammals and birds, both young and adult, to low temperatures. He recorded great reductions of body temperature in the young of various species, and lesser changes even in the adults. "Guinea-pigs, and adult birds [when subjected to a temperature of 1°C.] lost, at the utmost, no more than two or three degrees." Adult mice, upon exposure to "a moderate cold in winter," underwent a reduction in temperature which "surprized" this author, though we are not given the figures. The observations of Edwards upon very young mammals will be considered more fully later.

Finkler<sup>4</sup> exposed guinea-pigs to varying atmospheric temperatures, thereby inducing constant small differences of body temperature. Finkler likewise called attention to the very considerable differences in the thermometer reading which resulted from inserting the bulb to various depths in the rectum of the animal. His figures may be summed up as follows:

TABLE 1

	THERMOMETER INSERTED TO		
	9 cm.	6 cm.	2.5 cm.
	<i>deg. C.</i>	<i>deg. C.</i>	<i>deg. C.</i>
(1) Animals kept at 15° to 18° C.....	38.96	38.42	36.45
(2) After several hours at 25° to 27°.....	39.06	38.74	36.60
(3) After several hours at 0° to 5°.....	38.75	38.60	36.10

<sup>3</sup> On the influence of physical agents on life (translation from the French). London, 1832, pp. 488.

<sup>4</sup> Archiv für die gesammte Physiologie, Bd. 29, 1882, S. 98-244 (particularly 111-125). Taf. II.

Thus, if the 9-cm. readings be the ones employed, the body temperature, after exposure to the cold atmosphere, is  $0.31^{\circ}$  lower than after exposure to the warm. If the 2.5-cm. readings be considered, the difference is  $0.50^{\circ}$ .

Pembrey<sup>5</sup> experimented at considerable length upon mice and other animals, chiefly in relation to the effects of external temperature upon the output of carbon dioxide. His temperature figures for adult mice under different atmospheric conditions are so few and so variable that they can hardly be introduced as evidence in the present discussion. As regards young animals, however, Pembrey offers more valuable data, and these will be referred to again in the course of this paper.

Hill and Macleod<sup>6</sup> found that in a current of very moist air, even at temperatures as high as  $20^{\circ}\text{C}$ ., mice lost the power of heat regulation and acquired a temperature little, if any, higher than that of the atmosphere. Such phenomena are to be regarded as pathological, however, and throw little light on the present subject (see p. 343 below).

Macleod<sup>7</sup> subjected rats to relatively high temperatures, both in moist and dry air. As the result of a sojourn of a half hour at a temperature between  $36^{\circ}$  and  $39^{\circ}\text{C}$ ., rats were found to undergo a rise of several degrees (nearly  $4^{\circ}$  in one case). This, likewise, cannot be regarded as a normal phenomenon, and the conditions were certainly not comparable with those which obtained in any of my own experiments.

Congdon,<sup>8</sup> working in Przibram's laboratory, recently attacked this problem with the same object as the present writer, namely to determine the possibility of a direct effect of atmospheric temperature upon the germ-cells of mammals. Rats and mice were used for these experiments. Congdon's own summary of his results is quoted herewith:

1. Adult rats (*Mus decumanus*) reared at  $33^{\circ}$  have a rectal temperature of  $37.2^{\circ}$ ; at  $16^{\circ}$ ,  $36.2^{\circ}$ . There is thus a difference of  $1^{\circ}$ .

<sup>5</sup> Journal of Physiology, vol. 18, 1895, pp. 363-379.

<sup>6</sup> Journal of Physiology, vol. 29, 1903, pp. 492-510.

<sup>7</sup> American Journal of Physiology, vol. 18, 1907, pp. 1-13.

<sup>8</sup> Archiv für Entwicklungsmechanik der Organismen, 33 Bd., 1912, S. 703-715.

2. Rats and mice (*Mus musculus*) approaching puberty show no difference of temperature whether reared at 33° or 16°. The common temperature is for young rats 37.9°, i.e., 0.7° higher than for adults reared at 33°. For the young mice it was 35.7° to 35.9°, also evidently higher than the adults would under like conditions show.

3. Adult rats and mice if changed from 16° to 25° or to 33° undergo a rise of average temperature for the 10 to 20 days following the change of 1.5° to 2°. At 16° the temperature was 36.1° for the rats and at 25°, 37.8°. The mice had a temperature of 34.2° at 16° and of 35.7° at 33°. [35.3° at 25°.]

Mice approaching puberty changed from 33° to 16° and vice versa showed temperature changes of about 1°.

4. Adult rats removed from 16° to 5° undergo a fall of 1.8° to 34.4° for a period of 19 days. Adult mice, some of which may have been earlier at 33°, fell 3° after a light change of external temperature. Their temperature was then 31.2°. They were measured during a period of 19 days . . . .

5. *Myoxus glis*, a hibernating animal, on removal from the 14° room to the 25° room rose in rectal temperature 0.8° to 35.9°.

The average temperature for the white rat is about 37° and for the white mouse about 34.9° according to the criterion adopted above.

The results of some experiments of my own, both with adult and with young mice, are described in the present paper. These experiments were incidental to certain studies of heredity upon which I shall report as soon as practicable. The data which I here offer are, I realize, insufficient for any very comprehensive account of temperature control in mice. The number of individuals in any one group is unfortunately too small for the strict application of statistical methods, while many of the results are equivocal, inasmuch as the various factors influencing body heat were not, at all times, clearly distinguished. But as regards the main problem which I set myself, namely the influence of atmospheric temperature upon body temperature, under the conditions of my own experiments, I think that an answer of some definiteness is forthcoming.

No apology is needed, I trust, for publishing these data in full. The fact that neither the individual variability nor the details of experimental procedure have been recorded by some previous writers renders it difficult to properly appraise their work.

## 2. TEMPERATURE TESTS OF ADULT AND NEARLY ADULT MICE

1. *Methods*

In all, 198 temperature determinations were made in the course of this series of experiments. Fifty-one white mice were used for the purpose, of which 43 were males and 8 females. All of the females and most of the males were fully mature, having an age ranging from eight to twelve months. For certain experiments there were included some males (15 in all) which were about three and one-half months old. These last were, however, of good size, averaging about five-sixths of the weight of the adult males.

Twenty-three of the mice which were used belonged to a lot which had been kept for eight to sixteen weeks in an artificially heated room, the remainder having been kept during the same period in a much colder room. The mean temperature of the former, up to the time of the first experiment (January 14),<sup>9</sup> was 22.3°C., the mean daily range being 9°C. The mean temperature of the cold room up to the time of the first experiment (January 16) was 5.8°C.,<sup>10</sup> the mean daily range being 7°.

Unfortunately, my control over the temperature of the warm room was at times quite inadequate. The extreme temperatures recorded for the entire period were 5° and 36.7°C., but both of these conditions were very exceptional and of brief duration. The temperature of the cold room did not ordinarily differ much from that of the air outside. The extremes for this room were - 11.1° and + 17.8°, but these conditions were likewise exceptional. The temperature tests were made during the colder months of the year when the atmosphere of the two rooms naturally differed most widely.

The temperatures of the mice were determined by means of specially constructed mercury thermometers, inserted in the rectum. The instruments were made to order by the Randall-

<sup>9</sup> If computed to the time of the last experiment (April 1) the mean temperature would be almost exactly the same, viz., 22.4°.

<sup>10</sup> If computed to the time of the last experiment (March 1) the mean would be 4.4°.

Faichney Company, of Boston. They were, in appearance, much like ordinary clinical thermometers, but differed from these in several important details. The scale was adapted to cover a wider range of temperatures; the bulb was more slender and contained a much smaller amount of mercury, and was connected with the graduated stem by a long narrow neck. Furthermore, the mercury in the stem did not detach itself, so as to register the maximum point reached, as in the case of an ordinary clinical thermometer, but was free to fluctuate up and down. It was thus possible for the observer to follow the negative as well as the positive temperature changes.

Two thermometers, referred to as 'number 1' and 'number 2,' were used in taking the temperatures of the full-grown (or nearly grown) mice. 'Number 1' was graduated in the Fahrenheit scale, 'number 2' in the Centigrade. The latter instrument was used in the majority of these determinations. Its total length was 95 mm.; length of bulb, 10 mm.; length of neck, 12 mm.; length of scale, 50 mm., ranging from 32° to 42°C. The diameter of the bulb was 2 mm., the diameter of the neck being from 1.5 to 2 mm.

In practically all of the experiments a rubber ring or collar was placed on the narrow neck connecting the bulb and the graduated portion of the stem. This served, on the one hand, to insure a uniform depth of insertion (a point of considerable importance), on the other to reinforce the anal sphincter in closing the entrance to the rectum. This collar, in 'number 1,' was placed 13 mm. from the tip of the bulb (about 5 mm. from its proximal end); in 'number 2' it was placed 16 to 18 mm. from the tip of the bulb (about 7 mm. from its proximal end).

A third thermometer ('number 3'), having a scale ranging from 20° to 40°C., was used almost exclusively for the very young mice (Section 3). In the present series, it was used only in certain cases where sub-normal temperatures were obtained.

The errors of these various thermometers were determined by careful comparison, in water of different temperatures, with a standard thermometer which was borrowed from the Bureau of Standards. The necessary corrections have, of course, been applied to the original readings.



It seemed possible that, in a very cold room, an appreciable amount of heat might be conducted from the bulb of the thermometer to the stem, and from the latter radiated to the surrounding air. Apparent differences of body temperature in the two rooms might thus result. Tests were accordingly made by dipping the thermometers into water of about body temperature, at the time when the surrounding air was between 1° and 2°C. Thermometer 'number 2' gave a mean reading less than one tenth of a degree higher when the entire stem was immersed in the warm water than when it was immersed only as far as the rubber ring on the neck. With the other two thermometers no certain differences were detected, and it is plain that with all of them this source of error may be left out of consideration.

In taking the temperature of an adult mouse, I lifted the animal quietly, and placed it upon a table or other flat surface. With my left hand, I grasped the tail, raising the hind quarters somewhat from the table, but allowing the fore-feet to rest upon it. The thermometer bulb was coated with vaseline and inserted into the rectum. Animals varied greatly in their behavior under this treatment. Some, particularly females, remained nearly or quite passive. Others tugged hard in their endeavor to get away, or move about freely in various directions. This latter mode of behavior naturally rendered the observations difficult, and furthermore it was noted that the struggles of the animal often had a marked effect upon the temperature recorded.

It was found at the commencement of the experiments not only that different individuals varied greatly in their rectal temperatures under apparently identical conditions, but that the same individual, in the course of a very few minutes, might give readings which differed to a striking extent. Moreover, mice varied greatly in the way in which their temperature changed within the course of a single observation. In some cases, the mercury rose to a fixed point within a half minute or less, remaining stationary for a considerable period thereafter. In other cases, it continued to rise slowly and steadily after the first rapid ascent following insertion, and did not entirely come to rest, even within two or three minutes. Not infrequently, the mercury rose by

jumps, these being commonly associated with the struggles of the animal. On the other hand, the temperature sometimes remained stationary in individuals which struggled a good deal, or underwent a slow, steady rise in the case of some mice which remained very quiet. Finally, in some cases, particularly in the cold room, the mercury fell somewhat after the maximum reading was reached, this fall amounting sometimes to a considerable fraction of a degree. And the records likewise show that a fall, instead of a rise resulted at times from the struggles of the animal.

Considering all the circumstances, it may appear to the reader as extremely doubtful whether any figure could be recorded which fairly represented the 'normal' temperature of a given mouse. And indeed it must be conceded that, amid so much variability, individual figures are of little value, but that recourse must be had to averages based upon as many determinations as possible.

It was my original intention during these tests to leave the thermometer inserted until the mercury reached a point at which it remained stationary for at least half a minute. This, in many cases, proved to be impracticable, since the temperature oftentimes refused to remain stationary for so long a period. Moreover, the point at which the mercury came to rest was sometimes so high that it could not have represented the normal temperature of the animal prior to the excitement and struggle of the test.

I also recorded in all cases the maximum temperature attained, and in some of the experiments the temperature which was indicated thirty seconds after the insertion of the thermometer. This last I regard as representing much more truly the temperature of the undisturbed animal, since, on the one hand, the interval was more than sufficient to allow the mercury to become warmed to the body temperature,<sup>11</sup> while on the other hand it was not long enough to permit of any considerable rise due to excitement or struggle. In the tables (7-12) I have given in separate columns the maximum temperature recorded, as well as the thirty-second figure when this was available. Unfortunately, this last was not systematically recorded during the earlier tests, though it is

<sup>11</sup> In water, the final reading was obtained within 10 or 15 seconds at the utmost.

deducible in many cases from the notes made at the time.<sup>12</sup> In some of the later series, on the other hand, it was regularly recorded.

While the maximum figure, in exceptional instances, was more than a degree higher than the thirty-second figure, in a large proportion of cases the two were equal, while the mean difference between them, based upon 140 examples, was only  $0.24^{\circ}$ . In the ensuing pages, the thirty-second figures will be used so far as possible, but for some purposes the use of the maximum figures will be unavoidable. Such of the latter as are believed to be unreliable,<sup>13</sup> are designated by an asterisk. These have been rejected in the computations, unless the contrary is stated.

The number of temperature readings represented in tables 7 to 12 is large, and the total number of mice is likewise considerable. If it were permissible to average all of the warm-room figures on the one hand, and all of the cold-room figures on the other, a comparison of these averages would be statistically highly convincing. Unfortunately, however, this cannot be done. Several factors influencing body temperature manifested themselves, which had no relation to the temperature of the atmosphere and were therefore quite irrelevant to the chief problem at hand. Such factors were the degree of excitement or activity of the animal, its sex and apparently its age as well. It is possible, too, that the time of day must be considered in this relation, though I am not convinced that this factor had any influence apart from the degree of activity of the animals.

It is evident, therefore, that in order to arrive at a fair comparison of the body temperatures of the cold-room and the warm-room mice, we must eliminate these irrelevant factors. In other words, we can compare only mice of the same sex and roughly of the same age, and—temperature aside—we must compare them under conditions as far as possible identical for the two lots.

<sup>12</sup> Where this figure has merely been calculated or inferred, it has been designated by a dagger (†). Such figures are believed to be accurate, in all cases, within a very few tenths of a degree.

<sup>13</sup> For example, when they differ from the thirty-second figure by as much as  $0.5^{\circ}$ , or when doubt is cast upon their accuracy by the records of the experiment.

In thus restricting the groups between which comparisons are valid, it will be found that the number of available figures is never large. In respect to certain problems, indeed, the number is so small as to yield quite inconclusive results. This is due partly to the fact that my time was largely occupied with other matters during that part of the year when large differences of temperature were obtainable; partly to the fact that the scantiness of strictly comparable data was not realized by me until I had made a preliminary tabulation of the results, rather late in the winter. I believe, however, that we shall be able to distinguish those cases in which the evidence is sufficient from those in which it is insufficient.

*2. Differences of body temperature due to factors other than the temperature of the air*

In order to illustrate the effects of factors which are irrelevant to the main problem under discussion, I present herewith the results of 30 temperature readings of 5 adult males, taken in the course of twenty-seven to twenty-eight hours, under conditions of room temperature which varied but little during this period. The figures are taken from table 9. Here, the absolute temperatures are given for the first test only,<sup>14</sup> the differences being stated thereafter.

TABLE 2

	MARCH 31 4 P. M.	APRIL 1 9 A. M.	17 TO 19 MINUTES FROM LAST	1 HOUR FROM LAST	2 HOURS FROM LAST	7½ HOURS FROM LAST
A'1.....	37.8	-2.6	+2.3	-2.2	-0.3	+2.0
A'2.....	38.5	-1.6	+0.8	-0.1	-1.6	+1.9
A'3.....	38.8	-2.2	+1.4	-0.7	-0.9	+1.2
A'4.....	37.8	-1.2	+2.0	-1.1	-0.5	+1.4
A'5.....	37.0	-0.2	+2.2	-2.0	-0.1	+1.4
Mean.....	37.98	-1.56	+1.74	-1.22	-0.68	+1.58

<sup>14</sup> These are taken from the thirty-second column, but closely similar results are obtained from the maximum column.

We find a mean fall of  $1.56^{\circ}\text{C}.$ , between four o'clock, on the first afternoon and nine o'clock on the following morning.<sup>15</sup> This is succeeded by a rise of  $1.74^{\circ}$  during the quarter hour or thereabouts following the first test for the day. The temperature now declines by  $1.90^{\circ}$ , in two steps, during the ensuing three hours. Finally, there is a rise of  $1.58^{\circ}$  during the next seven and one-quarter hours, leaving the mean temperature of the five animals  $37.84^{\circ}$ , or very nearly the same as on the preceding afternoon.

It will be noted that all of the individuals agree in the character of the temperature change undergone in a given interval, differing only in the extent of the rise or fall (fig. 1). Such a close agreement in the behavior of a number of individuals, under identical treatment, is not, however, commonly to be met with, as will be realized from an inspection of tables 7 to 12.

It is likely that several factors are responsible for the temperature changes above revealed. One of these is the condition of excitement due to the temperature tests themselves, or perhaps, rather, a local congestion, due to the insertion of the thermometer.<sup>16</sup> It is only thus that we can explain the rise of  $1.74^{\circ}$  during the fifteen or twenty minutes following the first test of April 1. The subsequent fall of  $1.90^{\circ}$  probably represents merely a return to the previous normal condition, after the excitement has subsided.

Let us consider for a moment those temperature changes which are due to such excitement or irritation. In many cases, the temperatures of the same mice were taken two or more times in the course of a single day, sometimes after a brief interval, sometimes after the lapse of several hours. An analysis of such cases as are contained in the appended tables reveals the following facts:

1. In those cases where the interval between the first and second temperature tests was less than one hour (twelve to fifty-seven minutes), a mean rise in temperature is shown for both

<sup>15</sup> This despite a rise of  $4^{\circ}$  in the air temperature.

<sup>16</sup> That the rectum was irritated in some cases was shown by the occasional presence of blood on the thermometer.

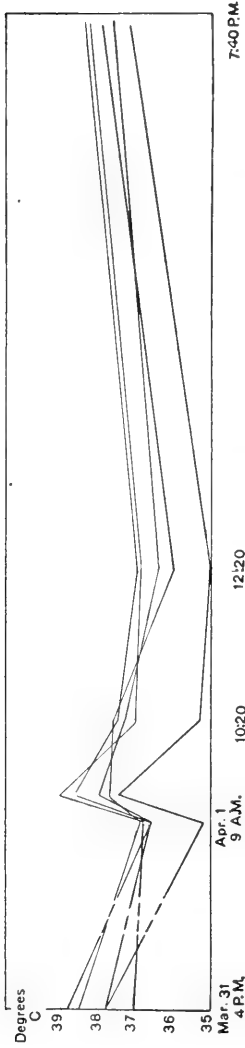


Fig. 1 Illustrating temperature changes undergone by 5 adult male mice, in the course of a series of tests (table 9). These changes had no relation to changes of atmospheric temperature.

sexes. If we reject the less reliable (starred) figures in the table, we have six cases where such a comparison is possible among the males, and an equal number among the females. The mean rise in temperature for the former is  $1.55^{\circ}\text{C}$ ., for the latter  $0.47^{\circ}$ . If we include the rejected figures (4 for the males and two for the females), the former average becomes  $1.51^{\circ}$ , the latter  $0.76^{\circ}$ . Only a single instance of a fall in temperature between the two tests is recorded.

2. When the interval between the two tests was more than one hour (eighty to one hundred and thirty-five minutes), there was much less agreement among the results. In 15 comparisons among the male mice, there was a rise in 10 cases, and a fall in 4 cases. The mean rise was  $0.53^{\circ}$ . Among the 8 available figures for females 2 show an increase and 5 a decrease, there being a mean decrease of  $0.05^{\circ}$  (i.e., no significant change).

3. When more than two tests were made during the same day, there was a mean fall of  $1.22^{\circ}$  between the second and third tests for 5 males (table 9), and a mean fall of  $0.4^{\circ}$  for two females (table 7). The time interval was one hour for the former, fifteen to thirty minutes for the latter.

From the foregoing considerations, it is plain that the most serviceable temperature data in my records are those which resulted from the first determinations for the day. These are consequently the ones which have been employed exclusively in the comparisons between the warm-room and cold-room animals.

Certain other conditions besides the excitement or irritation resulting from these tests were of probable influence in producing changes of temperature among the mice. When the animals were transferred to new and unfamiliar surroundings they were nearly always seen to be agitated for some time thereafter, and it is likely that this agitation resulted in a rise of body temperature. Referring again to table 9, it will be seen that the mean temperature of these 5 mice, on the afternoon of March 31, shortly after a transfer to new boxes<sup>17</sup> was  $37.98^{\circ}$  (thirty seconds) or  $38.30^{\circ}$  (maximum). Unfortunately, we have no afternoon

<sup>17</sup> My object, indeed, in changing the cages in the present instance, was to test this very point. I should have taken the second reading on the *afternoon* of the following day.

figures for these same individuals which it is allowable to use for comparison, but the table indicates a mean fall of  $1.56^{\circ}$  before the following morning. Other observations render it unlikely that this difference is due to a difference in the time of day. If we consider the six<sup>18</sup> available afternoon readings for the other adult male mice of the warm-room group (table 7, January 14 and 19), we find a mean temperature of  $35.83^{\circ}$  (maximum), or  $2.47^{\circ}$  lower than the earliest figures in table 9. It seems likely therefore, that these abnormally high temperatures were due to cause suggested, but the experiment should have been repeated with these and other mice.

Various writers have found evidence of a regular diurnal temperature rhythm in man,<sup>19</sup> though this probably depends upon the usual daily routine of work and rest. Unfortunately, few data are available in my tables which permit of a comparison of 'forenoon' and 'afternoon' figures under otherwise identical conditions, but such as there are furnish no evidence of a generally higher afternoon temperature. Indeed, there would be little point in comparing the 'A.M.' and 'P.M.' figures of my tables, even if this were permissible, since the observations were made, for most part, late in the morning and early in the afternoon, in some cases extending through the noon hour. In only two sets of tests (tables 9 and 11) were any of the determinations made in the evening. On both of these occasions, abnormally high temperatures were met with. In table 9, no other explanation can be suggested than a tendency for the temperature to rise in the evening, since it had fallen after the third and fourth tests for the day. In table 11, however, this tendency (?) is complicated by another factor, namely, the previous transfer of these mice to a much warmer atmosphere. In any case, these evening figures will not be used in the general comparisons of warm-room and cold-room mice.

It has been stated by some writers that the sexes differ somewhat in their mean body temperatures. In tables 7 and 10 we

<sup>18</sup> Two of these readings are characterized as unreliable, but they probably err in being too high rather than too low. Furthermore, the room temperatures are considerably higher in the case of the table 7 mice.

<sup>19</sup> See Pembrey, in Schäfer's *Physiology*, pp. 798-803.



find data for a comparison of male and female mice, under closely similar conditions. For statistical purposes, I have divided these into groups such that individuals of one sex may be compared with individuals of the other sex whose temperatures were taken at the same time and in the same room.<sup>20</sup>

We have thus in the warm room three groups of figures (those of January 14, 16 and 19), within which comparisons are possible between the sexes. For each of these groups, the mean of the male temperatures has been found, and the difference obtained between this and the mean of the female temperatures. These differences, for the three groups, are  $1.95^{\circ}$ ,  $1.60^{\circ}$  and  $1.38^{\circ}$ , respectively. In each case the figure for the females is the higher.

Among the cold-room lot, we have four groups, for which the differences between the male and female averages are  $0.95^{\circ}$ ,  $0.95^{\circ}$ ,  $0.02^{\circ}$  and  $0.80^{\circ}$ , respectively. For each of these except the first, the female figure is the larger.

If we compute the averages of these various group differences,<sup>21</sup> we find that the mean temperature of the females is higher by  $0.76^{\circ}$  than that of the males.

It is further stated that the temperature of boys and girls is higher than that of adult men and women;<sup>22</sup> and for mice Congdon gives the mean temperature of males "approaching puberty"<sup>23</sup> as  $1.7^{\circ}$  higher (at  $16^{\circ}\text{C}.$ ) than that of old mice. My own experiments are in general harmony with those of Congdon on this point, though my "nearly adult" males were evidently somewhat older than his. Comparisons of old and young individuals are possible in tables 8, 11 and 12, where we have about 38 available figures<sup>24</sup> derived from 24 mice, or 12 of each age. The mean difference, computed as for males and females, is  $0.66^{\circ}$  in favor of the younger mice.

<sup>20</sup> Only the first figures for the day are included in these computations. The 'maximum' readings are here employed, those being rejected which are regarded as unreliable (starred). Altogether, we have 17 figures representing 8 males, and 19 figures, representing 8 females.

<sup>21</sup> I have 'weighted' the various figures according to the method described below.

<sup>22</sup> Pembrey, in Schäfer's *Physiology*, p. 805.

<sup>23</sup> Dr. Congdon informs me that these mice were two to three months old.

<sup>24</sup> 'Thirty-second' figures only being used.

3. *Effects of considerable changes in the air temperature upon both warm-room and cold-room mice*

Before endeavoring to compare the mean body temperatures of the warm-room and cold-room mice, under conditions which were approximately normal for the two lots, it may be well to consider some results illustrating the effects upon these animals of sudden and considerable changes in the temperature of the air.

In table 8, we observe the effects of changing 10 male mice (5 young and 5 old) to a much colder atmosphere than that to which they were accustomed. Through causes beyond my control, they had already experienced a large and fairly rapid fall of temperature during the preceding night. But at the time of the first test, the room had been tolerably warm for an hour or more, though considerably below its average temperature. After the first test, the mice were transferred to the cold room, then at a temperature of about  $-2.5^{\circ}\text{C}$ . The temperatures of the mice were taken about five hours later (air at  $0^{\circ}$ ), and again on the following morning, all but two of the animals having been kept for twenty-four hours in the cold room. By the time of the last test, the atmosphere of this room had risen to  $6.5^{\circ}$ .

Considering first the figures for the adult mice,<sup>25</sup> we have at the outset a mean temperature of  $36.30^{\circ}$ , but it must be noted that the variability is very great. Twenty-four hours later, the average for these five was  $35.82^{\circ}$ , all but one of the animals having undergone a reduction of temperature during this interval. The individual changes during this period, as revealed by the second and third tests, are highly variable.

Passing to the five nearly adult males (three and one-half months old), it is to be recorded that in two cases subnormal temperatures resulted, one mouse reaching the surprising temperature of about  $12.5^{\circ}$ . These two individuals evidently lost, for the time being, their power of heat regulation, and will therefore be left out of consideration in the present computations. Considering the other three, we find a mean temperature, at the

<sup>25</sup> 'Thirty-second' figures.

first reading, of  $37.14^{\circ}$ , the single figures ranging from  $35.3^{\circ}$  to  $38.2^{\circ}$ .<sup>26</sup> Five hours later, the temperature of two of these mice had fallen considerably, that of the third showing no change. The mean fall for the three was  $1.90^{\circ}$ . There next followed a considerable rise on the part of all three of these mice, the mean increase, at the time of third test being  $1.6^{\circ}$  (fig. 2).

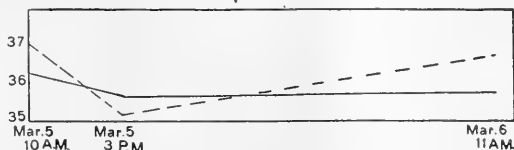


Fig. 2 Reduction of body temperature in 5 adult and 3 nearly grown male mice (table 8), resulting from a sudden reduction of  $13^{\circ}$  to  $14^{\circ}$  in atmospheric temperature, following the first test. The continuous line represents adults; the dotted line represents the younger lot.

Of these 8 male mice, accordingly, 6 underwent a net reduction in temperature during the twenty-four hours following the first test, while the mean temperature for the entire lot was  $0.42^{\circ}$  lower in the last test than in the first. It is also of interest that in the majority of individuals the temperature reduction was confined to the interval between the first and second measurements (five hours), while the majority manifested an actual rise between the second and third measurements.<sup>27</sup> Indeed this rise was very pronounced in all three of the young mice. These last, in comparison with the older animals, were at first much more seriously affected by the fall of temperature, two of them, indeed, passing far beyond the limits of physiological adaptability. On the other hand, the three which endured the test recovered their normal temperature more nearly in the end. With data so few and variability so high, it would, however, be rash to generalize in this direction.

Further data regarding the effects of a sudden cooling of the atmosphere upon mice of the warm-room group are to be found

<sup>26</sup> The mean for all five mice was  $36.78^{\circ}$ .

<sup>27</sup> It is true that a rise of  $4^{\circ}$  to  $6^{\circ}$  in air temperature occurred during this interval.

in table 7. The tests of January 16, were made during a 'cold wave', the temperature of the warm room falling  $19^{\circ}\text{C}$ . in the course of fifteen hours. Data from 2 males and 2 females are available. The former show an average temperature (maximum figures) of  $34.75^{\circ}$ , on the cold morning, the latter an average of  $36.35^{\circ}$ . The mean figures for the same mice, on two other days when the atmosphere was normal, are  $35.50^{\circ}$  for the males, and  $37.37^{\circ}$  for the females. The extent of the fall in body temperature in this case appears to have been about  $0.75^{\circ}$  for the former and  $1.0^{\circ}$  for the latter.<sup>28</sup>

The converse question, that of the effect of a very considerable rise in temperature upon cold-room animals, was tested in one experiment (table 11), in which the ten mice in question were subjected to an abrupt change of about  $23^{\circ}$ . The significance of the considerable rise in body temperature shown by these mice five hours after the transfer is confused by the fact, already recorded, that evening temperatures as a rule appear to be decidedly higher than those manifested earlier in the day. Both sets of animals (the younger and the older males) show a decline during the night; the older still retaining a considerably higher temperature than at the commencement of the experiment (higher also, by about  $0.7^{\circ}$ , than that found for the warm-room males in general), while the younger ones give about the same mean figure as at the outset (slightly lower, in fact). It is perhaps worth recalling here that the younger mice in table 8 likewise returned more nearly to their normal temperature than did the older ones.

Another opportunity for comparing the effects of widely differing temperatures upon the same mice is afforded by table 10. The tests of January 16, 17 and 18 were all made when the air temperature was several degrees below  $0^{\circ}$ . The tests of January 26, on the contrary, were made when the air temperature was  $11^{\circ}$  above zero, although there was no sudden rise to this point. If we average the figures for the first three dates, on the one hand,

<sup>28</sup> It is true that these cold day tests were made in the morning, while the only other available figures for these same mice were obtained early in the afternoon. But comparison with morning figures for other mice does not justify the belief that this circumstance has anything to do with the results stated.

and on the other those for the later date,<sup>29</sup> we find that for the four males the mean figure for January 26 is  $0.54^{\circ}$  lower than that for the colder days. For the females, on the contrary, the former figure is  $0.68^{\circ}$  higher than the latter. As in some other cases, our evidence is conflicting. The two figures, if averaged, would very nearly neutralize one another.

The writer is painfully aware of the insufficiency of the data included in this section. The figures are few and the variability high. Moreover, it has not always been possible to distinguish among the causes which may have been responsible for a given change. About all that we are justified in concluding is that the mice of the warm-room set underwent an appreciable reduction in body temperature when subjected to a greatly lowered atmospheric temperature, and that there was probably a tendency for the body temperature to resume its normal level later, even while the atmosphere remained cold. As regards the effect of a considerable rise in the atmospheric temperature, our evidence is conflicting. Among the table 10 individuals, the results from the two sexes were not in harmony, while among the table 11 mice, the old and the young animals gave discrepant results. At best, the data cited above merely serve to strengthen the belief, to which we shall be led later, that the effects of even large differences of atmospheric temperature are relatively slight and inconstant.

*4. Comparison of the temperatures of the two lots of mice, under conditions which were as normal as possible for each*

It is evident that we can fairly compare only male mice with males, and females with females, while among the males, we must separate the older ones from the younger. It was my first intention to further restrict the comparisons so that morning figures should be compared only with morning figures, and afternoon ones with afternoon ones. This intention was abandoned, however, since such a minute subdivision of the comparable groups seemed undesirable. Moreover, as already stated, the greater

<sup>29</sup> Employing only the first figures for the day (thirty-second readings).

part of the temperature tests here considered were made either late in the morning or early in the afternoon, some extending through the noon hour. A classification of such tests into 'morning' and 'afternoon' ones would be arbitrary. Finally, the mean time of observation did not differ widely for the two contrasted lots, being 11.30 A.M. for the warm-room experiments and 1.40 P.M. for the cold-room ones. That this difference of two hours could materially affect the trend of my results would hardly be contended.

In making the comparisons now to be considered, I have included only the first temperature reading for a given day. I have excluded such of the tests with warm-room mice (table 7, January 16; table 8, March 6) as were made at times of abnormally low atmospheric temperature<sup>30</sup> and such of the tests with cold-room mice (table 10, January 26; table 11, March 1) as were made at times of abnormally high temperature. I have also excluded certain other tests (table 9, March 31) which were made under conditions of abnormal excitement on the part of the mice.

It so happens that nearly all of the cold-room determinations were made on days when the temperature was some degrees below zero, since it was my object at that time to determine the greatest possible changes in body temperature which might be attributable to atmospheric conditions. Thus, the mean air temperature, at the time of the cold-room tests, was about  $-3^{\circ}\text{C}.$ , while the mean temperature to which the animals had been subjected for the preceding ten days was  $+7^{\circ}$ , in the case of the table 10 mice, and  $+1^{\circ}$ , in the case of those of tables 11 and 12.<sup>31</sup>

<sup>30</sup> In reality, the March 5 tests of table 8 were made at a time when the room temperature was considerably below the average. This, for the preceding ten days had been  $22.6^{\circ}$ , i.e.  $8^{\circ}$  or  $9^{\circ}$  above the temperature when the March 5 readings were made. To exclude these results, however, would be to seriously reduce the number of available figures, and their inclusion is further justified by the fact that the mean of those for the five adult males is very nearly the same as (in reality a little *higher* than) the mean figure for the 9 other adult males of the warm-room set. And it must be borne in mind that all of the cold-room tests were made at times of unusually low temperature.

<sup>31</sup> The mean temperature of the cold room, during the entire period of my experiments, was about  $+6^{\circ}\text{C}.$

A strictly fair comparison would include only tests which were made under average or 'normal' conditions for each lot. It is plain, however, that if any error has resulted from the course pursued by me, it must be in the nature of an exaggeration of the temperature differences between the warm-room and cold-room animals, rather than the reverse.

Considering first the adult male mice, the results of my experiments may be tabulated as follows:

TABLE 3  
*Adult males*

	MEAN AIR TEMP.	TESTS INCLUDED	TIME OF DAY	NUMBER OF MICE	30-SECOND FIGURES		MAXIMUM FIGURES (more reliable)		MAXIMUM FIGURES (all)	
					Number available	Mean	Number available	Mean	Number available	Mean
	<i>deg. C.</i>					<i>deg. C.</i>		<i>deg. C.</i>		<i>deg. C.</i>
Warm	21.2	Table 7 Jan. 14, 19 Table 8 Mar. 5 Table 9 Apr. 1 Table 10 Jan. 16, 17, 18	8.55 A.M. to 2.48 P.M.  10.35 A.M. to 3.25 P.M.	14	13	36.24	10	36.15	16	36.39
Cold	-3.3	Table 11 Feb. 28 Table 12 Feb. 28		14	15	36.19	16	36.10	20	36.15

If we consider the thirty-second figures alone—these I regard as the more reliable—the mean difference between the two contrasted lots of individuals is 0.05° in favor of the warm-room animals. In view of the small number of individuals and the high variability, such a difference is certainly negligible. The standard deviation of the warm-room average is 0.81°, that of the cold-room average being 0.75°. (The probable errors of these averages are 0.15° and 0.13° respectively)

Considering such of the 'maximum' figures as have not been designated in the tables as unreliable, we likewise find a mean

difference of only  $0.05^{\circ}$  in favor of the warm-room lot. If we include these less reliable readings, the difference becomes  $0.24^{\circ}$ , in favor of the warm-room lot.

TABLE 4  
*Nearly adult males ( $3\frac{1}{2}$  months)*

	MEAN AIR TEMP.	TESTS INCLUDED	TIME OF DAY	NUMBER OF MICE	30-SECOND FIGURES		MAXIMUM FIGURES (more reliable)		MAXIMUM FIGURES (all)	
					Number available	Mean	Number available	Mean	Number available	Mean
	<i>deg. C.</i>					<i>deg. C.</i>		<i>deg. C.</i>		<i>deg. C.</i>
Warm	15.0	Table 8 Mar. 5	10.30 to 10.50 A.M.	5	5	36.78	3	36.93	5	37.12
Cold	-2.5	Table 11 Feb. 28 Table 12 Feb. 28	12.25 to 3.47 P.M.	10	6	36.95	7	36.96	10	37.04

Passing to the younger males, we find a close agreement between the warm-room and cold-room figures, whichever pair of averages is chosen. The difference of  $0.17^{\circ}$  between the thirty-second figures is surely not significant, in view of the small number and the variability of the readings. These last range from  $35.3^{\circ}$  to  $38.2^{\circ}$  for the warm-room lot, and from  $36.2^{\circ}$  to  $37.5^{\circ}$  for the cold-room lot. Indeed, this series, by itself, would have no significance for our present purposes.

TABLE 5  
*Females*

	MEAN AIR TEMP.	TESTS INCLUDED	TIME OF DAY	NUMBER OF MICE	30-SECOND FIGURES		MAXIMUM FIGURES (more reliable)		MAXIMUM FIGURES (all)	
					Number available	Mean	Number available	Mean	Number available	Mean
	<i>deg. C.</i>					<i>deg. C.</i>		<i>deg. C.</i>		<i>deg. C.</i>
Warm	28.3	Table 7 Jan. 14, 19	1.30 to 2.53 P.M.	4	6	37.28	6	37.32	6	37.32
Cold	-4.0	Table 10 Jan. 16, 17, 18	10.30 A.M. to 3.08 P.M.	4	9	36.52	9	36.37	10	36.44



The females alone show us a considerably lower mean figure for the cold-room lot, the difference being  $0.76^{\circ}$ ,  $0.95^{\circ}$ , or  $0.88^{\circ}$ , according to which pair of averages is used for the comparison. Moreover, the variability is here comparatively small, the standard deviations being only  $0.28^{\circ}$  for the warm-room lot and  $0.5^{\circ}$  for the cold-room lot. It may be pointed out that the mean difference in the temperature of the two rooms, during the tests in which these females were used was about  $32^{\circ}\text{C}.$ , or  $8^{\circ}$  greater than that during the tests with the adult males. It is possible, however, that female mice are more readily affected by differences of atmospheric temperature than are males. Some slight evidence of this has already appeared. But the data at our disposal are far too limited to decide the question.

It is obviously not allowable to throw together all of the readings from the warm-room individuals (older and younger males, and females), and to compare the average thus derived with a similar figure derived from all of the cold-room determinations. We may, however, obtain a value which will represent, in a rough way, the mean difference between the two lots as a whole. For the separate groups of individuals, we have found the following differences, based upon the 'thirty-second' figures:

*Differences (warm—cold).*

Adult males.....	+0.05°
Younger males (three and one-half months).....	-0.17°
Females.....	+0.76°

To give these last figures equal value in finding their average, would not be allowable, since the first depends upon results derived from 13 warm-room and 15 cold-room tests, the second upon results from 5+6 tests, and the third from 6+9 tests. We may, however, 'weight' these figures in accordance with the number of tests which they represent, and then proceed to obtain the average.<sup>32</sup> The result is a difference of  $0.16^{\circ}$ , in favor of the warm-room set. This, it will be noted, is based upon 24 warm-room tests and 30 cold-room tests. The mean air temperature

<sup>32</sup> That is, multiply the first by  $13 \times 15 (= 195)$ , the second by  $5 \times 6 (= 30)$ , and the third by  $6 \times 9 (= 54)$ . The sum of these products is divided by 279.

of the former room, at the time of these tests, was  $21.7^{\circ}\text{C}$ ., that of the cold-room being  $-3.3^{\circ}$ . The difference of atmospheric temperature to which these mice were subjected was thus 25.

If we base our computations upon the 'maximum' figures (rejecting those designated as unreliable), our difference is  $0.25^{\circ}$ , while if we employ all of the 'maximum' readings, including the less reliable ones, the mean difference becomes  $0.31^{\circ}$ .

It thus appears that if any reduction of temperature occurred among these mice, it was a slight one, probably not exceeding a fourth or a third of a degree. It may be, however, that a more extended series of observations, under more satisfactory experimental conditions, would have revealed a greater mean difference than is here shown. It is quite possible, also, that the difference in reaction between the sexes, implied by the greater change shown among the females of my experiments, would be found to be constant. In that case, we could not fairly combine all of the figures into a single average.

Considering these experiments in relation to the main purpose of the investigation—that of determining the effect of the actual temperature conditions of my two rooms upon the body temperatures of the respective sets of mice—it will be seen that the course adopted was calculated to reveal the maximum effect of these conditions, rather than the average effect for the entire period. In view of the considerably smaller differences of atmospheric temperature which obtained for the period as a whole, it may well be doubted whether any appreciable differences of mean body temperature would have been manifested at most times.

##### *5. Discussion*

As stated earlier (p. 316), Finkler found a difference of several tenths of a degree between the temperature of guinea-pigs in an atmosphere of  $25^{\circ}$  to  $27^{\circ}$ , on the one hand and in an atmosphere of  $0^{\circ}$  to  $5^{\circ}$ , on the other. It is not stated how many individuals were used. The exposure lasted several hours.

The results of certain other investigators are mentioned in an earlier section of this paper. So far as I am aware, Congdon has conducted the most extended previous series of investigations

upon rodents, with a view to determining the influence of air temperature upon body temperature. Unfortunately, this writer has published only his bare averages, giving no clue to the variability of his readings,<sup>33</sup> and has furnished but a brief statement of the methods employed by him. On the other hand, the number of his temperature readings, belonging to each homogeneous group, was evidently much greater than in my own experiments.

Congdon's summary of his results has already been quoted (pp. 317-318). The striking differences between his results and my own are evident: (1) a much greater difference in body temperature, resulting from differences in atmospheric temperature, and (2) a much lower mean body temperature for the mice in general. I shall consider these two points in order.

Congdon states (p. 707), on the basis of experiments upon ten adult mice, that transfer from the warm room in which they had been reared (16°C.) to a temperature of 5° resulted in a mean fall of 3° in rectal temperature; while, in another lot of ten mice, transfer from 16° to 33°, in two steps, led to a total rise of 1.5° in body temperature. These mice, after the change, were each tested on five different days, extending through a total period of nineteen days, and the effect stated was found to persist throughout this period. No figures are given for adult mice which were reared (and tested) in the warm and cold rooms respectively, but such figures are given for adult rats (p. 705). Ten of these animals, kept at a temperature of 33°C., showed a mean temperature 1° higher than ten which were kept at 16°.

In my own experiments, no temperature reduction as great as 3° was found in any single case, even with changes of air temperature much greater than those referred to by Congdon.<sup>34</sup> In table 7, for example, two adult males and two females were subjected to a fall in atmospheric temperature of 19° in the course of 15 hours. The mean reduction in body temperature was 0.75°

<sup>33</sup> Dr. Congdon has recently been so kind as to show me some of his original notes.

<sup>34</sup> Exception being made of the two mice which lost their power of temperature regulation.

for the males and  $1.0^{\circ}$  for the females.<sup>35</sup> In table 8, five adult males exhibited a mean reduction of temperature of  $0.58^{\circ}$ , five hours after a change from  $14^{\circ}$  to about  $0^{\circ}$ . Indeed only three of the five showed any reduction at all in this interval.<sup>36</sup> In table 10, two males and two females, following a fall of 19 degrees in twenty-four hours, showed body temperatures averaging only  $1.4^{\circ}$  lower than they were ten days later, when the air temperature was  $18^{\circ}$  higher. (Here, again, one of the males showed no reduction whatever.) These cases, taken singly, are not fully convincing, in view of the variability of the figures, but their cumulative weight is considerable.

It is, however, the question of permanent differences between the two respective sets of mice which especially concern us here. The evidence regarding these has been summarized so recently that I need not repeat it. No difference of any significance, statistically considered, was demonstrated for the males (either young or old); while the females showed a difference of only about  $0.75^{\circ}$ , when subjected to a mean difference in air temperature of  $32^{\circ}$ . Congdon's rats showed a mean difference of  $1^{\circ}$ , as a result of living in temperatures differing only by  $17^{\circ}$

Congdon gives as the result of averaging "the temperatures of animals at puberty and of older animals, living at medium temperatures," the mean figure of  $34.9^{\circ}$  for the white mouse. More surprising still, the mice which were transferred by Congdon to a temperature of  $5^{\circ}$  showed a mean body temperature of  $31.2^{\circ}$ , while in no case did I observe a temperature as low as this by several degrees.<sup>37</sup>

While doubtless my warm-room temperatures were more nearly 'medium' than the cold-room ones, I have computed the mean of the six averages for the 'thirty-second' figures, given on pp. 335-336 above,<sup>38</sup> the result being  $36.66^{\circ}$ . This figure, which is based on 54 readings, is higher than Congdon's by  $1.8^{\circ}\text{C}$ .

<sup>35</sup> Compared with the first tests for the day on two other days at the normal temperature of the room.

<sup>36</sup> No mention is here made of the young males, since Congdon himself found much smaller changes among these.

<sup>37</sup> Except for the extreme cases of subnormal temperature, already mentioned.

It seems to me more than possible that these differences between Congdon's results and my own depend upon differences in the methods employed. Congdon states that "the thermometer was inserted just deep enough so that its neck was at the level of the anal sphincter muscles" (p. 705). It has been stated above that the proximal end of the bulb, in my experiments, was 5 to 7 mm. within the anal sphincter, and that the latter was further blocked by a rubber ring or collar, fixed at a definite point on the elongated 'neck' of the thermometer. Thus I obtained the temperature of a deeper lying part of the body than if the bulb had been just within the anal sphincter.

Now this difference in method would account for both of the principal differences between Congdon's results and my own, namely for the greater changes following changes of atmospheric temperature, and for the lower averages given by Congdon throughout. It would also account for the fact that the changes of body temperature, in his experiments, persisted, apparently, as long as the animals were kept at the temperatures in question.

That the depth of insertion of the thermometer has a marked influence upon the thermometer readings was shown by Finkler's experiments upon guinea-pigs, already cited. This writer found differences as great as  $36.1^{\circ}$  and  $38.9^{\circ}$  at depths of 2.5 and 9 cm., respectively. Further, the difference between the readings for animals kept at widely different temperatures was greater when the thermometer was inserted to the former depth than to the latter. In my own experiments, the dependence of temperature upon depth of insertion was observed, though not in a systematic way. Record is made of one such observation in table 11 (mouse No. 24-3). (See, also, Supplementary Note, p. 354.)

That the mean temperature given by Congdon for white mice in general is abnormally low is confirmed by the fact that Pembrey gives a figure even higher than mine. He states<sup>39</sup> that "the rectal temperature of tame mice was found to be  $37.4^{\circ}$  in 27

<sup>38</sup> Giving equal value to the warm-room and cold-room figures, and to those for the old males, young males and females, respectively.

observations upon eight normal animals and the range was 36.1 to 38.6°." Pembrey tells us neither the age nor sex of these animals, nor the atmospheric temperatures in which they were kept. His mean figure is about 0.7° higher than mine, but it is scarcely higher than my figure for the warm-room females.

It might be claimed that certain faulty conditions of my own experiments partly account for these differences between my results and Congdon's. For example, I had no constant temperature rooms at my disposal, and both lots of mice were accustomed to wide ranges of temperature. Therefore, it might be claimed their powers of temperature regulation were better developed. It is possible that this is true in some degree, but surely Congdon's mice should have rallied after 19 days exposure to changed temperature conditions.

Again Congdon states that he put all of his mice through a preliminary period of practice or habituation with the temperature-taking operations before making his final observations. This was not attempted in my own experiments, although a few preliminary records were rejected as untrustworthy. It might be claimed that the generally higher temperatures of my mice were due to excitement, resulting from their unfamiliarity with the operation. That this is not true I believe for several reasons: (1) In the thirty-second readings of my experiments, it is not likely that the effects of excitement are commonly manifest to any considerable extent. Yet these readings differ from the maximum ones by only about 0.25°. (2) There was no invariable association between body temperature and degree of struggle, although the two were often seen to be related. (3) So far as may be judged from tables 7 and 10, in which it seen that certain mice were tested on several different days, there seems to be no general tendency toward a reduction in the later figures. (4) Finally, the habituation of Congdon's own mice to the temperature tests does not appear to have greatly reduced the variability of his figures. Unfortunately, the individual thermometer readings have not been pub-

<sup>23</sup> Journal of Physiology, vol. 45, 1912, p. 83.

lished, but Dr. Congdon has kindly shown me some of his original notes, and the variability among his figures appears to be nearly or quite as great as among my own.

6. *Exceptionally low body temperatures*

The temperatures hitherto discussed have been within strictly physiological limits. The changes have, at most, amounted to  $1^{\circ}$  or  $2^{\circ}$ , and it has not been necessary to return the animals to a more normal atmosphere in order that death should be averted.

In two instances, however (27-1 and 20-1, in table 8), the mice quite lost their power of temperature control, and would doubtless have died unless restored to a much warmer environment. The body temperature fell to  $20.6^{\circ}$ , in one case, and to about  $12.5^{\circ}$  in the other, after an exposure of about five hours to an atmosphere ranging from  $-2^{\circ}$  to  $+2^{\circ}$ . The mouse which recorded the lower temperature was scarcely living at the end of this period, the only signs of life being certain reflexes and occasional gasping. Nevertheless, even this animal became fairly active after one and one-half hours in the warm room (then  $28.5^{\circ}$ ), and was seemingly well after four hours. No harmful effects were noted later.

Edwards<sup>40</sup> and some subsequent writers, notably Pembrey and White,<sup>41</sup> have shown that hibernating mammals normally reach extraordinarily low temperatures (even  $2^{\circ}$ ) during the winter. In the experiments of Hill and Macleod, already cited, a temperature as low as  $17^{\circ}$  was obtained by subjecting a mouse to moist air, which, however, was not cold, in the sense in which the word has been employed in the foregoing discussion, but had the very moderate temperature of  $18^{\circ}$ . Even human beings, exposed to the cold, during intoxication have been reduced to a temperature of  $24^{\circ}\text{C}$ ., without fatal consequences.

<sup>40</sup> Op. cit.

<sup>41</sup> Proceedings of the Physiological Society (bound with the Journal of Physiology, vol. 18, 1895), pp. xxxv-xxxvii.

## 3. OBSERVATIONS UPON VERY YOUNG MICE

It has long been known that the young of many species of mammals and birds behave, in respect to their body temperatures, like the so-called 'cold-blooded' or poikilothermous animals. W. F. Edwards,<sup>42</sup> as early as 1832, recognized that "the young of mammalia appear to be distinguished into two groups in relation to animal heat. Some are born, as it were, cold-blooded, others warm-blooded." A similar distinction was drawn among birds. Among the mammals which are born in a warm-blooded condition he mentions the guinea-pig; among those born in a cold-blooded condition he includes cats and dogs. These two groups of animals differ likewise in the degree of their structural development at the time of birth, those which are born in a 'warm-blooded' condition being much farther advanced. Edwards cites experiments of his own in which the body temperature of kittens became reduced to 17° or 18°, and of puppies to 13° or 14°C.

The general accuracy of Edwards's observations is still recognized. Subsequent investigators have found similar wide fluctuations in the temperature of various young mammals, following changes of atmospheric temperature. For example, Pembrey<sup>43</sup> determined the temperatures of young mice at the ages of one to ten days. At the former age, the temperature of three young mice was 32° when taken from the nest, but after forty minutes at a temperature of 23°, the body temperature fell to that of the air.<sup>44</sup> In the case of a mouse ten days old, the (groin) temperature was 33° at the beginning of the experiment, 32.25° after thirty minutes at about 22°. Pembrey concludes that for mice the power of heat regulation "appears to be well developed by the tenth day after birth" (p. 375). It will be shown below that this conclusion is not warranted except for the comparatively small differences of air temperature which were employed by Pembrey

<sup>42</sup> Op. cit.

<sup>43</sup> *Journal of Physiology*, vol. 18, 1895, pp. 363-379.

<sup>44</sup> Pembrey's methods of determining body temperature were extremely crude. According to his own statement, "with such small animals as young mice and rats the temperature was only roughly measured by covering the bulb of the thermometer with the bodies of two or three young animals, or by placing it in the groin when the animals were bigger" (p. 364).



In the course of endeavoring to rear young mice in the cold room used in my experiments, it was often found that the mother threw the young out of the nest and allowed them to die. This I explained on the supposition that the mice became so cold, during the absence of the mother from the nest, that they occasioned her discomfort upon her return, and were on that account rejected. The truth of this belief was strengthened by the fact that such young mice at first felt unpleasantly cold to the hand, but they could quickly be warmed to body temperature, and were then accepted by the mother. During the earlier days of their life, the young could endure more severe and more prolonged exposure to cold than later. For example, I have recorded that four mice, only a day old, were left at a maximum temperature of  $13.5^{\circ}\text{C}$ . (probably much colder part of the time) for a period of ten to twelve hours, during which time their body temperature was undoubtedly not far above that of the air. When warmed up, these young mice were accepted by the mother and lived for four days thereafter.

In the course of the present series of experiments, exactly 100 temperature tests were made upon young mice, varying in age from less than one day to twenty-one days. The number of individuals used was only slightly less than this, since the process of taking the temperature almost invariably involved the killing of the animal.

On the whole, the results derived from this series of experiments are to be regarded as more satisfactory than those derived from the experiments with adults. This is partly for the reason that the temperature changes undergone by the young in consequence of atmospheric changes are as a rule very much more pronounced than in the case of adults; partly for the reason that the young, at least in the earlier stages, are not affected by the excitement and struggle by which the older animals are affected. Likewise the available number of comparable figures is greater for the former.

With the exception of the first four mice tested, thermometer 'number 3' (p. 320) was employed in these experiments, owing to

the fact that the graduation of this one extended to a lower point (20°C.) than that of either of the others.

In a few cases, among the older of these young mice, the rectal temperature was taken (the mice were invariably killed in the end, however); in all other cases, the following procedure was employed.

The mouse was cut into very deeply, in the throat or chest region. Such an incision necessarily severed the large blood-vessels and soon led to the death of the animal. After the cut was made, the thermometer was inserted with all speed far into the abdominal viscera. The mouse was held, in nearly all cases, by a large piece of cotton-batting, and care was taken not to touch or bring the hand very near the body. The cotton was left loose, however, so as not to wrap up the young animal.

The mercury always rose abruptly to a point not far from the first stopping-place, then more slowly for a few tenths of a degree, when it became nearly or quite stationary. In some cases, it now remained almost perfectly motionless for a minute or more, in others, it began to rise again, sometimes reaching a point 1° or 2° higher. In others still, the final decline commenced much more quickly.

The results of my temperature determinations upon young mice are recorded in tables 13 to 20 where the figures given are the maximum reading for the thermometer and the first temperature recorded, the latter being that attained at the end of the first sudden rise of the mercury, following insertion. It was probably reached within 10 or 15 seconds.

That temperature readings obtained in the way described are a fair index of the body temperatures of the living animals is rendered very probable by a number of cases in which the rectal temperature was taken immediately before killing. The two were found to agree within close limits (tables 13 and 16).

In the course of my observations, I frequently recorded the temperature changes for several minutes, at half-minute intervals. These later readings, of course, throw no light upon the normal temperature of the body, but are of interest in showing temperature conditions after somatic death.

The young mice which were employed in these experiments belonged both to broods born and kept in the warm room and to broods born and kept in the cold room. For the purposes of the experiments the warm-room individuals were either (1) taken directly from the nest, or (2) completely exposed for varying periods in the warm room, or (3) exposed for varying periods in the cold room. The cold-room individuals were either (1) taken directly from the nest, the mother being present, or (2) taken directly from the nest, the mother being absent, or (3) fully exposed for varying periods in the cold atmosphere.

From tables 13 to 20 the synopsis on the following page has been prepared. Each figure in the latter is the mean of those for all individuals of the same age which were subjected to approximately the same conditions. Only the 'first temperature recorded' has been used for this purpose. In the synopsis, I have thought it expedient to throw together the figures from tables 14 and 15, also those from tables 16 and 17, and those from tables 19 and 20, since the differences between the groups thus combined were not great enough to warrant their separation.

From these figures curves have been constructed (fig. 3), showing the correlation between age and ability to regulate body temperature under different conditions of external temperature. These curves show, in a striking way, the development of homothermy in the lifetime of the individual mouse, culminating in a practically perfect power of temperature regulation (within the limits of the experiments) at about the age of three weeks. From the tenth day onward, mice exposed to the temperature of the warm room of my experiments (usually between  $20^{\circ}$  and  $25^{\circ}$ ) seem to have maintained the same temperature as those taken directly from the nest. This accords well with Pembrey's experiments, in which the young animals were subjected to an atmospheric temperature about  $10^{\circ}$  lower than that of the body. But it is plain that for greater differences, the power of heat regulation is not developed until long after the 10th day. Even at the age of eighteen days, the mean temperature of 3 mice, exposed to an atmospheric temperature of about  $7^{\circ}$ , was  $23.3^{\circ}$ , or  $13^{\circ}$  lower than that of mice of the same age kept in the warm room. The temperatures of

TABLE 6  
*Mean temperatures of young mice (one to twenty-one days old) under various conditions.*

	AGE IN DAYS																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
MEAN AIR TEMP.																					
<i>deg. C.</i>																					
1. Warm-room lot directly from nest....	23.5	32.4	32.6																		
2. Warm-room lot after exposure in warm room.....	25.1	31.0					35.0	36.6	34.6	34.6	34.7	36.0	35.6	35.7				36.4	37.2		37.0
3. Warm-room lot after exposure in cold room.....							30.8	29.4	30.3	33.5	35.9	35.9		36.5		37.5					
4. Cold-room lot directly from mother....							24.4	19.9	25.7	24.2	23.9	27.6		30.7		36.2		35.3			35.5
5. Cold-room lot after exposure in cold room, including those from nest, without mother...					34.0						31.4		35.6		35.0			32.1		35.3	
					21.2					20.6	25.2	24.7	33.2					23.3		37.0	37.3

\* No distinction made after 14 days.

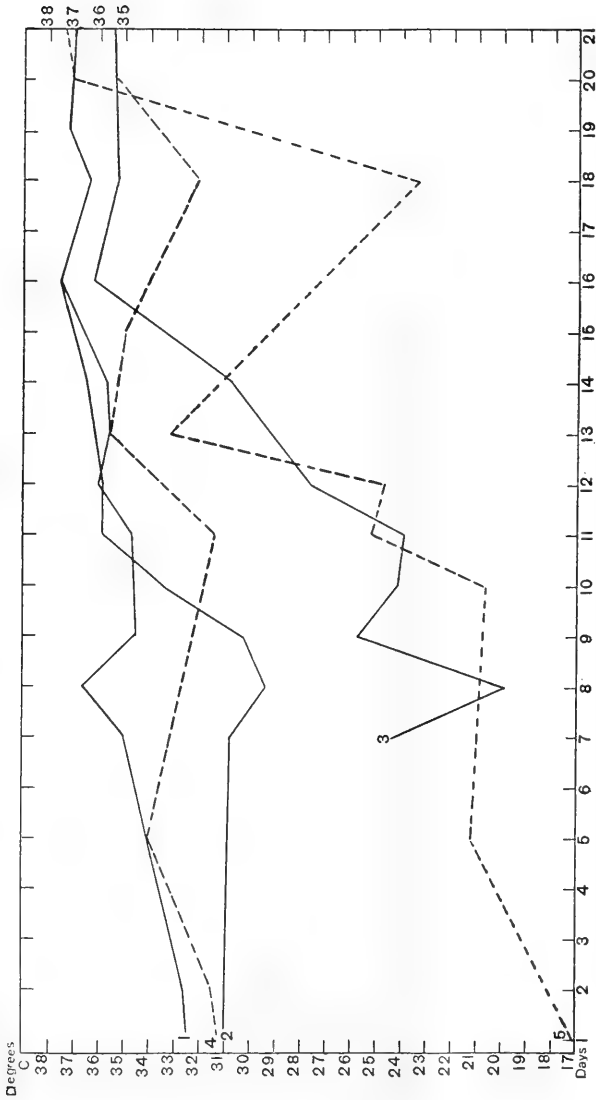


Fig. 3 Curves showing relation of body temperature to air temperature, based upon 100 tests of young mice, from one to twenty-one days old. The continuous lines represent young born in the warm-room; the dotted lines represent young born in the cold-room. The numbers (1 to 5) refer to the groups comprised in the table on p. 348, which must be consulted as an explanation of these curves.

these three mice seen, it is true, abnormally low, as will be realized by inspecting the curve for this group as a whole, or by comparing it with the other curves of the same series.

The warm-room mice which were transferred to the cold room seem to have nearly or quite attained their power of heat regulation at the age of sixteen days, though a small, constant difference is thereafter manifested between this group and those tested in the warm room.

The very great variability shown even by mice of exactly the same age is due in part to the size of the animals. Within each experimental group, I have compared, for each age, the smallest and the largest individual, providing that the difference in weight is as much as 0.5 gram or more. I find a mean temperature difference of over 2° in favor of the heavier mice. But considerable differences are met with, even among mice of the same age and size.

Certain phenomena which were manifested during the death of the young animals are of interest. In the warm room, the maximum was not commonly reached until several minutes after the fatal incision was made. In some cases, indeed, this maximum temperature was manifested some time after the animal ceased to move. Here is an example of a complete record for a 9-day mouse:

Mercury rose at once to.....	26.5°
After one-half minute.....	26.9°
After one minute.....	27.2°
After two minutes.....	28.2°
After three minutes.....	28.6°
After four minutes.....	28.8°
After five minutes.....	28.6°
After nine minutes.....	28.0°

It is recorded that this mouse ceased to move before the end of two minutes, except for one convulsive gasp, which was noticed at the end of five minutes.

In the cold room it likewise frequently happened that a rise of temperature followed incision, so that the maximum was not

reached until two or three minutes after the insertion of the thermometer. This rise was less marked, however, than in the warm room.

In general, the fall in temperature, following the fatal incision, was perhaps more rapid in a mouse two or three weeks old than in one five to ten days old, but there was no constancy in this regard.

#### SUMMARY

For adult and nearly adult mice the following conclusions may be offered:

1. The most striking feature of a set of temperature records of these animals is the great variability among the figures. Different animals, under seemingly identical conditions, differed as much as  $1^{\circ}$  or  $2^{\circ}\text{C}$ . in their body temperatures, while the same individual, in the course of a few minutes, might exhibit differences nearly or quite as great.

2. One of the chief causes of such temperature differences is the degree of excitement or agitation of the animal. To this must be added, perhaps, local congestion, due to the insertion of the thermometer into the rectum. Thus, a comparison of the first and second temperature readings for the day, when the interval between the two was less than an hour, showed a mean rise of about  $1.5^{\circ}$ , in the case of the adult males.

3. The mean temperature of 8 female mice was found to be  $0.76^{\circ}$  higher than that of 8 male mice, in a series of tests in which animals of opposite sex were compared with one another under identical conditions.

4. Nearly adult males, three and one-half months of age, were found to have a temperature of  $0.66^{\circ}$  higher than fully mature males (eight to twelve months old), the conditions being identical for the contrasted sets. This conclusion is based upon a consideration of 38 tests in which 24 mice were used.

5. When we have eliminated these various irrelevant factors, and selected from each group of figures only those which are strictly comparable, we are in position to test the effects of atmospheric upon body temperature in the case of animals which have become habituated to widely different temperature conditions. From a consideration of 36 tests, upon 28 adult males, we find a mean difference of at most  $0.25^{\circ}$  (considerably less, if certain doubtful readings be rejected) between the warm-room and cold-room lots, the figure for the former being the higher. The mean difference of atmospheric temperature during the period of these tests, was  $24.5^{\circ}\text{C}$ .

For the much smaller number of tests with males three and one-half months old, a mean difference was obtained which is statistically negligible. When the comparisons are restricted to the more reliable figures, indeed, the mean is slightly higher for the cold-room animals.

Adult females of the warm-room lot gave a mean figure which was  $0.76^{\circ}$  to  $0.95^{\circ}$  higher than that for the cold-room lot (depending upon which readings were employed in the computations. See text). These computations are derived from a consideration of 16 temperature tests, made upon 8 individuals. The mean difference of atmospheric temperature to which the two lots were subjected during these tests was  $32^{\circ}\text{C}$ .

If we seek a single average difference, representing the mean condition of the three groups just considered (for method of computation, see p. 337), we find that this lies between  $0.16^{\circ}$  and  $0.31^{\circ}$ , depending upon which readings are chosen for comparison. The mean difference in air temperature during these thermometer tests was  $25^{\circ}\text{C}$ ., which was very much greater than the mean difference between the temperature of two respective rooms for the experimental period as a whole. My methods have thus tended to exaggerate, rather than to minimize, any differences of body temperature which may have obtained throughout the course of my breeding experiments.

6. Some fairly convincing evidence was obtained pointing to a reduction of body temperature in individuals of either the warm-room or the cold-room lot, resulting from a very considerable fall



in air temperature. Less certain (owing to scarcity of data) is the conclusion that, after such a reduction, the mice tended to recover their normal temperature, even while the external cold persisted.

7. The mean figure obtained by averaging the warm-room and cold-room averages, for both males and females, is  $36.66^{\circ}\text{C}$ . This is based upon 54 readings, comprizing only those which are not believed to have been affected by abnormal conditions.

8. Two mice in these experiments lost their power of heat regulation, and displayed subnormal temperatures, the body temperature of one falling to about  $12.5^{\circ}\text{C}$ .

For very young mice (from less than one day to twenty-one days), the following conclusions may be offered, based upon 100 tests:

1. Very young mice, when exposed to a cold atmosphere, soon assume body temperatures very much lower than those which are normal for adult animals. In no case, however, did the body temperature, in my own experiments, fall nearly to that of the air, the lowest figure recorded being about  $15.5^{\circ}\text{C}$ ., which resulted from the exposure of a twelve-day mouse for thirty minutes to an atmospheric temperature of  $6^{\circ}$ . Since the period of exposure, in these experiments, never exceeded one and one-half hours, it is not at all likely that my figures represent the limit to which the body temperature of young mice may fall without fatal results.

2. The power of heat regulation, for moderate air temperatures ( $20^{\circ}$  to  $25^{\circ}$ ), is pretty well established at about the age of ten days. For lower temperatures, however, the power of regulation is not developed until much later, apparently not until the age of twenty days, under the conditions of my own experiments. Three mice, eighteen days old, exposed for thirty to sixty minutes to an atmosphere of about  $7^{\circ}\text{C}$ ., reached a mean body temperature of  $23.3^{\circ}$ , or  $13^{\circ}$  lower than that of mice of the same age kept in the warm room.

3. Young mice of the same age differed greatly in their power of heat regulation. The extent of this power depended partly upon the weight of the young animals, heavier individuals showing a higher degree of regulation.

Berkeley, California  
May 14, 1913/

#### SUPPLEMENTARY NOTE

Opportunity has very recently been afforded me to repeat certain of the experiments described in the preceding pages, and the results seem important enough to make it desirable for me to include them in the present paper. These supplementary experiments were conducted at the University of California, between August 31 and September 16, 1913. For the loan of some of his mice, as well as for the privilege of using his animal room, I am indebted to Dr. J. A. Long, of the Zoölogy Department of the University. My thanks are also due to Dr. J. F. Mitchell, of the Veterinary Department, for the privilege of keeping the mice for a number of days in the cold-storage room of the hog-serum laboratory.

Five male white mice, averaging 24.5 grams in weight, and five females, averaging 21.4 grams were used. These mice were probably all between five and ten months old.

The temperatures of the animals were first taken throughout a period of seven days, in the attic room in which they had been reared. For at least a month prior to my experiments, the temperature of this room had ranged between 20 and 26°C.

The methods employed were the same as those described for my earlier experiments. The figures here given are in all cases 30-second ones. The tests were made, for the most part, in the forenoon. Readings have been rejected which were affected by the struggles of the animal or by any other known disturbing influence.

In the attic room (mean air temperature, 21.5°; relative humidity probably 40 to 60 per cent, most of the time), 22 tests of five males, on six different days, gave a mean body temperature of

36.59°C. Twenty-four readings of five females, on six different days, gave a mean of 37.44°C.

In order to determine the effect of varying the depth of insertion of the thermometer, the rubber collar (ordinarily 17 mm. from the tip of the bulb) was moved so as to lie 10 mm. from the tip. Four readings of four males gave a mean figure of 35.28°, while four readings of four females gave 35.58°. These temperatures thus averaged 1.58° lower than the deeper ones.

The mice, after an interval of two days in a basement room of intermediate temperature, were moved to the cold-storage room. Here the temperature ranged from 11.5° to 14.5°, the mean being close to 13°. The relative humidity was about 70 per cent on the only occasion when it was tested. The mice were kept in this room for a period of six days.

Fourteen readings of four males, on five different days, gave a mean temperature of 36.54°. Twenty-one readings of five females during the same period, gave a mean of 36.82°.

When the rubber ring was shifted to a point 10 mm. from the tip, three readings of three males gave a mean of 33.71°, while five readings of five females gave 35.18°. These temperatures thus averaged 2.24° lower than the deeper ones.

Shortly after the return of the animals to the attic room, a period of very warm weather ensued. On September 15, at an air temperature of 31°, two readings of two males gave a mean of 36.83°, while four readings of four females gave a mean of 37.50°.

On September 16, in an air temperature of 35° (humidity, 32 per cent), two readings of two males gave a mean of 38.18°, while five readings of five females gave a mean of 38.50°.

These newer experiments thus furnish ample confirmation of some of the most important results presented in the body of this paper. The temperatures of the females are 0.48° to 0.85° higher than those of the males. A reduction 8.5°C. in air temperature, accompanied by increased humidity, resulted in a reduction in body temperature which was insignificant for the males, but which amounted to about 0.4° for the females. The extremely high temperatures to which the mice were subjected

on the last two days of the experiments seem to have resulted in a marked increase in body temperature, though the small number of figures and certain other circumstances render this interpretation somewhat doubtful. It must be noted, moreover, that the mean increase which followed the change from  $13^{\circ}$  to  $31^{\circ}$  was only about  $0.4^{\circ}$  for the two sexes. On the next day, the limits of normal adjustment were perhaps overstepped. Indeed, several rats which were kept in the same room died, apparently as a result of the heat.

Berkeley, California  
September 16, 1913

TABLE 7

Four male and four female adult mice (eight to ten months old); which had been kept during the preceding two months in the warm room (mean temperature to time of experiment, 22.3°C.). During the night of January 15, the room temperature fell from 25.6°, at 5 P. M., to 6.7° at 8 A. M., on the following morning, owing to a change of wind, accompanied by cold weather. The body temperatures of four of the mice which had been tested two days previously were taken on the morning of January 16, in order to determine the effect of this change in the atmosphere. The tests were repeated three days later, when four other mice, which had not been previously tested, were added to the lot. Thermometer number 1 was used, inserted as far as a rubber collar, placed 13 mm. from the tip of the bulb.

IDENTIFICATION NUMBER	SEX	DATE	HOUR	AIR TEMP. deg. C.	BODY TEMP.		DURATION OF INSERTION	REMARKS
					30 sec. deg. C.	Max. deg. C.		
44	♂	Jan. 14	2.20 P. M.	25.0		36.2*	1'	Mouse struggled and thermometer was removed twice before final test. Temperature not recorded till mercury was stationary.
44	♂	Jan. 14	2.40 P. M.	25.0	37.4	37.4	1'	Not much struggle. Mercury rose rapidly to maximum, and remained there about ½ minute.
44	♂	Jan. 14	3.08 P. M.	25.0		37.4*	1'±	Not very satisfactory. Mouse struggled so much thermometer was removed once. Maximum probably about as indicated.
44	♂	Jan. 16	8.50 A. M.	6.7		34.1	2'30"	Mouse did not struggle much. Mercury nearly stationary, last ½ minute.
44	♂	Jan. 16	9.40 A. M.	7.2		36.6*	2'30"	Mercury rose to between 35.0° and 35.5°, where it remained till mouse began to struggle; then it rose rapidly to 36.5°, where it remained at least ½ minute, reaching 36.6° the last part of the time.
44	♂	Jan. 19	1.25 P. M.	30.0		35.4	1'25"	Mouse moved considerably. Mercury rose to 35.4°, then fell slightly. As recorded for perhaps 30 seconds.
44	♂	Jan. 19	3.28 P. M.	28.3		36.3	1'	Little struggle. Temperature as recorded for last ½ minute.
42	♂	Jan. 14	2.48 P. M.	24.4		35.5	1'30"	Little struggle. Mercury rose for ½ minute or more; stationary the last ¼ minute.
42	♂	Jan. 14	3.00 P. M.	24.4		38.1*	1'35"	Mouse moved considerably. Mercury attained fixed level once, then rose to 38.1°, then fell about ½ degree. Close to 37.5° for about ½ minute at end.
42	♂	Jan. 14	3.25 P. M.	24.4		38.6	1'±	Mouse struggled a good deal. Mercury reached maximum very rapidly, and remained there ½ minute or more at end, fluctuating considerably in meantime.
42	♂	Jan. 16	9.05 A. M.	6.7		35.4	1'30"	Mouse struggled. Mercury reached maximum after ½ minute or so; probably stationary for ½ minute.
42	♂	Jan. 16	9.25 A. M.	7.2		37.5*	2'+	Mouse struggled a good deal. Thermometer taken out once; gave same reading both tests. Stationary ½ to ¾ minute.

TABLE 7—Continued

IDENTIFICATION NUMBER	SEX	DATE	HOUR	AIR TEMP.	BODY TEMP.		DURATION OF INSERTION	REMARKS
					30 sec.	Max.		
				<i>deg. C.</i>	<i>deg. C.</i>	<i>deg. C.</i>		
42	♂	Jan. 19	1.35 P.M.	30.0	35.6	35.6	1'	As recorded for about $\frac{1}{2}$ minute, despite struggle at end.
42	♂	Jan. 19	3.30 P.M.	28.3	35.9	35.9	1'30"	Little movement on the part of mouse. Temperature as recorded for last 40 seconds.
40	♂	Jan. 19	1.40 P.M.	30.0	36.4†	36.6	1'45"	The period (11 minutes) interrupted once by withdrawal of bulb. Mercury rose to 36.2° in 10 seconds, and did not go above 36.4° until struggle and removal of thermometer. After this, it rose to 36.6°, and remained stationary at least $\frac{1}{4}$ minute.
40	♂	Jan. 19	3.37 P.M.	28.3	35.9	35.9	1'10"	Mouse moved about considerably. Thermometer varied from 35.6° to 35.9° during last 40 seconds.
36	♂	Jan. 19	1.50 P.M.	30.0	35.7*	35.7*	3'	Mercury first rose to about 34.9° and became stationary; then to 35.1° and became stationary; then (the mouse moving somewhat) it rose slowly to 35.7° and remained thus for at least $\frac{1}{2}$ minute.
36	♂	Jan. 19	3.43 P.M.	28.3	35.5	35.5	1'30"	Mercury rose to this level once and remained some time, then fell, during struggles of mouse rose again to 35.4° and stayed for some time; then to 35.5° for 15 or 20 seconds.
38-1	♀	Jan. 14	2.25 P.M.	25.0	37.9†	37.9	1'10"	No struggle. Mercury rose within a very few seconds to 37.6°. No change during last $\frac{1}{2}$ minute.
38-1	♀	Jan. 14	2.45 P.M.	25.0	37.8	37.8	1'	No struggle. Same remarks as foregoing. If anything, temperature fell slightly at the end.
38-1	♀	Jan. 14	3.12 P.M.	24.4	37.1	37.1	1'30"	Mouse moved more than previously. Mercury stationary for about $\frac{1}{2}$ minute, and moved little within 1 minute.
38-1	♀	Jan. 16	8.55 A.M.	6.7	36.0	36.0	1'40"	Mouse quiet. Mercury rose to maximum (36.0°) in 30 seconds or less; then fell about 0.3°.
38-1	♀	Jan. 16	9.45 A.M.	6.7	37.1	37.1	1'	No struggle. Mercury rose to maximum in 30 seconds; then remained stationary for 30 seconds.
38-1	♀	Jan. 19	1.30 P.M.	30.0	37.3	37.3	1'	Very little struggle. Temperature as recorded for perhaps $\frac{1}{2}$ minute.
38-1	♀	Jan. 19	3.25 P.M.	28.3	36.5	36.5	1'	Little struggle. Temperature as recorded for last $\frac{1}{2}$ minute; little change after first 15 seconds.
18-4	♀	Jan. 14	2.53 P.M.	24.4	37.0	37.0	1'15"	Very little struggle. Mercury rose very soon to maximum; stationary for last $\frac{1}{2}$ minute.
18-4	♀	Jan. 14	3.05 P.M.	24.4	37.6	37.6	1'	Mouse did not stir for last $\frac{1}{2}$ minute. Mercury stationary for $\frac{1}{2}$ minute or more.
18-4	♀	Jan. 14	3.20 P.M.	23.9	37.5	37.5	1'30"	Mouse quiet for last minute or more. Mercury stationary for 1 minute.
18-4	♀	Jan. 16	9.00 A.M.	6.7	36.7	36.7	1'45"	Mercury rose to maximum in about $\frac{1}{2}$ minute; stationary $\frac{1}{2}$ minute; perhaps a fall of .1° at end.
18-4	♀	Jan. 16	9.30 A.M.	7.2	37.1	37.1	2"	Mouse moved somewhat. Mercury rose to 37.1° in 20 seconds, and remained there for some time; then rose to 37.3°, but returned to 37.2° and remained about 1 minute.

TABLE 7—Continued

IDENTIFICATION NUMBER	SEX	DATE	HOUR	AIR TEMP. <i>deg. C.</i>	BODY TEMP.		DURATION OF INSERTION	REMARKS
					30 sec. <i>deg. C.</i>	Max. <i>deg. C.</i>		
18-4	♀	Jan. 19	1.37 P.M.	30.0	37.24	37.3	1'20"	Mercury rose to 37.1° in 15 seconds; as recorded for about 1/2 minute.
18-4	♀	Jan. 19	3.33 P.M.	28.3	37.2	37.3	1'	Mercury rose only from 37.2° to 37.3° during last 45 seconds.
6-1	♀	Jan. 19	1.43 P.M.	30	37.1	37.2	1'	Not much struggle, but steady pulling. Mercury rose to 37.2°, then fell slightly. At 37.1° for last 1/2 minute or more.
6-1	♀	Jan. 19	3.40 P.M.	28.3	36.9	36.9	1'	Very little movement. Mercury rose to this point in about 15 seconds, and remained almost absolutely stationary for last 45 seconds.
16-1	♀	Jan. 19	1.55 P.M.	30	37.2	37.2	1'	Little struggle. Mercury rose to 37.1° in 15 or 20 seconds; as recorded for at least 30 seconds.
16-1	♀	Jan. 19	3.45 P.M.	28.3	37.1	37.1	1'	Mercury perfectly stationary at point indicated during last minute. No struggle during this period, though there was at outset.

TABLE 8

Five adult males (numbers 2 to 12), 9 to 11 months old, and five males (numbers 23-3 to 20-1), about three and one-half months old, all belonging to the warm-room lot (mean temperature to date, 22.3°C.). The first test of each mouse was made on a morning following a cold wave. The temperature had fallen from 27°C. at 10 P.M., March 4, to 5°, at 8 A.M., March 5. A small window which had been left open was then closed and the temperature began to rise at once. After the first temperature test with the entire lot, the mice were transferred to the cold room, then at a temperature of -2.5°C., and placed in separate small cages with cotton for nests. The second test was made after the mice had remained nearly five hours at about this temperature. The mice, with two exceptions, were kept for about twenty-four hours in the cold room, the temperature ranging from 1.5° to 6.5°C. The third test was made at the end of this period. Two of the younger mice (27-1 and 20-1) were so seriously affected by the cold, at the end of the five-hour period, that they were returned to the warm room (22° to 30° during the ensuing twenty-four hours), at 4 P.M., March 5, the third temperature test with them being made in this room. These two mice would doubtless have died in the cold room, though even number 20-1, which was nearly overcome by the cold, became fairly active within one and one-half hours after return to the warm room, and was seemingly well in four hours. At the end of the experiment, certain of the mice were observed to cough, though no permanent injury was noted. All of these determinations, except where stated, were made with thermometer number 2, the bulb being inserted as far as a rubber collar placed 16 to 18 mm. from the tip.

IDENTIFICATION NUMBER	WEIGHT	DATE	HOUR	AIR TEMP.	BODY TEMP.		DURATION OF INSERTION	REMARKS
					30 sec.	Max.		
2	24.0	Mar. 5	10 A.M.	deg. C. 14.0	deg. C. 37.0	deg. C. 37.2	1'40"	Thermometer 37.0° at end of ½ minute, 37.2° at end of 1 minute; mouse then began to stir considerably, and mercury fell some tenths below 37°, rising again to latter point when mouse quieted.
2		Mar. 5	3.05 P.M.	0	36.1	36.5	1'+	Two trials made. First time, thermometer stood at 36.1° after ½ minute; then struggle followed and mercury rose to 36.5°, and thermometer was withdrawn. On second trial, it rose to 36.1° in ½ minute and remained stationary ½ minute.
2		Mar. 6	10.50 A.M.	6.5	35.8	35.9	1'	Thermometer at 35.8° in ½ minute, fluctuating between 35.7° and 35.9° for next ½ minute. At the end, a trifle below 35.8°.
6	24.0	Mar. 5	10.12 A.M.	14.0	37.8	37.8	1'5"	Mouse moved considerably at first, but became more quiet. Mercury rose only 0.5° during last 35 seconds.
6		Mar. 5	3.12 P.M.	1.0	35.4	36.0*	1'30"	Not very satisfactory. Mercury rose to 36.0°, but thermometer removed, owing to movements of mouse. When reinserted, 35.4° in ½ minute, 35.6° in 1 minute, falling almost immediately to 35.4°.



TABLE 8—Continued

IDENTIFICATION NUMBER	WEIGHT	DATE	HOUR	AIR TEMP.	BODY TEMP.		DURATION OF INSERTION	REMARKS
					30 sec.	Max.		
	grams			deg. C.	deg. C.			
6		Mar. 6	10.55 A.M.	6.5	36.2	36.4	1'30"	Not much struggle during first ½ of time. Thermometer 36.2° after first ½ minute, 36.4° at close of 1 minute, same after 1½ minutes.
8	23.5	Mar. 5	10.17 A.M.	14.0	34.7	35.1	1'30"	Mercury rose slowly to 34.7° in ½ minute, then rose about 0.3° suddenly, when mouse began to struggle. On latter's quieting down, mercury fell, but rose again, remaining between 35.0° and 35.1° during last ½ minute.
8		Mar. 5	3.15 P.M.	1.0	34.3	34.3	1'20"	Mercury rose to 34.3° in ½ minute, falling to 34.2° at end of 1 minute; continued to fall slightly, indicating 34.1° in 1½ minute, when thermometer was withdrawn owing to movements of mouse.
8		Mar. 6	11.00 A.M.	6.5	34.3	34.8*	2'	Thermometer recorded 34.3° at end of ½ minute and 34.8° at end of 1 minute; then mercury fell; about 34.5° for ½ minute at end.
10	25.0	Mar. 5	10.22 A.M.	14	35.9	36.7*	1'30"	Thermometer recorded 35.9° at first ½ minute. A few violent tugs by mouse sent mercury up suddenly to 36.5°. It stood at 36.7° at end of 1 minute, 0.05° higher after 1½ minutes.
10		Mar. 5	3.20 P.M.	1	36.3	36.6	2'	Recorded 36.3° at end of ½ minute; 36.4° at end of 1 minute; rose during struggle to 36.6°, but fell to 36.5° and remained stationary for some time.
10		Mar. 6	11.05 A.M.	6.5	36.8	37.0	2'	Moderate struggle. Mercury rose to 36.8° in ½ minute, standing at 36.9° after 1 minute, 37.0° after 1½ minutes and after 2 minutes.
12	25.0	Mar. 5	10.25 A.M.	14	36.1	36.2	1'10"	Mouse very quiet during entire period. Thermometer at 36.1° after ½ minute, and 36.2° for last ½ minute.
12		Mar. 5	3.25 P.M.	1.5	36.5	37.2*	1'30"	Mercury rose to 36.5° in ½ minute, thereafter undergoing very considerable fluctuations during movements of mouse; 37.2° for last ½ minute.
12		Mar. 6	11.10 A.M.	6.5	36.0	36.0	1'	Reading given is for second trial. Thermometer recorded 36.0° at end of ½ minute and at end of 1 minute.
23-3	20.0	Mar. 5	10.30 A.M.	15	35.3	36.0*	2'	Thermometer indicated 35.3° at end of ½ minute, 35.7° at end of 1 minute; rose to maximum of about 36.0°, then fell, remaining 35.8° for about ½ minute at end.
23-3		Mar. 5	3.28 P.M.	2	35.3	35.5	1'30"	Little movement. Thermometer recorded 35.3° at end of ½ minute; 35.5° at end of 1 minute and at end of 1½ minutes.
23-3		Mar. 6	11.13 A.M.	6.5	36.3	36.6	1'40"	Mouse tugged strongly. Thermometer recorded 36.3° at end of ½ minute; 36.5° at end of 1 minute; then rose to 36.6° and back to 36.4° at end.
27-1	20.0	Mar. 5	10.36 A.M.	15	36.9	37.1	1'30"	Mouse very quiet and thermometer inserted easily. Thermometer recorded 36.9° at end of ½ minute, 37.1° at end of 1 minute and at end of 1½ minutes.

TABLE 8—Continued

IDENTIFICATION NUMBER	WEIGHT	DATE	HOUR	AIR TEMP.	BODY TEMP.		DURATION OF INSERTION	REMARKS
					30 sec.	Max.		
	grams			deg. C.	deg. C.	deg. C.		
27-1		Mar. 5	3.52 P.M.	2	[20.6]	[20.6]	1'	Mouse "dumpy" and rather feeble, though far from exhausted. Mercury far below scale of thermometer no. 2, so no. 3 was substituted. Latter recorded 20.6° at end of $\frac{1}{2}$ minute and 1 minute.
27-1		Mar. 6	12 M.	28.5	35.5	35.6	1'	Little movement by mouse. Thermometer recorded 35.5° after about 15 seconds; rose only 0.1° in next $\frac{1}{2}$ minute.
24-2	23.0	Mar. 5	10.43 A.M.	15	37.9	37.9	1'	Moderate amount of movement. Thermometer recorded 37.9° at end of $\frac{1}{2}$ minute. About 0.05° higher at end of 1 minute.
24-2		Mar. 5	3.35 P.M.	2	35.7	35.7	1'	Thermometer recorded 35.7° at end of $\frac{1}{2}$ minute and same at end of 1 minute. In interval mercury had gone down once or twice, owing to tugging of mouse.
24-2		Mar. 6	11.18 A.M.	6.5	36.8	36.8	1'	Considerable tugging, though not very hard. Temperature same after $\frac{1}{2}$ minute and after 1 minute.
22-2	22.5	Mar. 5	10.47 A.M.	15	38.2	38.8*	2'	Thermometer went in easily and mouse did not struggle till latter part of time. Mercury rose to 38.2° in $\frac{1}{2}$ minute, 38.5° in 1 minute, 38.8° in $\frac{1}{2}$ minutes, remaining about the same at end of 2 minutes.
22-2		Mar. 5	3.40 P.M.	2	34.7	35.4*	2'	Mouse struggled little, though there was considerable blood on bulb. Thermometer recorded 34.7° at end of $\frac{1}{2}$ minute, 35.4° for last 40 seconds or thereabouts.
22-2		Mar. 6	11.22 A.M.	6.5	37.4	37.6	1'30"	Mouse tugged considerably. Thermometer recorded 37.4° at end of $\frac{1}{2}$ minute, 37.5° at end of 1 minute, during next $\frac{1}{2}$ minute, it rose nearly 0.1°.
20-1	16.0	Mar. 5	10.50 A.M.	15	35.6	35.8	1'30"	Thermometer inserted easily; little struggle. Mercury rose to 35.6° in $\frac{1}{2}$ minute, 35.8° in 1 minute, and same at end of $\frac{1}{2}$ minutes.
20-1		Mar. 5	3.50 P.M.	2	[12.5=]	[12.5=]	1'	Mouse scarcely living; gasping occasionally and showing some reflexes. Thermometer no. 3 used. Mercury not much above bulb and far below scale. If stem was uniformly calibrated, temperature was between 11.5° and 13.5°.
20-1		Mar. 6	12.05 P.M.	28.5	36.7	37.5*	2'	Considerable movement. Thermometer recorded 36.7° at end of $\frac{1}{2}$ minute, and 37.1° at end of 1 minute, thereafter rising about 0.2° each $\frac{1}{2}$ minute.

TABLE 9

*Five adult males, less than a year old, which had been kept over four months in the warm room (22.4°C.). Since these mice had been transferred to new boxes with fresh sawdust, from one-half to three-quarters of an hour before the first temperature test, the abnormally high temperatures then recorded are probably due to excitement resulting from the strange surroundings. The mice were then left undisturbed until the following day, when five tests were made at varying intervals. Thermometer number 2 was used as above described.*

IDENTIFICATION NUMBER	WEIGHT grams	DATE	HOUR	AIR TEMP. deg. C.	BODY TEMP.		DURATION INSER- TION	REMARKS
					30 sec. deg. C.	Max. deg. C.		
A'1	21.0	Mar. 31	3.45 P.M.	16	37.8	38.0	1'5"	Mercury rose to 37.8° in 35 seconds, then fluctuated between 38.0° and 37.7° during next ½ minute.
A'1		Apr. 1	8.55 A.M.	20	35.2	35.5	1'30"	Mouse unusually quiet. Thermometer recorded 35.2° at end of ½ minute, 35.5° at end of 1 minute and at end of 1½ minutes.
A'1		Apr. 1	9.14 A.M.	20	37.5†	37.5	1'15"	Considerable tugging. Temperature 37.5° for last ½ minute.
A'1		Apr. 1	10.14 A.M.	21	35.3	35.3	1'	Mouse fairly quiet. Thermometer recorded 35.3° at end of ½ minute, or earlier, and remained stationary till end of 1 minute.
A'1		Apr. 1	12.14 P.M.	19	35.0	35.0	45"	Mouse very quiet. Thermometer recorded 35.0° at end of 15 seconds and same for ½ minute thereafter.
A'1		Apr. 1	7.25 P.M.	21	37.0	37.2	1'30"	Mouse fairly quiet. Thermometer recorded 37.0° at end of ½ minute. During next minute, mercury fluctuated between 37.1° and 37.2°.
A'2	27.0	Mar. 31	3.48 P.M.	16	38.5	38.6	1'	Considerable movement. Mercury rose to 38.5° in 20 seconds, remaining about thus for next 40 seconds, rising at one time to 38.6°.
A'2		Apr. 1	9 A.M.	20	36.9	37.7*	2'	Considerable tugging. Thermometer recorded 36.9° at end of ½ minute, 37.4° at end of 1 minute, 37.6° to 37.7° for last ½ minute or so.
A'2		Apr. 1	9.18 A.M.	20	37.7	38.0	1'30"	Considerable tugging. Thermometer recorded 37.7° at end of ½ minute. For last ½ minute it fluctuated between 37.8° and 38.0°.
A'2		Apr. 1	10.16 A.M.	20	37.6	38.0	1'45"	Thermometer recorded 37.6° in ½ minute, 37.8° in 1 minute, 38.0° in 1½ minutes, remaining stationary for last ½ minute.
A'2		Apr. 1	12.16 P.M.	20	36.0	36.1	1'	Thermometer recorded 36.0° before end of ½ minute, remaining at this point for about ½ minute, but rising 0.1° at end.
A'2		Apr. 1	7.30 P.M.	20	37.9	38.8*	2'30"	Mercury rose to 37.9° in ½ minute, 38.3° in 1 minute, 38.5° in 1½ minutes, 38.7° in 2 minutes, 38.8° in 2½ minutes. Continued rising throughout observations.

TABLE 9—Continued

IDENTIFICATION NUMBER	WEIGHT	DATE	HOUR	AIR TEMP.	BODY TEMP.		DURATION OF INSERTION	REMARKS
					30 sec.	Max.		
	grams			deg. C.	deg. C.	deg. C.		
A 3	24.5	Mar. 31	3.52 P.M.	16	38.8	39.2	2'	Mercury rose to 38.8° in $\frac{1}{2}$ minute (after previous brief insertion), 38.9° in 1 minute, 39.1° in $\frac{1}{2}$ minutes, remaining thus for nearly $\frac{1}{2}$ minute, 39.2° at end.
A 3		Apr. 1	9.03 A.M.	20	36.6	36.6	1'	Mouse fairly quiet. Temperature as recorded at end of $\frac{1}{2}$ minute; at end of 1 minute about 0.06° higher.
A 3		Apr. 1	9.22 A.M.	21	38.0	38.6*	2'5"	Thermometer recorded 38.0° in $\frac{1}{2}$ minute, 38.3° in 1 minute, 38.5° in $\frac{1}{2}$ minutes. Between latter point and 38.6° during last 35 seconds.
A 3		Apr. 1	10.20 A.M.	20	37.3	38.0*	1'45"	Considerable movement. Mercury rose to 37.3° in $\frac{1}{2}$ minute, 37.6° in 1 minute, 37.8° in $\frac{1}{2}$ minutes. Stayed at latter point nearly $\frac{1}{2}$ minute, then rose to 38.0°.
A 3		Apr. 1	12.20 P.M.	20	36.4	37.3*	2'30"	Mercury rose to 36.4° in $\frac{1}{2}$ minute, then kept on steadily rising. Stationary at 37.3° for 15 or 20 seconds, but rose again at end.
A 3		Apr. 1	7.35 P.M.	20	37.6	39.0*	2'30"	Thermometer recorded 37.6° at end of $\frac{1}{2}$ minute, 38.0° at end of 1 minute, 38.3° at end of $\frac{1}{2}$ minutes, 38.7° at end of 2 minutes, 39.0° at end of $\frac{1}{2}$ minutes.
A 4	24.0	Mar. 31	3.55 P.M.	16	37.8	38.2	1'30"	Thermometer recorded 37.8° in $\frac{1}{2}$ minute, 38.0° in $\frac{1}{2}$ minutes, 38.2° in 1 minute, and same in $\frac{1}{2}$ minutes.
A 4		Apr. 1	9.08 A.M.	20	36.6	37.1*	2'	Mouse fairly quiet. Thermometer recorded 36.6° at end of $\frac{1}{2}$ minute, 36.8° at end of 1 minute, 37.1° at end of $\frac{1}{2}$ minutes, and same at end of 2 minutes.
A 4		Apr. 1	9.25 A.M.	21	38.6	38.8	1'20"	Mercury rose to 38.6° in $\frac{1}{2}$ minute, and 38.8° in 50 seconds, rising slightly beyond that figure at the end.
A 4		Apr. 1	10.25 A.M.	21	37.5	38.5*	3'	Test not very satisfactory. Thermometer recorded 37.5° in $\frac{1}{2}$ minute, then rose slowly and steadily, but remained at about maximum point recorded for last $\frac{1}{2}$ minute.
A 4		Apr. 1	12.25 P.M.	18	37.0	37.7*	2'50"	Thermometer recorded 37.0° in $\frac{1}{2}$ minute. Mercury rose steadily, arriving at no stationary point.
A 4		Apr. 1	7.40 P.M.	20	38.4	38.4	1'45"	Temperature as recorded at end of $\frac{1}{2}$ minute and same at end of 1 minute; fell once, but rose again.
A 5	24.0	Mar. 31	4.05 P.M.	16	37.0	37.5*	1'30"	Test not very satisfactory, owing to movement of mouse. Thermometer inserted twice. The first time, mercury rose to 37.0° in $\frac{1}{2}$ minute, the second time 37.4° in $\frac{1}{2}$ minute, and about 37.5° in 1 minute.
A 5		Apr. 1	9.10 A.M.	20	36.8	37.4*	2'	Thermometer recorded 36.8° at end of $\frac{1}{2}$ minute, 37.1° at end of 1 minute, 37.3° at end of $\frac{1}{2}$ minutes, rising to 37.4° toward close.

TABLE 9—Continued

IDENTIFICATION NUMBER	WEIGHT	DATE	HOUR	AIR TEMP.	BODY TEMP.		DURATION OF INSERTION	REMARKS
					30 sec.	Max.		
A 5	grams	Apr. 1	9:29 A.M.	deg. C. 21	deg. C. 39.0	deg. C. 39.0	1'	Thermometer recorded 39.0° at end of ½ minute and at end of 1 minute, little change after first rise. Slight bleeding from rectum.
A 5		Apr. 1	10:30 A.M.	21	37.0	37.8*	2'	Test not satisfactory. Mercury reached 37.0° in ½ minute, then rose steadily till it passed 37.8° in 2 minutes.
A 5		Apr. 1	12:30 P.M.	17	36.9	37.0	1'15''	Thermometer recorded 39.9 in ½ minute, 37.0 for last ½ minute.
A 5		Apr. 1	7:43 P.M.	20	38.3	39.0*	2'	Mercury rose to 38.3° in ½ minute, 38.6° in 1 minute, 39.0° in 1½ minutes, falling to 38.8° at end of 2 minutes.

TABLE 10

*Eight adult male and female mice, eight to ten months old, which had been kept during the preceding two months in the cold room (mean temperature 5.8°C.). During the day preceding the first test, the air temperature fell from +12.2°C., at 10.30 A.M., January 15, to -6.7°C., at 8 A.M., January 16. In the case of four of the mice, the tests were commenced on the morning of January 16, with the others the first test was not made until January 17. Thermometer number 1 was used, inserted as far as a rubber collar placed 13 mm. from the tip of the bulb.*

IDENTIFICATION NUMBER	SEX	DATE	HOUR	AIR TEMP. deg. C.	BODY TEMP.		DURATION OF INSERTION	REMARKS
					30 sec.	Max.		
47	♂	Jan. 16	10.35 A.M.	-6.7	deg. C.	34.5	1'30"	Mouse struggled somewhat. Maximum 34.8°. Mercury stationary at 34.6° for last $\frac{1}{2}$ minute.
47	♂	Jan. 16	11.15 A.M.	-6.7	deg. C.	37.1*	2'30"	Test not very satisfactory. Mercury rose to 36.4° and became stationary for some time. Then, during struggles of mouse, it rose nearly 37.1° and fell again somewhat. Stood at about 36.8° for last 15 or 20 seconds.
47	♂	Jan. 17	1.45 P.M.	-5.6	deg. C.	35.4	1'30"	Considerable movement on part of mouse. Mercury fluctuated during last minute between 35.1° and 35.4°.
47	♂	Jan. 17	2.40 P.M.	-4.4	deg. C.	36.0*	2'45"	Test not very satisfactory. Mouse struggled a good deal. Mercury stood at 35.1° for about $\frac{1}{2}$ minute, then rose to 36.0° and even more. Stood at 35.9° for last 15 or 20 seconds.
47	♂	Jan. 18	2.25 P.M.	-2.2	deg. C.	37.1†	1'45"	Much struggle. Mercury rose quickly to 37.1° or more, then, as mouse struggled, it rose to successive new levels. Perhaps would have gone higher still.
47	♂	Jan. 18	3.45 P.M.	-1.1	deg. C.	37.6*	1'+	Mouse struggled so much that I could not be sure maximum was indicated. Had to withdraw thermometer.
47	♂	Jan. 26	10.20 A.M.	11.1	deg. C.	35.4	3'30"	Record very unsatisfactory. Mercury rose to 35.4° in 30 seconds or less, and remained there nearly 30 seconds, then mouse began to struggle and mercury rose in jumps, remaining for a while at each new level.
47	♂	Jan. 26	12.05 P.M.	10.6	deg. C.	36.8	45"	Much struggle and thermometer withdrawn once. Mercury rose at one time to 36.8°, but fell. About 36.6° for last 15 or 20 seconds.
43	♂	Jan. 16	10.40 A.M.	-6.7	deg. C.	37.0	1'20"	Little struggle. Mercury rose in $\frac{1}{2}$ minute to 37.0°, then fell and remained at 36.5° for last $\frac{1}{2}$ minute.
43	♂	Jan. 16	11.30 A.M.	-6.7	deg. C.	37.6	1'45"	Mouse struggled considerably. Mercury fluctuated slightly above and below level indicated for about a minute.
43	♂	Jan. 17	1.55 P.M.	-5.6	deg. C.	36.5	1'30"±	Mercury fluctuated between 36.3° and 36.5° for last minute or more; the larger part of time at 36.4°. (Slight bleeding from rectum).

TABLE 10—Continued

IDENTIFICATION NUMBER	SEX	DATE	HOUR	AIR TEMP. <i>deg. C.</i>	BODY TEMP.		DURATION OF INSERTION	REMARKS
					30 sec. <i>deg. C.</i>	Max. <i>deg. C.</i>		
43	♂	Jan. 17	2.50 P.M.	-4.4		37.5*	2'	Much struggle. Record not very satisfactory. Mercury rose to 37.5° or even higher, and then fluctuated between this and 37.1°. For some time at 37.4°.
43	♂	Jan. 18	2.30 P.M.	-2.2	36.4	36.4	1'	Little struggle. Mercury rose to level indicated in about 20 seconds, remaining there for 45 seconds.
43	♂	Jan. 18	3.55 P.M.	-1.1		38.2	1'10"	Considerable struggle. Mercury rose to 38.1° and even 38.2°, but only for a short time; stood at 38.0° for 20 or 30 seconds.
43	♂	Jan. 26	10.10 A.M.	11.1	36.4	37.7*	3'15"	Record very unsatisfactory. Mouse struggled continuously. Mercury rose to 36.4° in 30 seconds remaining there 15 or 20 seconds, then went up in stages, remaining stationary for brief periods. At 37.7° for 20 to 30 seconds; very likely would have risen higher.
43	♂	Jan. 26	11.55 A.M.	10.6		36.4*	1'45"	Much struggle. Mercury fluctuated between 36.2° and 36.4° for at least $\frac{1}{2}$ minute; had earlier come to a stationary level of 35.7°.
41	♂	Jan. 17	3.05 P.M.	-3.9	36.8	36.8	1'	Almost no struggle. Temperature as recorded for last $\frac{1}{2}$ minute at least, though fluctuating slightly.
41	♂	Jan. 18	2.40 P.M.	-2.2	36.3	36.4	1'15"	Little struggle. Mercury rose to 36.3° in $\frac{1}{2}$ minute; then went to 36.4° and back to 36.3°.
41	♂	Jan. 18	4.00 P.M.	-1.1		38.5	1'30"	Mouse struggled somewhat. Mercury rose to 38.2° pretty quickly. Maximum about as indicated, but mercury stationary at 38.4° probably $\frac{1}{2}$ minute.
41	♂	Jan. 26	9.58 A.M.	11.1	36.6	36.7	1'	Mouse fairly quiet. Mercury rose to 36.6° in $\frac{1}{2}$ minute or less. Possibly still rising slightly at the end.
41	♂	Jan. 26	11.50 A.M.	10.6	35.9	36.0	55"	Mouse in almost continuous movement. Thermometer dislodged once, but remained in place 55 seconds after reinsertion. Mercury rose to 35.9° in 15 seconds, rising only 0.1° in remaining time.
25	♂	Jan. 27	3.10 P.M.	-3.9	35.4	35.4	1'15"	Little struggle. Mouse very quiet at the end. Mercury remained absolutely stationary at 35.4° for $\frac{1}{2}$ minute. This occurred again when test was repeated 1 minute later.
25	♂	Jan. 18	2.50 P.M.	-1.7	37.0†	37.0	1'40"	Thermometer removed once owing to struggle. Mercury rose each time to about point indicated (possibly to 37.1°) and remained almost stationary.
25	♂	Jan. 18	4.10 P.M.	-1.1	37.2	37.5	1'30"±	Mouse moved considerably. Mercury rose to 37.2° in about $\frac{1}{2}$ minute, paused for a time, then rose again. Remained at 37.5° for 20 to 30 seconds.
25	♂	Jan. 26	9.50 A.M.	11.1	35.6	35.7	1'30"	Mercury rose to 35.6° in $\frac{1}{2}$ minute or less, then to 35.7° in about a minute. Possibly still rising at end, but very slowly.

TABLE 10—Continued

IDENTIFICATION NUMBER	SEX	DATE	HOUR	AIR TEMP.	BODY TEMP.		DURATION OF INSERTION	REMARKS
					30 sec.	Max.		
				<i>deg. C.</i>	<i>deg. C.</i>	<i>deg. C.</i>		
25	♂	Jan. 26	11.40 A.M.	10.6	34.6	34.9	1'30"	Considerable movement. Mercury did not go above 34.6° for first ½ minute; at 34.9° for last ½ minute.
47-1	♀	Jan. 16	10.30 A.M.	-6.7	35.4	35.5	1'	Mouse did not struggle. Maximum reached in about 15 seconds. Temperature stationary at 35.4° for last ½ minute or more.
47-1	♀	Jan. 16	11.20 A.M.	-6.7	37.0*	37.0*	2'30"	During struggles of mouse, mercury rose to 37.0° and remained there for some time; stood at 36.4° for last ½ minute or more.
47-1	♀	Jan. 17	1.50 P.M.	-5.6	36.8	36.9	1'10"	Mouse struggled little, latter part of time not at all. Mercury between 36.8° and 36.9° for ½ minute.
47-1	♀	Jan. 17	2.45 P.M.	-4.4	37.5	37.5	1'	Mercury rose rapidly to maximum. Remained stationary at least ½ minute, fluctuating little, though mouse moved considerably.
47-1	♀	Jan. 18	2.28 P.M.	-2.2	37.0	37.0	1'15"	Mouse struggled somewhat, without much effect on temperature. Mercury rose to point recorded within ½ minute, remaining thus about ¾ minute.
47-1	♀	Jan. 18	3.50 P.M.	-1.1	37.3	37.3	1'10"	Mouse struggled considerably, but mercury kept at point indicated over ½ minute.
47-1	♀	Jan. 26	10.25 A.M.	11.1	36.8†	36.9	1'15"	Mercury rose to 36.5° in 10 seconds, then to point recorded, remaining thus for over ½ minute, though fluctuating slightly.
47-1	♀	Jan. 26	12.10 P.M.	10.6	37.0*	37.0*	2'±	Record unsatisfactory, owing to almost constant struggle of mouse. Mercury rose to 37.0° at one time, then fell to 36.5° and fluctuated within a few tenths of this for ½ minute.
29-2	♀	Jan. 16	10.45 A.M.	-6.7	34.4	34.4	1'30"	Mouse moved little. Temperature nearly stationary for last ½ minute, though fluctuating slightly.
29-2	♀	Jan. 16	11.25 A.M.	-6.7	36.2*	36.2*	2'	Mouse moved considerably most of time. Mercury rose to 35.7° and remained there for some seconds, then rose to 36.2° and remained stationary, then fell to 35.7° and remained 20 or 30 seconds despite struggle.
29-2	♀	Jan. 17	1.53 P.M.	-5.6	36.5	36.5	1'	Very little struggle. Mercury at point recorded for at least ½ minute.
29-2	♀	Jan. 17	2.55 P.M.	-3.9	36.5	36.5	1'15"	Mouse struggled somewhat. Mercury rose to 36.5° in 20 seconds and remained stationary for some time, then fell to 36.4° and remained thus for about ½ minute.
29-2	♀	Jan. 18	2.35 P.M.	-2.2	36.2	36.4	1'10"	Little movement at end. Mercury rose from 36.2° to 36.4° in last ½ minute.
29-2	♀	Jan. 18	3.57 P.M.	-1.1	36.3	36.3	1'30"	Mouse moved considerably, causing mercury to fall. When quiet, mercury rose each time to about point indicated, finally remaining stationary nearly ½ minute.
29-2	♀	Jan. 26	10.15 A.M.	11.1	37.0	37.1	1'	Mercury reached 37.0° in 15 seconds; rose 0.1 degree at one time but fell again.
29-2	♀	Jan. 26	12 M.	10.6	36.7	36.7	1'	Little movement. Temperature as indicated for last ½ minute.



TABLE 10—(Continued)

IDENTIFICATION NUMBER	SEX	DATE	HOUR	AIR TEMP.	BODY TEMP.		DURATION OF INSERTION	REMARKS
					30 sec.	Max.		
49-3	♀	Jan. 17	3.00 P.M.	-3.9	deg. C. 36.6	deg. C. 37.1*	2'30"	Mercury rose to 36.6° in 15 seconds and remained there perhaps 30 seconds; then, when mouse struggled, it rose to 37.1°, then fell to 36.6° and later to 36.3°. (Slight bleeding from rectum).
49-3	♀	Jan. 18	2.45 P.M.	-1.7	36.2	36.2	1'	Mouse struggled somewhat. Mercury rose to temperature recorded in 20 seconds, remaining there for last 40 seconds.
49-3	♀	Jan. 18	4.05 P.M.	-1.1	37.0	37.0	1'05"	Mouse struggled considerably, but mercury remained pretty steadily at point reached for last 20 to 30 seconds.
49-3	♀	Jan. 26	10.03 A.M.	11.1	37.4†	37.9*	2'	Mouse in almost continuous movement. Mercury rose to 37.4° in about 15 seconds, paused for a time, then rose to 37.9°, remaining there a short time, and began to fall.
49-3	♀	Jan. 26	11.53 A.M.	10.0		37.0*	1'15"	Remained at 37.4° for 20 or 30 seconds.
1-3	♀	Jan. 17	3.08 P.M.	-3.9	37.1	37.5	1'15"	Much struggle. Mercury fell slightly after reaching maximum.
1-3	♀	Jan. 18	2.53 P.M.	-1.7	36.9†	36.9	1'15"	Mercury rose to 37.1° and remained stationary perhaps 15 seconds. The mouse then struggled and mercury suddenly rose to 37.4°, standing at 37.5° for last 30 seconds.
1-3	♀	Jan. 18	4.15 P.M.	-1.1	38.1*	38.1*	2'30"	Little struggle. Mercury as recorded for last ½ minute or more.
1-3	♀	Jan. 26	9.55 A.M.	11.1		37.5*	1'45"	Mercury rose to 37.9° and even 38.1° for a moment, but 37.3° was the only point at which it remained stationary for any length of time. Little struggle for last ½ minute or so.
1-3	♀	Jan. 26	11.45 A.M.	10.6	36.0	36.2	1'15"	Mouse struggled considerably. Thermometer removed once. When inserted again, mercury rose for over 1 minute, but fell slightly during last ½ minute.

TABLE 11

Male mice which had lived about three months in the cold room (mean temperature to date, 4.4°C.), partly from the old lot, partly from a lot about three and one-half months old. The first two tests were made in the cold room, on a day following a rather sudden fall in temperature. The thermometer had recorded +8°C. at 2 P.M. on February 27, falling to -6° at 6 A.M. on the following day. The mice were taken from their nests for the first test; after that, they were placed singly in cages without nests. The last two tests were made after the mice had been transferred (at 3 P.M., February 28) to the warm room (temperature 18° to 20°C. at the time. Thermometer number 2 was used throughout. In the last three tests of each mouse, it was inserted to the rubber collar, 16 mm. from the tip of the bulb, in the first trial, the washer axis lacking, the thermometer being inserted to about the same depth, however.

IDENTIFICATION NUMBER	WEIGHT	DATE	HOUR	AIR TEMP.	BODY TEMP.		DURATION OF INSERTION	REMARKS
					30 sec.	Max.		
	grams			deg. C.	deg. C.	deg. C.		
5	23.5	Feb. 28	11.50 A.M.	-3	35.9	35.9	1'40"	Mercury rose to point indicated in 15 or 20 seconds, remained there about ½ minute, then fell slightly, standing at about 35.7° at the end.
5		Feb. 28	2.05 P.M.	-3	37.1†	37.7*	1'40"	Mouse moved considerably. Mercury rose from 37.0°, at end of 15 or 20 seconds, to 37.7°, where it remained about ½ minute at the end.
5		Feb. 28	7.52 P.M.	20	37.7	37.8	1'	Considerable struggle. Thermometer recorded 37.7° for nearly ½ minute, rising to 37.8° at the end.
5		Mar. 1	3.00 P.M.	23	37.6	37.6	1'05"	Little struggle. Temperature above 37.4° for last ⅓ minute.
7	23.5	Feb. 28	11.55 A.M.	-3	35.0	35.3	1'30"	Mercury rose to 35.0° and remained there nearly ½ minute, then fell a few tenths, then rose to 35.3° and remained about ⅓ minute, rising slightly at the end.
7		Feb. 28	2.10 P.M.	-2.5		37.1	1'20"	Mouse struggled much. Mercury between 37.0° and 37.1° for over ½ minute at the end.
7		Feb. 28	7.57 P.M.	20		38.2	1'	Mouse struggled so much that thermometer was withdrawn at end of 1 minute. A little blood on bulb. During last ⅓ minute, mercury rose only from 38.1° to 38.2°.
7		Mar. 1	3.07 P.M.	23		35.5	1'30"	Mercury rose to maximum of 35.5° and remained some time, then (when mouse struggled) it fell considerably, then rose again, remaining at 35.4° for last ⅓ minute.
13	23.5	Feb. 28	12 M.	-3	34.6	34.8	1'(-)	Mercury rose to 34.8° about ¼ minute, then fell to 34.6° and remaining thus for over ¼ minute.
13		Feb. 28	2.15 P.M.	-2.5	37.5	37.6	1'5"	Mouse fairly quiet. Mercury stood at 37.5° for over ¼ minute at the end, having previously risen to 37.6°.
13		Feb. 28	8.05 P.M.	20		38.4	1'30"	Mouse moved about considerably. Mercury rose to 38.4°, then fell 0.1 degree at the end. Between 38.3° and 38.4° for last ⅓ minute or so.

TABLE 11—Continued

IDENTIFICATION NUMBER	WEIGHT	DATE	HOUR	AIR TEMP.	BODY TEMP.		DURATION OF INSERTION	REMARKS
					30 sec.	Max.		
	grams			deg. C.	deg. C.	deg. C.		
13		Mar. 1	3.12 P.M.	23		36.8*	1'40"	Considerable movement and results not very satisfactory. Mercury fluctuated between 36.5° and 36.8° for about a minute, though not remaining long at any point. Recorded 36.8° at the end.
15	25.0	Feb. 28	12.07 P.M.	-3	35.5	35.5*	1'	Mouse struggled much and record not very satisfactory. Mercury rose to 35.0° and remained there some time, then to point indicated, and remained at about this for 20 or 30 seconds.
15		Feb. 28	2.18 P.M.	-2.5		36.0	1'15"	Mouse moved much at the end. Maximum 36.0°. Mercury remained stationary at 35.9° for 15 or 20 seconds, then fell to 35.7° and remained stationary for a short time.
15		Feb. 28	8.40 P.M.	20		38.0	1'30"	Little struggle and thermometer went in easily. Mercury almost stationary for the last ½ minute.
15		Mar. 1	3.15 P.M.	23	37.1†	37.2	1'15"	Little struggle. Temperature between 37.1° and 37.2° for over ½ minute; 37.1° at end.
17	23.0	Feb. 28	12.42 P.M.	-3.5	36.3	36.3	1'	Mouse fairly quiet. Mercury rose to 36.1° in 15 seconds, and 36.3° in ½ minute, remained at that point till very near end of minute, then fell 0.1 degree.
17		Feb. 28	2.20 P.M.	-2.5		37.8	1'30" ±	After reaching maximum of 37.8°, mercury began to fall and remained at 37.7° for nearly half minute; fell slightly after this.
17		Feb. 28	8.14 P.M.	20		38.5	1'40"	Considerable movement. Mercury rose to 38.1° and remained stationary; then to 38.5° where it remained for last ½ minute.
17		Mar. 1	3.20 P.M.	23		37.2	1'30"	Mouse fairly quiet. Mercury rose during first half minute, then remained stationary for ½ minute or more.
20-2	18.5	Feb. 28	12.25 P.M.	-3	36.2	36.2	1'15"	Mercury rose to 35.7° and remained stationary for some seconds. Then, as mouse struggled, it rose nearly a degree in a few seconds, then became stationary despite struggles.
20-2		Feb. 28	2.30 P.M.	-2.5		36.6*	1'30" ±	Mercury rose to 35.7° and remained stationary for some seconds. Then, as mouse struggled, it rose nearly a degree in a few seconds, then became stationary despite struggles.
20-2		Feb. 28	8.20 P.M.	20	37.5	37.5	1'	Mouse very still. Mercury almost absolutely stationary during last ¼ minute.
20-2		Mar. 1	3.25 P.M.	23	36.1	36.1	1'30"	Thermometer recorded 36.1° in ¼ minute, 36.4° in 1 minute; rose only 0.2 degree in next ¼ minute. Considerable blood on bulb.
27-1	20.5	Feb. 28	12.30 P.M.	-3	36.4†	36.7	1'30"	For last minute mercury fluctuated between 36.4° and 36.6°. At one time reached 36.7° but only momentarily.
27-1		Feb. 28	2.32 P.M.	-2.5		36.5	1'	Mercury at maximum (36.5°) for last ¼ minute.
27-1		Feb. 28	8.23 P.M.	20	38.1	38.1	1'05"	Considerable movement. Temperature as recorded for last ¼ minute.

TABLE 11—Continued

IDENTIFICATION NUMBER	WEIGHT	DATE	HOUR	AIR TEMP.	BODY TEMP.		DURATION OF INSERTION	REMARKS
					30 sec.	Max.		
27-1	gram	Mar. 1	3.30 P.M.	23	deg. C. 36.6	deg. C. 36.7	1'	Thermometer recorded 36.6° at end of ½ minute and 36.7° at end of 1 minute; then mouse struggled so much that thermometer was removed.
18-3	19.5	Feb. 28	12.33 P.M.	-3	37.5	37.5	45"	Mouse pretty quiet. Mercury rose to 37.5° in 15 seconds, and remained about thus for 30 seconds, falling to 37.4° near end.
18-3		Feb. 28	2.35 P.M.	-2.5	35.1	35.1	1'20"	Little movement. Temperature as recorded for last ½ minute.
18-3		Feb. 28	8.27 P.M.	20	38.5*	38.5*	1'45"	Much struggle. Thermometer had to be removed at last. A little blood on the bulb. Mercury paused after reaching 38.1°.
18-3		Mar. 1	3.35 P.M.	23	36.1	36.3	1'	Mercury rose to 36.1° in ½ minute, remaining there till near the end of 1 minute. Then mouse began to move considerably and mercury rose to 36.3°.
24-3	19.0	Feb. 28	12.40 P.M.	-3	37.3‡	37.8*	1'+	Record not very satisfactory. Temperature 37.3° when thermometer was inserted the proper distance. When pushed in further, it rose to 37.8°, falling again on withdrawal.
24-3		Feb. 28	2.40 P.M.	-2.5		36.4	1'20"	After reaching maximum of 36.4°, mercury fell to about 36.1°, but remained most of time about 36.3°.
24-3		Feb. 28	8.33 P.M.	20		38.1	1'30"	Mouse struggled somewhat. Mercury rose only from 38.0° to 38.1° in last 40 seconds.
24-3		Mar. 1	3.40 P.M.	23		37.2	1'30"	Mouse fairly quiet. Thermometer recorded 37.1° at end of 1 minute, rising only 0.1 degree in last ½ minute.
25-2	24.5	Feb. 28	12.45 P.M.	-3		37.3	1'30"	Mercury fluctuated within 1 or 2 tenths of a degree of 37.2° for last ½ minute at last.
25-2		Feb. 28	2.45 P.M.	-2.5		36.8*	1'15"	Mouse struggled much during latter part of time. Mercury reached 36.5° in about 1 minute, remaining here for nearly ½ minute, later rising to 36.8°.
25-2		Feb. 28	8.38 P.M.	20	37.1	37.5	1'35"	Mercury reached 37.1° in ½ minute, then slowly rose again. Mercury near the maximum (37.5°) for more than ½ minute.
25-2		Mar. 1	3.42 P.M.	23	35.7	36.2*	1'30"	Mercury reached 35.7° in ½ minute and 36.0° in 1 minute, then rose to 36.2° and fell to 36.1° in the next ½ minute.

TABLE 12

Ten cold-room males, both old and young (three and one-half months) lots, subjected to a single measurement each on the afternoon of February 28, shortly after the second test with the preceding series. The mice were removed directly from the nests; those of the younger set ("B") were running about more or less. Thermometer number 2 used, with rubber collar as before.

IDENTIFICATION NUMBER	DATE	HOUR	AIR TEMP. deg. C.	BODY TEMP.		DURATION OF INSERTION	REMARKS
				30 sec. deg. C.	Max. deg. C.		
21	Feb. 28	3.05 P.M.	-2	35.0*	35.0*	2'	Mercury reached 34.5° at end of 2/3 minute and remained there for some time, when it suddenly went up again, remaining at 35.0° for last 1/3 minute.
27	Feb. 28	3.12 P.M.	-2	36.1	36.1	1'	Temperature as recorded at end of 25 seconds. Rose no higher by end of 1 minute, despite much struggling.
29	Feb. 28	3.15 P.M.	-2	37.1	37.1	1'	Not much struggle. Temperature as recorded for last 1/3 minute. Little change during last 2/3 minute.
31	Feb. 28	3.22 P.M.	-2	36.8*	36.8*	2'30"	Maximum of 36.8° reached after struggles of mouse. Fell soon, however, remaining about 36.3° for last 1/3 minute.
33	Feb. 28	3.25 P.M.	-2	36.4	36.4	1'30"	Considerable movement. Temperature as recorded for last 1/3 minute; very little change during last 2/3 minute.
B 1	Feb. 28	3.35 P.M.	-2	36.4	36.4	1'20"	Mercury fluctuated within 0.1 of 36.3° for over 1/3 minute.
B 2	Feb. 28	3.40 P.M.	-2	36.8*	36.8*	2'	Much struggle. Mercury rose to 36.8°, then fell; did not remain at any point for as long as 1/3 minute.
B 3	Feb. 28	3.43 P.M.	-2	37.1*	37.1*	1'±	Mouse struggled with unusual violence. Mercury did not remain long at the maximum point.
B 4	Feb. 28	3.45 P.M.	-2	37.4	37.5	55"	Much tugging. Maximum 37.5°, but mercury at 37.4° for most of last 1/3 minute.
B 5	Feb. 28	3.47 P.M.	-2	36.9†	37.1	1'	Mercury remained between 36.9° and 37.1° for last half minute; was falling slightly at the end.

TABLE 13

*Warm-room young. Mice fourteen days old, or younger, were taken directly from the nest. Older animals may or may not have come from nest.*

IDENTIFICATION NUMBER	AGE	WEIGHT	AIR TEMP.	BODY TEMP.		REMARKS
				First rec.	Max.	
	hours	grams	deg. C.	deg. C.	deg. C.	
1-1	24 (-) days		27.0	32.4	32.6	
1-3	2-3		20-22	32.6	32.6	
2-1	7	2.9	25.0	35.0	35.6	Mother not in nest
2-2	7	2.9	25.0	35.0	35.0	Mother not in nest
14-1	8	3.0	22.0	36.6	36.6	Mother with brood
3-1	9	2.3	25.0	34.6	34.9	
3-2	9	2.6	25.0	34.6	35.1	
15-1	10	3.9	22.0	34.6	34.6	Mother with brood
1 (bis)-1	11	3.4	24.5	33.4	35.2	
8-1	11	5.1	29.0	36.1	36.1	
9-1	12	6.8	29.0	36.0	36.0	
6-1	13	4.8	26.0	35.8	35.8	Rectal temp. = 35.8°, visceral = 35.6°
6-2	13	4.6	25.0	34.3	34.3	
6-3	13	about as last	25.0	36.0	36.0	Rectal temperature
6-4	13	about as last	25.0	36.2	36.2	Rectal temperature
3-7	14	3.2	27.0	34.9	35.2	Mouse nursing
12-2	14	3.8	27.0	36.5	36.5	
6-3 (or 4)	16		29.0	37.5	37.5	Rectal temperature
7-1	18	3.7	25.0	36.0	36.0	Rectal and visceral temperatures identical
25-1	18	5.2	16.0	36.3	36.3	
26-1	18	10.3	16.0	36.6	36.6	
27.1	18	7.9	16.0	36.0	36.3	
28-1	18	4.9	16.0	35.8	35.8	Mother absent
28-2	18	4.9	16.0	37.6	37.6	
16-1	19	3.9	22.0	37.2	37.3	Rectal temperatures given. Visceral temp. = 36.5
16-2	19	3.5	22.0	35.6	35.6	
17-1	19	8.3	23.0	38.2	38.3	Rectal and visceral temperatures identical
17-2	19	9.2	23.0	37.9	38.5	Rectal temperatures. Visceral temp. = 38.3 to 38.4
7-2	21		29.0	37.0	37.1	Rectal temperature

TABLE 14

*Warm-room young. After exposure in warm room for fifteen to thirty minutes.*

IDENTIFICATION NUMBER	AGE	WEIGHT	AIR TEMP.	DURATION OF EXPOSURE	BODY TEMP.		REMARKS
					First rec.	Max.	
	<i>hours</i>	<i>grams</i>	<i>deg. C.</i>	<i>minutes</i>	<i>deg. C.</i>	<i>deg. C.</i>	
1-2	24 (-) <i>days</i>		27.0	15	31.0	31.8	Mercury did not rise in stem ("Number 1")
1-4	2-3		20-22	15			
2-3	7	2.9	25.0	30	30.1	31.5	
15-2	10	4.0	22.0	30	33.3	33.3	
8-2	11	5.0	29.0	30	35.9	35.9	
9-2	12	7.4	29.0	30	35.9	36.2	

TABLE 15

*Warm-room young. After exposure in warm room for one-half hour to one and one-half hours.*

IDENTIFICATION NUMBER	AGE	WEIGHT	AIR TEMP.	DURATION OF EXPOSURE	BODY TEMP.	
					First rec.	Max.
	<i>days</i>	<i>grams</i>	<i>deg. C.</i>	<i>minutes</i>	<i>deg. C.</i>	<i>deg. C.</i>
2-4	7	2.7	25	40	31.5	33.4
14-2	8	2.5	22	35	29.9	29.9
14-3	8	2.9	22	55	29.0	29.2
3-3	9	2.4	29	60	29.5	31.0
3-4	9	2.5	26	60 (+)	31.2	31.8
15-3	10	3.6	22	50	33.8	33.8
3-8	14	3.2	26-27	60	36.4	36.4
12-3	14	4.8	26-27	70	36.6	36.6

TABLE 16

*Warm-room young. After exposure in cold room for fifteen to thirty minutes*

IDENTIFICATION NUMBER	AGE	WEIGHT	AIR TEMP. (cold room)	DURATION OF EXPOSURE	BODY TEMP.		REMARKS
					First rec.	Max.	
	<i>days</i>	<i>grams</i>	<i>deg. C.</i>	<i>minutes</i>	<i>deg. C.</i>	<i>deg. C.</i>	
2-5	7	2.7	10.0	15	24.4	25.4	
14-4	8	2.8	9.5	15	23.2	23.6	
14-5	8	3.5	9.5	30	16.5±	18.0±	
4-1	9	4.1	14.0	20	29.1	31.6	
4-2	9	3.6	14.0	30	26.5	28.8	
4-3	9	3.7	10.0	20	22.1	26.1	
2-6	10	4.7	11-13	15	27.8	27.8	
15-4	10	4.3	9.5	15	25.6	25.6	
15-5	10	4.3	9.5	30	19.3	19.5	
1(bis)-2	11	3.3	10.0	20	21.1	21.1	
8-3	11	4.9	11-13	20	31.0	31.0	
10-1	12	3.9	11-13	25	28.3	28.4	
6-3 (or 4)	14		10.0	15	36.0	36.0	Rectal temperature
6-4 (or 3)	14	5.6	10.0	30	34.2	34.2	Rectal and visceral
3-5	14	3.2	6.0	15	28.4	28.4	
3-6	14	3.2	6.0	30	21.2	21.2	
12-1	14	6.2	6.0	25	33.6	33.6	
7-2	18		10.0	20	35.6	36.0	Rectal temperature
26-2	18	8.8	8.0	30	37.9	37.9	
28-3	18	4.7	7.5	30	35.3	35.3	

TABLE 17

*Warm-room young. After exposure in cold room for one-half to one and one-half hours*

IDENTIFICATION NUMBER	AGE	WEIGHT	AIR TEMP. (cold room)	DURATION OF EXPOSURE	BODY TEMP.		REMARKS
					First rec.	Max.	
	<i>days</i>	<i>grams</i>	<i>deg. C.</i>	<i>minutes</i>	<i>deg. C.</i>	<i>deg. C.</i>	
4-4	9	4.0	10	35	25.1	25.1	
1(bis)-3	11	3.4	10	40	19.6	22.1	
4-5	12	6.1	10-12	60	23.2	24.8	
9-3	12	7.4	10-12	40	31.4	31.9	
6-3 (or 4)	16		10-12	95	36.2	36.3	Rectal temperature
7-3	18	3.7	10	35	32.2	32.2	
27-2	18	7.2	8±	40	37.6	37.6	
28-4	18	4.3	7.5	40	33.4	33.4	
7-2 (?)	21		10-12	90	35.5	35.6	Rectal temperature



TABLE 18  
*Cold-room young. Directly from nest—the mother present*

IDENTIFICATION NUMBER	AGE	WEIGHT	AIR TEMP.	BODY TEMP.	
				First rec.	Max.
	<i>days</i>	<i>grams</i>	<i>deg. C.</i>	<i>deg. C.</i>	<i>deg. C.</i>
11-2	1 (+)	1.4	6	31.2	31.2
11-3	2 (+)	1.8	-1	31.5	31.5
5-1	5	1.7	10-10.5	34.0	34.1
13-1	11	3.4	1	31.4	31.4
20-1	13	5.2	8	35.6	35.6
21-1	15	4.6	7-8	35.0	35.0
22-1	18	4.1	7	32.1	32.1
23-1	20	5.9	7	35.3	35.3

TABLE 19  
*Cold-room young. Directly from nest—the mother absent*

IDENTIFICATION NUMBER	AGE	WEIGHT	AIR TEMP.	PERIOD OF ABSENCE	BODY TEMP.	
					First rec.	Max.
	<i>days</i>	<i>grams</i>	<i>deg. C.</i>	<i>minutes</i>	<i>deg. C.</i>	<i>deg. C.</i>
11-1	1 (+)	1.6	6	45 (+)	17.0±	18.5±
5-4	5	1.9	10	45	22.6	22.8
18-1	10	2.5	6	20	20.6	21.1
13-2	11	2.9	4	30	25.2	25.2
19-1	12	2.7	5-6	?	33.6	33.6
19-3	12	2.9	6	30 (+)	30.3	30.3
19-5	12	2.5	6	45 (+)	23.4	24.1
19-6	12	3.0	6	55 (+)	21.9	21.9
20-2	13	5.2	7-8	30	33.2	33.2
22-2	18	4.0	7	30	26.6	26.6
22-4	18	4.0	7-8	60	18.0±	18.0±

TABLE 20  
*Cold-room young. Exposed, uncovered, in cold room*

IDENTIFICATION NUMBER	AGE	WEIGHT	AIR TEMP.	DURATION OF EXPOSURE	BODY TEMP.		REMARKS
					First rec.	Max.	
	<i>days</i>	<i>grams</i>	<i>deg. C.</i>	<i>minutes</i>	<i>deg. C.</i>	<i>deg. C.</i>	
5-2	5	1.7	10.0	15(+)	23.4	24.0	
5-3	5	2.0	10.0	30(+)	17.5±	18.5±	
19-2	12	2.9	6.0	15	23.2	23.4	
19-4	12	3.0	6.0	30	15.5±	17.0±	
22-3	18	4.3	7-8	30	25.3	25.3	
23-2	20	5.4	7.5	30	37.0	37.0	
23-3	20	5.8	7-8	70	37.1	37.1	
24-1	21	8.8	7-8	3 or 4	36.8	37.2	Rectal temperature Rectal temperature (Visceral = 37.4)
24-1	21	8.8	7-8	30	37.8	37.8	



## FURTHER OBSERVATIONS ON ARTIFICIAL PARTHENOGENESIS IN FROGS

JACQUES LOEB AND F. W. BANCROFT

*The Rockefeller Institute for Medical Research, New York*

1. We have repeated our efforts to raise parthenogenetic frogs during the past season in order to be able to determine their sex, but with less success than we had last year. None of the parthenogenetic tadpoles which we produced this year lived as long as those of the previous year.

During the past two seasons we have punctured the eggs of *Rana sphenoccephala*, *R. pipiens*, *R. silvatica*, *Chorophilus feriarum* and of *Bufo americanus*. Only the eggs of the first two species could be utilized for our purpose, inasmuch as it is possible in them alone to obtain swimming parthenogenetic tadpoles from the punctured eggs. If the eggs of the other species mentioned are punctured they may begin to segment but they do not reach the tadpole stage. The frogs were caught in the open while copulating, the sexes separated and sent immediately to the laboratory. Some of the frogs (*R. pipiens*) were obtained in Long Island and some were sent from Chicago. The results were in both cases the same, in spite of the fact that the eggs of the frogs sent from Chicago must have remained in the uterus several days. From 600 to 1000 eggs of each female were punctured. The results were similar to those described in our last paper,<sup>1</sup> except that this spring all the swimming parthenogenetic tadpoles died when they were from twenty to thirty days old. Some of the eggs were smeared with blood before they were punctured. While

<sup>1</sup>Jour. Exp. Zool., vol. 14, p. 275.

the percentage of the eggs which underwent early segmentation was possibly augmented by this procedure, the percentage of the eggs which developed into tadpoles was not increased in this way. Practically all the eggs of the same females that had been fertilized with sperm developed into tadpoles, many of which are healthy and alive today.

2. We were anxious to obtain parthenogenetic frogs in order to test the statement of Kuschakewitsch,<sup>2</sup> to which Professor Morgan called our attention, that the young frogs are often hermaphrodites, or intermediate forms. If Kuschakewitsch's statement is correct, the presence of ova in the genital glands is not sufficient proof that the young frog is going to develop into a female. He gives some criteria for the discrimination between real females and the intermediate form which may develop into males. On the basis of his statements we examined once more the sections of the sexual glands of last year's parthenogenetic frog. We also utilized the slides we had made from the sexual glands of a young frog produced by fertilization with sperm from an egg of the same female from which the parthenogenetic tadpole and frog originated.

The gonads of the parthenogenetic frog which died at the close of metamorphosis were fairly well preserved. An examination showed that there were eight or nine genital spaces. According to Kuschakewitsch eight genital spaces are characteristic for the intermediate form while six are characteristic for the pure female. In addition there were many degenerating ova, which Kuschakewitsch mentions as a characteristic for the intermediate form. Comparatively few of the oöcytes had entered the growth period. The right gonad, which was smaller and in an earlier developmental stage than the left one, had nineteen larger oöcytes while the left gonad had only fifteen. Most of the oöcyte nests (or oögemmae as Kuschakewitsch calls them) did not contain any large oöcytes. If Kuschakewitsch's criteria are correct, the parthenogenetic frog which we described is a hermaphrodite or intermediate.

<sup>2</sup> Festschr. f. Richard Hertwig, Bd. 2, p. 61.

According to Kuschakewitsch these intermediates may develop into either males or females, and there are indications in the sections of the parthenogenetic frog that this transformation may have been under way. In the anterior part of the left gonad of the parthenogenetic frog the sections show all the appearances which Kuschakewitsch (p. 145) considers characteristic for the early stages of the transformation of the intermediate form into males. The endothelial lining of the secondary genital spaces is thicker than usual, more convoluted, and mitotic figures may be seen in places. In some regions considerable masses of undifferentiated embryonic tissue have been formed which are continuous with the endothelium of the secondary genital spaces and occupy almost the whole cross section of the gonad except for a few patches of germinative endothelium. If we may rely upon the statements of Kuschakewitsch, our frog would have developed into a male if it had survived.

The gonads of the parthenogenetic tadpole were so poorly fixed that nothing could be made out about them except that they contained many more large oöcytes than those of the frog. It could not be determined whether this individual was a female or belonged to the intermediate form.

The frog which had originated from a fertilized egg of the same female from which the parthenogenetic tadpole and frog were obtained, was killed about four months after metamorphosis. It had grown rapidly. The gonads were evidently those of a male. The spermatoc follicles contained cells in all stages of spermatogenesis from spermatogonia to spermatids with greatly elongated nuclei. In addition almost every section showed follicles which were nearly filled with a large oöcyte in the early growth period. Thus it appears that this male may have developed from a hermaphrodite or intermediate, if Kuschakewitsch's statements are correct.

In the European species, *R. esculenta*, the formation of spermatozoa does not take place for several years after metamorphosis while in the control frog this process was well under way about four

months after metamorphosis. It would seem, then, that in *Rana sphenoccephala*, which is a southern species, sexual differentiation and sexual maturity set in a little more rapidly than in *Rana esculenta*.

It is obvious that further observations are needed to determine definitely the sex of parthenogenetic frogs.

# HELIOtropISM, DIFFERENTIAL SENSIBILITY,<sup>1</sup> AND GALVANOTRISM IN EUGLENA

FRANK W. BANCROFT

*Department of Experimental Biology, Rockefeller Institute for Medical Research, New York*

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<sup>1</sup>Differential sensibility is used as synonymous with Unterschiedsempfindlichkeit.

## 1. INTRODUCTION

In 1904 Jennings published a paper in which he came to the conclusion that *Euglena*, among other light sensitive protista, did not react to light in accordance with the tropism theory, but by the method of trial and error. This view has been criticised by Holmes ('05), Torrey ('07) and Parker ('07, '11), none of whom, however, investigated especially the behavior of the organisms in question. On repeated occasions this view has been defended by Jennings and also by Mast who has reinvestigated the reactions of several *Euglenae* and of other protista studied by Jennings.

For reasons which will become clear in later parts of this paper it seemed important to compare galvanotropic and heliotropic<sup>2</sup> reactions in *Euglenae*. Accordingly cultures of *Euglena* were prepared in a number of different media in order to detect galvanotropism which has not yet been described for this genus. The reactions to both light and electricity of the *Euglenae* from some of these cultures were tested and a state of affairs was discovered, which, so far as I can see, is entirely incompatible with Jennings's ideas. For, instead of finding that, as Jennings supposed, the heliotropic turning takes place by a series of motor reactions, or that the heliotropic mechanism and the mechanism for the motor reactions were fundamentally similar, it appeared that in *Euglena* these two mechanisms are independent variables and can be modi-

<sup>2</sup>Heliotropism will be used here as it is used by Loeb, and most authors who use the word, to indicate a certain kind of reaction, entirely apart from the theory which may be adopted to explain the way in which the reaction takes place. Loeb makes his use of the term clear in many places. As an example may be quoted: "Unter den Tropismen der Tiere verstehen wir die zwangsmässige Orientierung gegen resp. die zwangsmässige Progressivbewegung zu oder von einer Energiequelle" ('10 p. 452). Jennings has finally ('09, p. 307) come to use the word in nearly the same way that Loeb does, saying "The tropism includes those reactions in which the organism takes and maintains a definite orientation." Mast, however, ('11) has not distinguished between the descriptive use of the term and the various theories and explanations that have been offered to account for it. Thus, in his section on "Various Definitions of Tropisms" (p.53) we find definitions of the term and theories to account for the reaction inextricably mingled. In this paper, then Heliotropism includes those reactions in which there is a compulsory orientation with respect to the light, no matter how that orientation may have been brought about.



fied independently. Thus, according to Jennings negatively heliotropic *Euglenae* always react by the motor reaction to sudden illumination, and not to sudden shading. Jennings's whole theory is based upon the association of negative heliotropism with motor reactions by illumination and of positive heliotropism with the motor reactions by shading. But it was found possible to produce at will negatively heliotropic *Euglenae* that react to sudden shading, and not to sudden illumination, by the motor reaction. Positively heliotropic *Euglenae* which gave the motor reaction only upon sudden illumination were also observed. These, as well as many other facts, are not at all in accord with Jennings's theories on the subject, but show that Torrey's ('07, p. 517) contention that *Euglena* is *unterschiedsempfindlich*, and nevertheless turns directly towards or away from light is correct. Thus it turns out that *Euglena* furnishes a beautiful example of both types of light reactions described by Loeb: and it will also be shown that the mechanism of these reactions is best explained by the theories on the subject advanced by Loeb.

## 2. HISTORICAL REVIEW

In this controversy concerning heliotropism in *Euglena* there are two questions which have sometimes been considered together, but which for clearness' sake it is essential to separate. They are:

1. Does *Euglena* become oriented to light as directly as its method of locomotion admits; or does it orient indirectly, by the method of trial and error? In either case the reaction will be heliotropic, but the method of orientation will be different?

2. Is heliotropism in *Euglena* brought about by response to temporal changes in light intensity, or is it caused by the continuous action of the light independent of changes in intensity?

It is only the first question that will be considered in this historical review as it is the answer to this that determines whether the heliotropism in *Euglena* is considered to take place after the manner advocated by Loeb and most of those investigators who consider the tropisms of great importance in the behavior of

lower organisms, or according to the trial and error method of Jennings. The second question constitutes another difference of opinion in the views of these two camps but it is of minor importance in comparison with the question to be considered under this heading.

This, as I conceive it, is the fundamental issue at stake, though it is barely possible that I may be doing Jennings an injustice in supposing that his conception of 'trial and error' excludes a direct heliotropic orientation. Thus, for instance, he says in summing up the heliotropism in infusoria ('04, p. 64), "It is not in accordance with the tropism theory above set forth. . . . The whole reaction is a strongly marked example of the type of behavior which may be called the 'method of trial and error.'" This seems perfectly clear, but the trouble is that in such cases Jennings is always combating the 'local action theory of tropisms' which it seems to me has not been held by most of the authors to whom it has been attributed by Jennings.

#### A. *The "local action tropism theory"*

Much as I dislike to digress for the correction of a misunderstanding, nevertheless a rather detailed discussion of the 'local action theory' is necessary, as silence on the subject has already been interpreted (Jennings, '08, p. 701) as assent; and in the absence of contradiction this misunderstanding is liable to continue to grow as it has, for instance, in the case of Mast ('11, pp. 41, 85).

Jennings says in summing up his section on the 'essential points of the tropism theory' ('04, p. 94), "We will here attempt to summarize these observations so far as they bear upon the essential points in the theory of tropisms. In particular we will ask, (1) Is the observed behavior brought about by orientation in the way the theory of tropisms demands? (2) Does the evidence show that the action of a stimulus is directly upon the motor organs of that part of the body on which the stimulus impinges?" It is this latter statement, that the tropism theory supposes the stimulus to act directly upon the motor organs that adherents of the

tropism theory object to. Parker ('07, p. 548) has objected to it, but Jennings thinks that he is not a good judge of what the general opinion on the question is ('08, p. 70). Loeb has objected to it ('07, p. 156, footnote), but Jennings thought that he referred to something entirely different ('08, p. 700, footnote). It is difficult to see how Jennings can have thought that such a man as Loeb, who entered the field of tropisms as a brain physiologist and worked mainly on the tropisms of animals with eyes, ever thought that "orientation is produced by the direct action of the stimulating agent on the motor organs of that side of the body on which it impinges" ('06, p. 266). Loeb certainly never thought anything of the kind, and has never written anything of the kind so far as I have been able to discover. He certainly does not make any such statement in any of the passages that Jennings quotes or refers to by page. Thus the passage from Loeb ('00, p. 7) that Jennings quotes on two occasions ('04, p. 92 and '06, p. 266) reads:

The explanation of them [the tropisms] depends first upon the specific irritability of certain elements of the body-surface, and, second, upon the relations of symmetry of the body. Symmetrical elements at the surface of the body have the same irritability; unsymmetrical elements have a different irritability. Those nearer the oral pole possess an irritability greater than those near the aboral pole. These circumstances force an animal to orient itself toward a source of stimulus in such a way that symmetrical points on the surface of the body are stimulated equally. In this way the animals are led without will of their own either toward the source of stimulus or away from it.

In this and many other<sup>3</sup> similar cases Jennings seems to think that 'certain elements of the body surface' means motor organs; though why he should think so I am at a loss to understand. Not only does Loeb refrain from supporting the 'local action

<sup>3</sup>In Jennings's ('06, p. 268) quotation from Davenport the meaning is less clear for Davenport speaks of darkened and illuminated muscles, though he does not say that it is the illumination of the muscles that directly causes their relaxation. But when the passage in Loeb ('93, p. 86) from which Davenport has taken his exposition is looked up we find: "Trifft das Licht eine Seite des Tieres, so gehen zunächst uns einstweilen unbekannte Veränderungen in den durchleuchteten Teilen vor sich. Die Folge ist eine Veränderung in der Spannung der Muskeln." So in this case "unbekannte Veränderungen" are taken by Jennings to mean direct

tropism theory' but on numerous occasions shows that the motor organs are not acted on directly by the stimulating agent. Thus on page 3 of the *Comparative Physiology of the Brain* from which work the passage quoted by Jennings is taken, Loeb says, "The flight of the moth into the flame is a typical reflex process. The light stimulates the peripheral sense organs, the stimulus passes to the central nervous system, and from there to the muscles of the wings and the moth is caused to fly into the flame. This reflex process agrees in every point with the heliotropic effect of light on plant organs. Since plants possess no nerves, this identity of animal with plant heliotropism can offer but one inference—these heliotropic effects must depend upon conditions which are common to both animals and plants." This quotation strikes the keynote of Loeb's attitude towards the tropisms. Since he believes the tropisms are fundamentally similar in animals and plants he has been careful to speak of the tropisms in general terms that might apply to either plants or animals. Jennings seems to have consistently misunderstood these general statements, and to have thought that they referred to only one of the things that were included in them.

With regard to Torrey, Jennings says ('08, p. 701) that he 'expressly defends the local action theory.' In his paper, however, Torrey never mentions the local action theory by name, and as I understand his paper the 'tropism hypothesis' that Torrey is writing about is the usual theory of tropisms and not the local action theory. I repeatedly discussed the question with Dr. Torrey both before and after his paper was published and know that he never believed in the local action theory.

In conclusion I would like to add that I am not a believer in the 'local action tropism theory.' I would even go farther than

stimulation of the muscles. I have looked over a number of the other papers to which Jennings refers ('06, p. 269) as advocating the local action tropism theory; and among the papers examined I found only one which Jennings has not misunderstood in a similar way. The single exception is the paper by Holt and Lee who do speak of the stimulation of the cilia by light. Thus, on the page referred to by Jennings, Loeb ('00, p. 186) says "The chemical effects of the diffusing molecules on certain elements of the skin influence the tension of the muscles." Here again Jennings takes "certain elements of the skin" to mean muscles, etc.

Jennings in my disapproval of it. For while he thinks that it holds in galvanotropism, I think it more probable that the cathodal reversal of the cilia in *Paramecium* is not brought about by the action of the current directly on the cilia, but on some intermediate mechanism. I do not wish to deny, however, that there may be some reactions such as those of *Ameba* mentioned by Jennings ('06, p. 306) for which the local action theory may hold.

*B. Position of Jennings and Mast on direct orientation*

Before proceeding to the experimental data it will be best to outline Jennings's and Mast's position so that the significance of my results may be correctly understood.

In his earlier papers Jennings found that most infusoria when encountering an obstacle react by what he called a motor reflex. The essential element of this reflex is the turning towards a structurally defined side of the organism. The direction of turning bore no relation to the part of the organism stimulated or the location of the source of the stimulus. The stronger the stimulus the greater the amount of turning; but the exact point at which the turning stopped and the usual forward locomotion began bore no relation to the location of the source of stimulation. Often the infusorian would be brought repeatedly in contact with the stimulus and repeated motor reflexes would result. In such a reaction the final direction of progression may be said to be due to the selection of random movements; for the direction of turning and the final direction of progression are with respect to the position of the stimulus determined only by chance. In subsequent papers this type of reaction was called by Jennings 'trial and error.'

Later Jennings found ('04, p. 31) that *Stentor* when subjected to an increase in illumination gave the typical motor reflex and that it also became oriented in a uniformly illuminated field by means of a series of motor reflexes. The turning was always towards a structurally defined side, and after each reflex the animals found themselves in the most various positions. But if the light was shining on their anterior ends the reflex was always repeated.

When by chance some reflex left the animals turned away from the light the motor reflexes stopped, and the forward swimming was resumed. This was called orientation by the method of trial and error.

But when Jennings, in the latter half of the same paper came to investigate the behavior of *Euglena* an essentially different reaction was observed. *Euglena* executed motor reflexes similar to those described for *Stentor*. But the organism also became oriented in a uniformly illuminated field by swimming in a sweeping curve which soon brought it into orientation without the selection of any random movements. However, in spite of the regular nature of the orienting process, Jennings puts this gradual orientation of *Euglena* also into the category of 'trial and error.' As it moves in its spiral course the swervings to or from the light are interpreted as trials, erroneous if away from, successful if towards the light.

Now it is to be noted that this is an entirely different kind of 'trial and error' from that previously discussed, and it has been criticised by a number of investigators. Thus Holmes ('05, p. 110) says:

But to view the matter in this way is to go far towards obliterating the distinction between orientation through trial and error and orientation by the direct method. In the mode of phototactic response here considered *Euglena* does not react by a number of indiscriminate movements until the right one is accidentally hit upon, but by a direct reflex whose effect is to bring the organism more nearly parallel to the direction of the rays.

Torrey also ('07, p. 317) has objected to this point of view. He says:

Jennings's figure indicates that *Euglena* is both unterschiedsempfindlich and heliotropic. At *a* (fig. 1) the reversal in the direction of the light which has been coming from the direction in which the creature has been swimming produces a sudden change in the intensity of stimulation, a shock which results in the swerving from the previous course as indicated between *a* and *c*. The organism recovers rapidly only to be subjected to the constant stimulus of a steady light from one direction to the end of the experiment. The result of the action of the constant stimulus is a path from *c* to 5 . . . . perfectly in harmony with the tropic schema.

In his reply to Torrey, Jennings does not meet Torrey's objection squarely, but still makes his position very plain. In his explanation of the figure to which Torrey refers, Jennings says that "At 3 [= *c*] the normal amount of swerving is restored." Now it is the part of the curve from *c* to 5 in which the normal amount of swerving obtains to which Torrey expressly refers, but Jennings in reply to him says ('08, p. 705): "The most direct way in which the organism, swimming in a spiral, could become oriented to the light would be by an increase in the swerving to the right and a decrease in the swerving to the left, and this is what the tropism theory would lead us to expect. But the fact is that there is an increase in the swerving both to the left and to the right, the spiral becoming a wider one; the increase to the right being, however, greater than that to the left, the organism becomes gradually pointed to the right. The increased swerving to the left is not accounted for by the tropism theory, and is indeed squarely opposed to it, while it is to be expected if the analysis I gave is correct." Here we see that Jennings in attempting to meet Torrey's objection concerning the part of the course from *c* to 5 points to the behavior during the previous part of the course from *a* to *c* the additional swerving in which Torrey has interpreted as due to *Unterschiedsempfindlichkeit* and not in accordance with the tropism schema. It is evident, however, that this does express clearly and fairly Jennings's views. He thinks that the part of the course from *c* to 5, in which there is the normal amount of swerving, is not direct orientation because in the previous part of the course there was additional swerving in both directions. Since this additional swerving was considered a motor reaction the later swervings which are only towards the light are interpreted as motor reactions also.

But even this evidence in favor of the trial and error conception has been removed by observations of Mast ('11, p. 104) who finds for *Euglena* that the orientation "takes place just as Jennings represents (fig. 12 [= fig. 1]) with the exception that if the direction of the rays is changed without any change of the intensity, orientation may take place without an increase in the diameter of the spiral course represented in fig. 12 (= fig. 1) *a-c*."

Mast, however, instead of interpreting his observations in favor of direct orientation, says ('11, p. 99) for the orientation of *Euglena* in the crawling state which he considers 'in all essentials' like that of the free-swimming individuals: "When the organism is not oriented every change from a position in which the light strikes the ventral surface to one in which it strikes the dorsal, and vice versa, due to rotation on the long axis, may be considered a 'trial movement.'"

To resume then: 'Trial and error' which at first was used to designate the selection of chance movements, has now been extended to include reactions in which the only elements of trial consist in the normal spiral swimming in an unoriented position. It might be justifiable to call such unoriented spiral swimming trial and error if it were not for the fact that in all cases trial and error is contrasted with direct orientation, and the two used as mutually exclusive terms. In this way trial and error has been extended to include the very mechanism of locomotion through direct orientation, if it were possible, would have to act; and the only way that *Euglena* could orient directly according to this view would be by growing some other locomotor mechanism that would permit of progression in a straight line.<sup>4</sup> I think that it is evident that these objections to Jennings's view are conclusive and that the criticism of Holmes, Torrey and Parker is well founded.

The comparison of galvanotropic with heliotropic orientation has also been used by Jennings to show that the heliotropic orientation is not direct. Thus, speaking of the infusoria in general he says ('09, p. 314):

In galvanotropism the organism usually does turn as directly as possible into the position of orientation. It turns directly towards the cathode (or anode as the case may be)—whatever the position of its body when the electric current comes into action. But in phototropism the position of orientation is attained *by the same organisms* in an entirely

<sup>4</sup>It seems to me that this attitude of Mast is just as unjustifiable as to maintain that a man who is walking towards some stairs does not ascend the stairs directly but by 'trial and error,' because in bipedal locomotion his head bobs up and down to a certain extent; and to maintain that this bobbing up and down represents a series of trials and errors one of which is followed up.



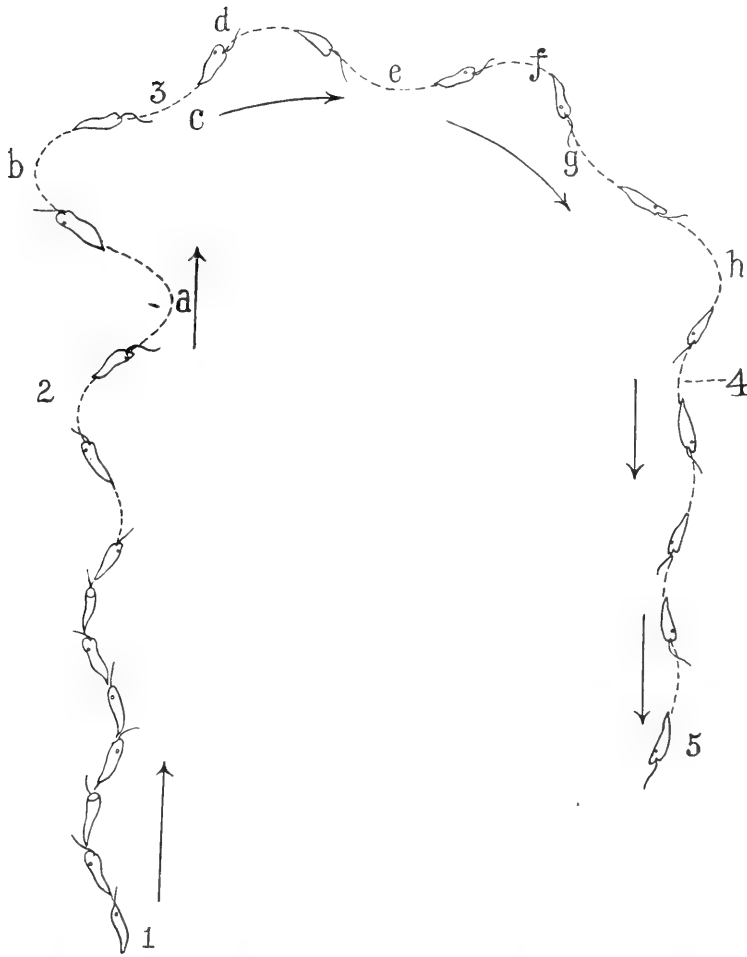


Fig. 1 After Jennings ('04, p. 57). "Path followed by Euglena when the direction of the light is changed. From 1 to 2 the organism swims forward in the usual spiral path. At 2 the position of the source of light is changed, so that it now comes from behind. The organism then begins to swerve farther than usual toward the dorsal side, owing to the decrease in the illumination of the anterior end. Thus the spiral becomes wider, *a* and *b* showing the limits of the swerving. At 3 the normal amount of swerving is restored. . . . Now the organism swerves at each turn of the spiral a short distance away from the source of light, as at *c*, *e*, *g*, and a longer distance towards the source of light, as at *d*, *f*, *h*. . . . At *h* it has in this manner become directed toward the source of light, and there is no further cause for swerving more to one side than the other; it therefore swims in a spiral with a straight axis toward the source of light."

different manner. The organisms do not turn as directly as possible into the oriented position. They reach the position of orientation by a complex spiral course, always turning towards the aboral side, never toward the opposite side. Yet it is not impossible for them to turn toward the opposite side, for in galvanotropism they do so.

In another place ('08, p. 706) Jennings says, "Since *Euglena* itself has not been shown to react to electricity we cannot make the comparison here."

We shall see, however, that by the proper procedure *Euglena* may be made to react to electricity; and that under these circumstances it always turns towards the dorsal side just as in the heliotropic orientation.

### 3. METHODS AND MATERIALS

The only species studied was *Euglena viridis*. Both material and specific diagnosis I owe to the kindness of Prof. G. N. Calkins of Columbia University, to whom I wish to express my most sincere thanks.

This material was cultivated in many of the different media described by Zumstein ('00), but the cultures most used were:

*Culture A.* Boiled timothy hay infusion which did not develop a putrefactive odor, but during the experiments always had a pleasant aroma, and was slightly acid to litmus paper. Kept in medium light. *Euglenae* taken from this culture were, unless specially treated, always negatively heliotropic to any intensity of light to which they responded at all.

*Culture B.* About 20 dried yellow peas boiled for several hours in one liter tap water. Citric acid added to make the whole decoction about  $\frac{M}{500}$ . Kept in strong light. In about two weeks the whole fluid was dark green with *Euglenae* which were always positively heliotropic to weak and negative to strong light.

Other decoctions of both hay and peas in which the initial concentration of citric acid was as high as  $\frac{M}{100}$  and  $\frac{M}{50}$  also gave good results, especially with galvanotropism.

The only treatment of the organisms that was attempted in order to show more clearly the various phenomena studied was dilution of the culture medium with tap water, and subjection to

darkness or light for varying periods. Subjection to darkness or light for over night was particularly satisfactory in bringing about reactions that were not obtained in other ways.

In order to prevent evaporation especially in those cases where the same individuals were observed for several days, the *Euglenae* were usually examined in hanging drop preparations.

In attempting to repeat these observations it should be borne in mind that, since I have not been working with chemically known culture media free from bacteria, it may frequently happen that several cultures prepared of the same material in the same way may give different results. Consequently, to obtain the results described, it may frequently be necessary to modify the procedure here described. But the main facts are so clear, and there are obviously so many different modifiable photochemical reactions involved, that I feel convinced that any one who will work with *Euglenae* possessing different kinds of photo-sensitiveness, and systemically subject them to other modification of the environment beside that of light, will be able to repeat all the results here described and to bring to light many additional phenomena which have so far eluded observation.

#### 4. NORMAL LOCOMOTION

In an unstimulated condition *Euglena viridis* swims through the water in a narrow spiral constantly swerving towards its dorsal side (larger lip) which thus faces approximately towards the outer side of the spiral. To many changes in its environment it reacts by the shock-movements<sup>5</sup> pointed out by Engelmann and Jennings which usually consist in stopping, swerving in the usual direction through an angle of 90 degrees or more, and then swimming ahead again. If the stimulus is very strong the organism may swim backwards a little before turning, and the body may also contract. In the weakest shock-movements merely a slight temporary widening of the spiral is seen.

All of these movements are produced by the contractions of the single flagellum which emerges from near the anterior end of the

<sup>5</sup>Used as synonymous with Engelmann's 'Schreckbewegung' and Jennings's 'motor reflex.'

body (fig. 2) and extends posteriorly along the ventral side; not anteriorly as indicated in Jennings's ('04, '06) figures. That this is the actual position of the flagellum was determined by watching the motion of india ink particles which had been added to the culture fluid (fig. 3); and also by observing the flagellum directly after adding the viscid Irish moss solution<sup>6</sup> (fig. 2, a). When the fluid was made so viscid that the *Euglenae* cou'd barely swim some of the flagellae ceased beating continuously and in these indi-

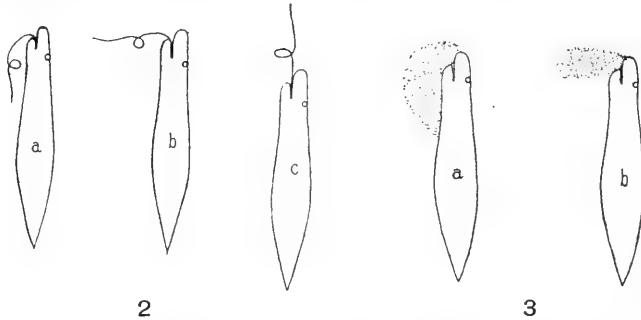


Fig. 2 Diagram showing the position of the flagellum as seen in a viscid medium; *a*, when *Euglena* is swimming forward in a narrow spiral; *b*, when swerving sharply towards the dorsal side; *c*, when moving backwards.

Fig. 3 Dotted area, shows the position of the moving india ink particles. *a*, when *Euglena* is swimming forward in a narrow spiral; *b*, when swerving towards the dorsal side during a shock-movement.

viduals it could occasionally be seen that loops were formed near the proximal end of the flagellum, which loops then rapidly progressed towards the tip. It seems, then, that the flagellum goes through about the same motions as a rope one end of which is free, and the other end held in a hand which from time to time describes small quick circles in a plane perpendicular to the rope (Bütschli). It will be seen that such a motion would furnish both a forward component and one which would tend to rotate the *Euglena* on its long axis. It is also evident that the *Euglena* can never swim

<sup>6</sup>Normally the flagellum seems to work as close to the ventral side of the organism as it is possible for it to do; for india ink particles that are in contact with the ventral surface are set in motion by its vibrations. When swimming in a narrow spiral unilateral stimulation does not diminish the slight swerving towards the dorsal side.

straight ahead but that it is only through the turning on its long axis that the resultant of all its various swervings may become a straight line.

But, while the motions of the rope appear to be identical with those of the flagellum, the mechanism is certainly different. For in the flagellum the loop may stop when it is half way down the flagellum and then go on again. It looks as if the contractile tissue of the flagellum were arranged in a spiral as it is in the stem of *Vorticella*. In this case, however, the whole of the spiral does not contract at once as in *Vorticella*, but a wave of contraction progresses from the proximal to the distal end.<sup>7</sup>

The shock-movements are produced by a very simple mechanism which acts merely by diminishing in various degrees the curvature of the proximal part of the flagellum. In the Irish moss and india ink solutions it could easily be seen that during the usual shock-movement the flagellum extended at right angles to the body (fig. 2, b); and that when the organism moved backwards the flagellum was extending forwards (fig. 2, c). The behavior of the flagellum during slight widenings of the spiral was not directly observed but there can be little doubt that it is caused by the flagellum being directed obliquely backwards. Thus we see that apart from the contraction of the body, the characteristic response of the locomotor mechanism to stimulation consists in a diminution in the curvature of the proximal part of the flagellum.<sup>8</sup>

It is clear then that *Euglena* turns towards the dorsal side because its locomotor mechanism is such that it cannot turn in any

<sup>7</sup>It should be noted that in the action of the flagellum of *Euglena* we have nothing of the nature of the stroke and recovery of an oar. Each loop of contraction as it reaches the tip of the flagellum leaves this organ ready for the next so that there is no need for a recovery. Thus the locomotion, in this species of *Euglena* at any rate, cannot be likened to that of a boat whose bow is provided with a single oar which is dipped first on one side and then on another as is done by Verworn ('03 p. 534). But its locomotion must rather be likened to that of a submarine propelled by a backward driving screw situated at one side of the bow.

<sup>8</sup>A similar locomotor mechanism has been described by Úlehlá ('11, p. 674) in *Euglena deses*. But in this species the flagellum is extended at right angles to the long axis during the normal forward swimming; and points forward in varying degrees in order to bring about the shock-movements.

other direction. But this does not mean that *Euglena* must necessarily orient by 'trial and error' and cannot orient directly for, as we shall see, this organism does orient as directly as its method of locomotion permits.

#### 5. THE FACTS OF HELIOTROPIC ORIENTATION

Jennings maintains that the gradual orientation of *Euglena* takes place by means of 'trial and error' and consists in a series of shock-movements. If this is true then we should expect that under various conditions the shock-movements and the gradual orientation would vary together, and be influenced similarly by the different factors of the environment. On the other hand, Torrey maintains ('07, p. 317) that we have here a complex of differential sensibility (*Unterschiedsempfindlichkeit*) and direct heliotropic orientation. If Torrey is correct then it ought to be possible by appropriate means to separate these two factors and to influence one without influencing the other. Consequently my plan of work consisted in subjecting *Euglenae* from various cultures to different environments and noting whether the gradual heliotropic turning and the shock-movements could be influenced independently of each other.

#### *A. Correlation between the sign of the heliotropism and the sign of the differential reaction<sup>9</sup>*

Jennings found that positively heliotropic *Euglenae* always give the motor reaction when suddenly shaded, and not when suddenly illuminated. He maintains that in positive heliotropism the effective stimulus consists in the increased shading of some photosensitive region while the infusorian is swerving away from the light. The response is a shock-movement which manifests itself as an increased swerving towards the light during the next half of the spiral revolution. Similarly in negative heliotropism the swerving away from the light is the shock-movement caused by

<sup>9</sup>By differential reactions are meant reactions caused by temporal change in the intensity of the stimulus. In this case they include reactions to both shading and illuminating.

the increased illumination produced by swerving towards the light during the previous half of the spiral revolution. Consequently, according to this view, positive heliotropism is conditioned by and should be accompanied by shock-movements produced by shading (= shading reaction), and negative heliotropism should always be accompanied by shock-movements produced by sudden illumination (= illumination reaction).

It has been found, however, that this usual association of shock-movements with tropism is not a necessary one, but that it can be destroyed if the proper means be taken. Consequently the view that the heliotropic swerving is a shock-movement must fall.

When *Euglenae* from Culture B were placed in the rays of the arc light, at a distance of four or five feet from the light, they were strongly positively heliotropic and gave the shading reaction ( $\frac{+}{S}$ , B., fig. 4). When, however, they were gradually brought nearer to the light a point was reached at which the heliotropism disappeared but the shading reaction persisted ( $\frac{0}{S}$ , B., fig. 4). When moved still closer to the light they became negatively heliotropic but still without any change of the shading reaction (fig. 4, B.,  $\frac{-}{S}$ ). When moved still closer to the light, there was a short time when no shock-movements could be obtained (Fig. 4, B.,  $\frac{0}{S}$ ), but soon the illumination reaction appeared ( $\frac{-}{I}$ ). At the same time the negative heliotropism became more prompt and precise. Finally, when the light was still further increased and allowed to act for a considerable time, even the illumination reactions frequently disappeared completely ( $\frac{-}{I}$ ), and a most pronounced and compelling negative heliotropism held full sway.

The difference between the intensity of the light required to reverse the heliotropism and that required to change the shading into the illumination reaction varied with different samples, and with the same sample at different times. With hanging drops of culture fluid and *Euglenae* which had just been taken from the jar this difference was sometimes quite small so that care was needed to reverse the sign of the tropism without changing the shading reaction, and even then the negative heliotropism was sometimes only poorly developed. But when the same prepara-





motor reaction when shaded, and then to become oriented again and to swim away from the light when the shading screen was removed.

It might be contended that when the individuals of Culture B are shaded, the weaker light resulting from the shading, makes them positively heliotropic, and so of course they give the shading reaction. While this objection can be met, it is hardly worth while to consider it at length here, for two other observations have been made to which this objection cannot possibly apply.

Ordinarily the only heliotropism that could be observed in individuals of Culture A was the negative, and the only differential reaction was the illuminating reaction (fig. 4 upper A). But in a few exceptional samples most of the *Euglenae*, while preserving their negative heliotropism, gave the illuminating reaction in a strong light, and a pronounced shading reaction in a weaker light (fig. 4 lower A). Since these individuals never become positively heliotropic the above objection will not hold.

In one exceptional sample from Culture B the individuals did not exhibit the usual negative heliotropism when subjected to the strong arc light; but became still more strongly positive. When they were shaded by interposing a screen, no shock-movements resulted. But when the shading screen was removed the *Euglenae* gave pronounced shock-movements, and then rapidly became oriented, and swam towards the light. Here also the above objection will not hold, because the light which produces the illumination reaction is the same in which they become oriented towards the light.

It is very evident, then, that the invariable correlation of positive heliotropism with the shading reaction, which is required by Jennings' theory, does not exist. But both kinds of heliotropism may be associated with either the shading or the illumination reaction. Accordingly, it must be concluded that the heliotropic mechanism does not depend upon the mechanism for the shock-movements, but that the two mechanisms are independent.

*B. Correlation of gradual heliotropic orientation and differential reactions with respect to the presence and absence of these two factors*

We have seen that there is no necessary correlation between the sign of the heliotropic and differential reactions which co-exist in the same individuals. We have now to consider whether heliotropic Euglenae always show some kind of a differential reaction (either a positive or a negative one) or whether we may have the one without the other. According to Jennings since the heliotropic orientation is a shock-movement of a somewhat modified kind it ought to be possible to find some kind of a constant relation between shock-movements and tropism.

Although usually the cultures studied were heliotropic in light of ordinary intensity it occasionally happened that some cultures when treated in certain ways exhibited no heliotropic reactions that I could be sure of. In one of these cases the medium was pea decoction in  $\frac{M}{1000}$  citric acid. The Euglenae showed no tropism or only doubtful traces of it in diffuse day light, and required a strong arc light to bring about certain heliotropism. In the weaker light, however, the shading reaction was at all times conspicuous.

With Euglenae from Culture B it was always possible to bring about the absence of heliotropism by subjecting them to light which was just too bright to cause the positive and too weak to cause the negative tropism. In this case, as we have already seen in the previous section, the shading reaction persisted with undiminished vigor. Consequently the absence of tropism in these two cases cannot be attributed to the failure of the shading reaction; and we see that it is possible for the differential reaction to exist without the tropic one. Similarly even in strong light in which over 99 per cent of the individuals are strongly negative a few individuals are often seen in which no heliotropism can be observed but which give pronounced illuminating reactions.

On account of lack of time only one experiment was made to test the correlation of tropism and differential reaction on the positively heliotropic Euglenae from Culture B. Two hanging

drop preparations were subjected to the light of an incandescent electric light bulb, removed foot by foot, and at each distance the diminution in tropism and shading reaction noted. The tropism and shading reaction were found to weaken and disappear together until at ten feet they were absent in one preparation and could just be made out in the other. Moreover, as the light diminished it could be seen that the individuals which were still heliotropic were the same ones which still gave the shading reaction. Here then we have exactly the kind of a result that Jennings's theory demands. But this does not mean that the shading reaction must always accompany such positive heliotropism. For if plants of this kind be put in the strong arc light for five or ten minutes and then brought back into the weak light, they become positive at once but fail to give the shading reaction for two or three minutes.

Most of the work on this correlation was done on the negatively heliotropic organisms of Culture A. From the outset they behaved entirely differently and not at all as required by Jennings's theory. They were not so sensitive to light but would show a feeble negative heliotropism at one or two feet from a glowing carbon filament. To light stronger than this they showed a decided negative heliotropism which became more pronounced as the light became stronger. But in bright diffuse daylight, and even occasionally in direct sunlight no illuminating reaction could be obtained. It was only in the arc light which had been strongly concentrated with a lens that shock-movements were produced by sudden illumination. When first subjected to this strong light all the individuals gave the illuminating reaction with the greatest precision. When, however, a strong light was allowed to act continuously for half an hour or an hour, the illuminating reaction was seen to become weaker and weaker until in some cases it entirely disappeared in nearly all of the individuals.

We see, then, that the shading reaction may be strongly developed where there is no heliotropism; and also that both positive and negative heliotropism can be regularly obtained without any differential reactions at all. It is obvious that no constant correlation exists between the heliotropism and the differential reac-

tions as is required by the theory of Jennings; but that we have to deal with two separate mechanisms that can be influenced separately to a certain extent. The correlation which does seem to exist between these two reactions in positively heliotropic *Euglenae* in weak light merely serves to emphasize the lack of any regular correlation for all conditions such as Jennings's theory demands.

### *C. Sensitization, fatigue and recovery*

Some photochemical systems require the action of the light for a certain time before they acquire the power to react to the light in their usual way. They are said to require sensitizing. Some substances are quickly destroyed by light, others more slowly. Some reactions are irreversible, and when they have been completed in an organism cannot take place again until the metabolism has furnished a new supply of the reacting substances. Other reactions are reversible and may take place in one direction in the light and in the other in the darkness, etc.

In the organism the phenomena of sensitization for the response to light, of the fatigue in reactions to light and of the recovery from fatigue are undoubtedly functions of some such reactions as those enumerated above. If, as Jennings supposes, the differential light reactions and heliotropic orientation are essentially the same reaction and differ only in degree, then we should expect that in both of them the same photochemical substances are involved, and both should behave in the same way in regard to sensitization, fatigue and recovery.

When preparations from Culture B were brought into the light after having been kept in the dark over night or longer the shading reaction often developed in less than a minute while no tropism could be made out for ten or fifteen minutes. But this is not conclusive because additional experiments are required to prove that the light intensity employed was not just between the intensity needed for positive and negative heliotropism. On one occasion, however, perfectly distinct heliotropism developed before any shading reaction was developed, and here we can be sure that sensitization has taken place more rapidly for the heliotropic mechanism than for the mechanism which produces the shading reaction.

The same is true for results obtained in the few preparations from Culture A that developed the shading reaction (p. 401). When these preparations were brought into a weak light the shading reaction was immediately observed but the heliotropism did not become distinct enough so that I could be sure that it was negative for five or ten minutes. Since there was no positive heliotropism in these individuals the objections urged above do not hold, and here we have another clear case of unequal sensitization.

It was only occasionally that anything like fatigue was seen for the heliotropic reactions. Thus in four or five cases some preparations from Culture A that had been kept in the dark developed positive heliotropism, while other preparations which, so far as possible, had been treated in exactly the same way failed to develop it.<sup>10</sup> In all of these cases the positive heliotropism was quite transient. It disappeared after five or ten minutes of illumination and could not be obtained again.

When positively heliotropic *Euglenae* from Culture B are subjected to stronger and stronger light the positive tropism disappears before the negative heliotropism comes on. It may be that this disappearance of the positive heliotropism should be interpreted as fatigue. But as no especial attempt was made to determine whether the usual positive and negative tropism are separate phenomena or simply portions of the same phenomenon it will be useless to discuss the matter here.<sup>11</sup>

For the negative heliotropism in individuals from all the cultures nothing of the nature of fatigue has been observed. The stronger the light, the stronger the tropism until the organisms are killed or become immobile as the result of the intense stimulation. But the fatigue of the illuminating reaction, which usually accompanies

<sup>10</sup>In one of these cases the positive heliotropism appeared most distinctly in the arc light and was changed to negative when the weaker incandescent light was used! This reversal was accomplished several times before the reaction became indistinct.

<sup>11</sup>In general I have written as if there were but a single mechanism for all kinds of gradual heliotropic orientation, and another one for the differential reactions. I should like to state, however, that this is only done to prevent circumlocution. I should not be at all surprised if it should turn out that there are different mechanisms for the shading and the illuminating reactions as well as for positive and negative heliotropism, or even for the different kinds of positive heliotropism. For

the negative tropism in strong light can be easily accomplished, as described on pages 400 and 402 above, by subjecting the plants to an intense light for half or three-quarters of an hour.

This fatiguing of the illuminating reaction was sometimes obtained with the hanging drop cultures, but was obtained better when the *Euglenae* were exposed to the light in an open trough of tap water. It is thus possible in this case that there may be a chemical or osmotic as well as a light effect.

We see, then, that in weak light the mechanism for the heliotropic and the shading reactions become sensitized at different rates; and that the negative heliotropism cannot be fatigued under conditions which easily fatigue the accompanying illuminating reaction. It is evident that here again Jennings's theory will not account for the facts.

#### *D. Orientation with and without shock-movements*

We have seen on page 391 that Jennings found that when beginning to orient to light from a new direction *Euglena* at first swims in a wider spiral which is evidently to be interpreted as one or more shock-movements.<sup>12</sup> With the negative material from Culture A I found it easy to obtain this reaction when an arc light was allowed to shine suddenly on weakly illuminated individuals. The first result of the bright light was a widening of the spiral which soon merged into the narrow spiral swimming which completed the orientation. We have seen (p. 391) that Mast found that orientation may take place without any widening of the spiral. This observation I confirmed for the negative individuals from Culture A in a weak light. In this light the illuminating reaction could not be demonstrated.

instance, it seems very probable that the photochemical substances responsible for the temporary positive tropism in individuals of *Culture A* described above are quite different from those responsible for the usual positive heliotropism which can be obtained at will with weak light.

<sup>12</sup>For mechanical, chemical and most other stimuli Jennings interprets such a widened spiral as a single shock-movement. But in positive heliotropism he interprets each part of the spiral in which the swerving is towards the light as a single shock-movement. It seems to me that in this case also the whole of the widened spiral represents a single shock movement.

I then tried to determine whether it was possible to obtain orientation without the widened spiral in the arc light. It was found that when *Euglenae* that were oriented in the arc light were subjected to a gradual change of direction by rotating the stage of the microscope on which the slide was resting, the gradual orientation was frequently seen without a trace of the initial widening of the spiral. In this way the most convincing demonstrations of gradual orientation without any shock-movements or widening of the spiral were possible. With the bright light the orientation is much more prompt and precise; so that in these cases 80 per cent or 90 per cent of the organisms were oriented. When the stage was turned through an angle of 90 degrees in three or four seconds the *Euglenae* would respond to the changed direction so quickly that they would remain oriented throughout the turning, or would become oriented within a few seconds after the turning stopped. In this way hundreds of *Euglenae* were seen to orient with only a very occasional shock-movement and in a light intensity which was capable of producing shock-movements in all of them when it was allowed to shine suddenly on weakly illuminated organisms. Thus, we see that also in bright light, which is capable of producing shock-movements, the mechanism underlying the gradual orientation is more active than in weaker light; and if sudden changes of intensity and direction are avoided this mechanism may cause prompt and precise orientation without the appearance of any shock-movements. We thus get additional evidence for considering the mechanism just discussed different from that producing the shock-movements.

#### *E. Time required for stimulation*

As we have seen, Jennings supposes that the stimulus for the heliotropic orientation consists in the changes of light intensity which take place as *Euglena* swerves towards or away from the light in each half of a spiral revolution. Each of these stimuli is then supposed to cause a short shock-movement lasting during the next half of the spiral revolution. If this view is correct then it should be possible to produce shock-movements by sudden il-

lumination lasting not longer than the time required for *Euglena* to make half a revolution on its long axis.

The negatively heliotropic *Euglenae* from Culture A (which were the only ones available when these experiments were performed) made on an average ten revolutions about their long axis in 5.8 seconds. Half a revolution then would require 0.29 second. It should be possible, then, to produce shock-movements by sudden illumination lasting not longer than 0.3 second.

The attempt was made to time the stimulus with a photographic focal plane shutter. But the longest illumination which the shutter would give automatically was 0.1 second; and it was never found possible to obtain shock-movements with such a short illumination. Consequently the method of procedure consisted in opening the shutter, leaving it open, and then timing the interval between the beginning of illumination and the first shock-movement with a stop watch. The method is unsatisfactory, but the results were so clear that further refinement was dispensed with.

With a comparatively weak arc light which was just strong enough to produce the illuminating reaction in most of the individuals, but which caused a pronounced negative heliotropism, it always required an illumination lasting at least 1.0 or 1.4 seconds before the first illuminating reactions appeared. With a much stronger light and sensitive unfatigued individuals the reaction was obtained after 0.5 and 0.4 second. It was probably obtained after still shorter illuminations, though I could not succeed in making the stop watch register less. However, the time could not have been less than 0.1 second for the automatic shutter failed to give results. It is evident that the time required for stimulation with the weaker light is much longer than the 0.3 second which Jennings's theory demands. Consequently, since effective stimulation must take place within the time interval of 0.3 second, it cannot be the shock-movement mechanism that is stimulated, but it must be some more sensitive mechanism that is responsible for the gradual heliotropic orientation. Furthermore, since the time required for effective stimulation evidently varies inversely with the light intensity and since *Euglena* is markedly heliotropic



to much weaker light than the weaker arc light used in these experiments, it is evident that Jennings's theory is still less able to account for the heliotropism in these weaker intensities.

*F. No blending of shock-movements and tropism as the light is diminished*

If, as Jennings believes, the gradual swerving is a series of shock-movements, then each swerve must be considered a much weaker shock-movement than the usual kind in which the change of direction is much greater. Presumably, then, each swerve towards or away from the light during the gradual orientation is produced by a smaller photic stimulus than the usual shock-movements. I therefore studied the shock-movements in very bright light and in light which was just able to cause the shock-movements, in order to see if, with the weaker light, the shock-movements tended to take on the character of the slight swerving which produces the gradual orientation. The results, however, were the reverse of what was to be expected according to Jennings's view.

Euglenae from Culture A were observed in the hanging drop in the light of the arc lamp. An incandescent electric light at some distance gave just enough illumination to see the Euglenae when the arc light was cut off by interposing a screen. The arc light was allowed to produce orientation, then the screen was interposed. An individual which had not changed its orientation was selected, and observed after the screen had been removed again.

With the weaker arc light, the sudden action of which sufficed to produce shock-movements in about half the Euglenae, the orientation was good in the majority of the individuals. When the oriented individuals were suddenly illuminated, in the way indicated above, the shock-movements usually threw them out of orientation, and the orientation was only gradually regained by the slow swerving process. Thus in 20 shock-movements that were closely observed only 7 left the organism oriented. In four cases two or three shock-movements instead of a single one followed the sudden illumination; and in two of these four cases the first shock-movement left the organism oriented, but was followed by another movement which destroyed the original orientation.

The shock-movements were in many cases not a mere widening of the spiral, but the motion seemed to be in a plane. The shock-movements also in most cases did not merge into the usual spiral swimming, so that it was easy to tell when the shock-movement stopped and the gradual swerving began. We see, then, that in this light intensity which just sufficed to produce the shock-movements the latter were incoördinated and variable, usually threw the plant out of orientation and showed but little tendency to merge into or resemble the usual spiral swerving.

When the same experiment was repeated on the same plants with a brighter light, the sudden action of which always produced a marked shock-movement in all the *Euglenae*, quite a different result was obtained. Here the orientation was more universal, rapid and precise. After sudden illumination the shock-movements of both oriented and unoriented individuals were of a more orderly character, and in almost every case consisted in nothing more than a pronounced widening of the spiral which rapidly merged into the oriented spiral swimming. Here then we have with the brighter light the phenomenon which, according to Jennings's point of view, was to be expected with the weaker light. The explanation would appear to be that the mechanism which underlies the direct gradual orientation responds more strongly to a bright light, so that it can largely counteract the excessive swerving which the shock-movements might otherwise produce. This masking of the shock-movements is probably also facilitated by the more rapid tiring of the shock-movement mechanism described above.

#### *G. How is Euglena kept oriented?*

Parker ('11, p. 462) in criticising Mast ('11) says "Nor is it anywhere made clear how an organism, after it has once become oriented, can continue to move in a straight line without involving the essential elements of the tropism theory." Mast ('12, p. 211) in reply to Parker says "I have stated my position regarding this matter referring to *Euglena* (p. 230) briefly but clearly as follows: 'The reactions caused by changes of intensity result in directing the organisms toward various points of the compass. As soon as

they reach a position in which the rotation on the long axis no longer causes a change of intensity on the sensitive region there is no longer any cause for turning; they therefore continue in this direction." The first point then to be determined about *Euglena* in this connection is whether it stays oriented because it has got started that way and there is no cause for turning, or whether it is kept oriented by the light.

It is easy to devise experiments which seem to answer this question in favor of Mast. If a light of medium intensity be used and a hanging drop free from débris then it can frequently be seen that, when the orienting light is cut out after the *Euglenae* are half way across the drop, the plants continue on their course and reach the farther side of the drop nearly as directly as if the orienting light were still shining.

But it is just as easy to devise experiments that prove that the light does keep the *Euglenae* oriented even in the presence of stimuli which are constantly tending to destroy the orientation. If a small open trough be filled with tap water and a drop of culture fluid containing débris be mixed with the tap water then a very uneven environment is produced in which the *Euglenae* swim about haltingly giving a constant series of shock-movements as they encounter the particles of débris and the dissolved substances diffusing from them. If now a strong enough arc light is directed along the trough the organisms are nevertheless compelled to orient themselves very accurately and to move away from the light. The foreign particles are so small that, when the *Euglenae* fail to respond to the mechanical and chemical stimuli, the particles are brushed aside and the organisms are not thrown out of orientation. When, however, the orienting light is now cut off, the orientation is rapidly lost on account of the shock-movements produced by the uneven environment. There is no reason for thinking that while the orienting light shines, the stimuli that cause these reactions both before and after suddenly cease. We must conclude, therefore, that the mechanism for heliotropism does actively maintain the orientation in the face of disturbing stimuli. Since the plants remain well oriented there are no temporal changes in illumination, and

so this mechanism cannot depend upon shock-movements which are the responses to temporal change of intensity. There remains then only the mechanism for direct heliotropic orientation which is capable of explaining these phenomena.

### *H. Galvanotropism and heliotropism*

Hitherto no one, so far as I know, has succeeded in demonstrating galvanotropism in *Euglena*. I also failed to get certain galvanotropism in many cultures but obtained good galvanotropism in others. *Euglenae* from Culture A gave a weak temporary anodal galvanotropism lasting only from three to twelve minutes, but only when the individuals were taken from dense gatherings. The scattered individuals gave nothing. Good permanent cathodal galvanotropism was obtained from the following cultures:

1. Hay infusion in  $\frac{M}{500}$  citric acid, ten days old.
2. Pea decoction in  $\frac{M}{100}$  citric acid, nineteen days old.
3. Pea decoction in  $\frac{M}{50}$  citric acid, thirty-five days old.

Good permanent anodal galvanotropism was also obtained with individuals from (2) and (3) which were examined in chondrus (Irish moss) jelly and tap water respectively. But no attempt was made to work out the conditions responsible for cathodal and anodal galvanotropism.

When these strongly cathodal *Euglenae* were first subjected to the current their response to the reversal of the current was more rapid and accurate than any heliotropism which I have seen. But as the experiment continued the rapidity of reversal became progressively less and less until soon they turned in sweeping curves just as *Euglena* becomes oriented to light of medium intensity. Later the galvanotropism became still weaker, individuals could no longer be seen to reverse when the current was reversed and it was only by watching for some time that it could be seen that most of the plants had turned towards the new cathode.

Cathodal individuals that were orienting in sweeping curves were examined in chondrus jelly under a 4 mm. Zeiss objective. Under these conditions the flagellae and all the details of the orienting reaction could be most distinctly seen. *Euglenae* moving

at right angles to the current lines were observed, and the current made when the dorsal side was towards the anode. No change was seen until the revolution on the long axis brought the dorsal side towards the cathode when a more vigorous motion of the particles around the flagellum could be seen, and at the same time a small abrupt turn towards the cathode (fig. 5). It took four or five of these turns to cause complete orientation. When the organism was nearly oriented each jerk of the flagellum seemed to

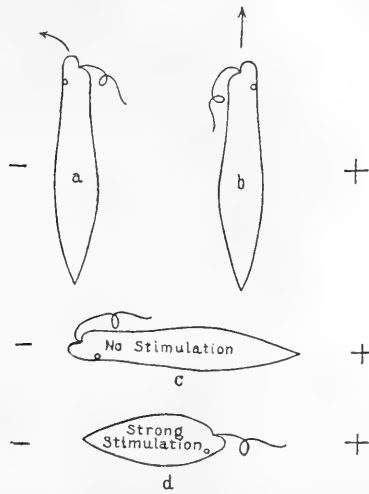


Fig. 5 Behavior of cathodally galvanotropic *Euglenae* in galvanic current. + = anode; - = cathode; a = dorsal surface towards cathode—slight stimulation of flagellum accompanied by stronger swerving towards the dorsal side; b = dorsal surface towards anode—no stimulation; c = anterior end towards cathode—no stimulation; d = anterior end towards anode—strong stimulation.

cause less turning than when it was at right angles to the current lines. When the dorsal side was towards the anode the organism still swerved a little towards the dorsal side, but not so strongly as when this side was towards the cathode. It is evident from this account that the details of the galvanotropic orientation are identical with the heliotropic orientation as described by Mast, and which I can confirm. *Euglena* has no more direct way of orienting than that employed in heliotropism. Jennings's con-

trast between heliotropic and galvanotropic orientation will not hold for this organism.

There remains to be made out the pole of the organism at which the current stimulates, and the location of the sensitive region, the stimulation of which brings about this reaction. The sensitive region is probably in the vicinity of the base of the flagellum. If the current is made when the *Euglena* has its anterior end towards the cathode very little if any change is produced. If, however, it is made when the anterior end is towards the anode there is intense stimulation (fig. 5, *c* and *d*). The flagellum extends forwards, the *Euglena* swims backwards a little and then contracts. Thus there can be no doubt that in this case the stimulation is at the anode just as it is in *Paramecium* that has been subjected to citrates for a short time (Bancroft, '06).

Consequently as the stimulation in the transverse *Euglena* takes place when the dorsal side is towards the cathode, we must suppose that at this moment the spot where stimulation occurs is anodal with respect to that part of the body from which it receives its stimulating ions. In this way we see that, given the normal locomotor mechanism, all that is necessary to make cathodal galvanotropism possible is the presence of some substance which is affected by certain anions in such a way as to increase the turning effect of the beat of the flagellum. But this substance must be so located that it is anodal to the stimulating anions when the dorsal side of the *Euglena* is turned towards the cathode.

### *I. Conclusions*

The facts of the light reactions of *Euglena* described by Jennings and Mast have been confirmed in all cases in which they were reinvestigated. No differences of opinion exist as regards these facts. But in interpreting the facts Jennings and Mast have explained them by means of a theory which does give a formal explanation but is supported by no evidence beyond the facts which it was intended to explain; and which, as we have seen, is not capable of standing the strain of explaining the additional facts brought forward by experiments especially devised to test the correctness of the theory.

I think it may be said, then, that the main facts concerning heliotropic orientation are:

1. The locomotor mechanism of *Euglena* is of such a nature that the organism always swims in a spiral and responds to all effective stimuli by additional swerving towards the dorsal side. It cannot turn in any other direction.

2. The mechanism for the gradual heliotropic orientation is different from and is independent of the mechanism that produces the differential light reactions.

3. This heliotropic mechanism, as we may call it, is of such a nature that, in unoriented negatively heliotropic *Euglenae*, it stimulates the flagellum when the dorsal side of the organism is turned from the light. In other words, we must suppose that the heliotropic mechanism like the galvanotropic mechanism is more easily excited when<sup>13</sup> the light is coming from one direction. In the case of negative *Euglenae* the stimulation takes place most easily when the light shines on the ventral surface; in positive individuals when the light shines on the dorsal surface.

It is to be noted that in this method of heliotropic orientation there is nothing of the nature of the selection of random movements. When the spiral swimming brings the ventral surface of negative *Euglenae* towards the light stimulation occurs and the plant turns from the light. In other positions this response cannot take place, hence the turning is always away from the light and orientation is thus accomplished. The reaction is perfectly definite and stereotyped. It contains nothing of the nature of 'trial and error' if by 'trial and error' is meant anything more than the normal spiral swimming by means of which the ventral surface is repeatedly brought to face the light. On the contrary, it is perfectly clear that during the gradual heliotropic turning

<sup>13</sup> Note that I do not say *by* light coming from one direction. It may be that the difference between positive and negative heliotropism is that in one case the light causes stimulation and in the other it prevents stimulation. There are many other hypotheses possible which are capable of experimental verification. Since the experiments are lacking the description of the heliotropic mechanism must be formulated in the most general way, and such a formulation does not mean any more than it says.

*Euglena* does orient as directly as its locomotor mechanism allows it.

But this locomotor mechanism is imperfect, it forces the organism to move in a spiral, and always to turn towards a structurally determined side. There are many organisms which swim in spirals and become oriented by turning towards a structurally defined side. Jennings and Mast include all such orientations under 'trail and error' and contrast them with the direct orientation of such animals as the amphipods in which the turning may be either towards the left or the right. Let us now consider whether the orientation of *Euglena* is more like the selection of random movements (which we would all agree may justifiably be called 'trial and error'), or whether it is more like the orientation of the terrestrial amphipods studied by Holmes ('01).

I think that all students of behavior including Jennings and Mast believe that in the case of these amphipods we have direct heliotropic orientation. If the right eye of such a positively heliotropic amphipod be covered with asphalt varnish it will execute circus movements towards the left. The usual explanation is that the main nervous connection is between the eye on one side and the legs on the opposite side of the body. The light shining on the uncovered eye brings about a condition of increased muscular tonus in the legs of the opposite side, which is not present in the legs connected with the covered eye. Consequently the right legs push more strongly and the amphipod turns towards the left.

Suppose now we remove some or all of the left legs from an amphipod of this kind so that it will always turn towards the left, and transfer it to water in which it must be supposed to swim in a spiral path. We will then have an organism which would become oriented in essentially the same way that *Euglena* does. The animal would always swerve towards the left. But, when the spiral course brings it into such a position that the light shines directly on the left eye, the muscular tonus of the right legs would be increased and the swerving towards the light would increase. Thus orientation would be effected in just the same way that it is in *Euglena*.



While these hypothetical changes that must be made in the amphipod, to make it react like *Euglena*, are considerable they concern only the details. The fundamental nature of the photochemical substances, the nature of their stimulation and the character of their connection with the locomotor organs have none of them been modified. All that has been done is to make an asymmetrical organism swimming in a spiral out of a bilateral one. These changes are much less fundamental than those which we would have to imagine in order to make an amphipod orient to light by the selection of random movements. In order to bring about this latter change the whole nature of the photochemical substances and their relations to the leg muscles would have to be modified. In the one case the required changes are all of a mechanical nature and so simple that the experiment might possibly succeed. In the other case the required changes are largely chemical, and so complex that we have no data for even imagining what ought to be done in order to bring them about.

Differences such as those between the intact amphipod, the mutilated amphipod and *Euglena* have always appeared as of minor importance to Loeb and to most of the advocates of the importance of the tropisms. Their fundamental similarity has appeared to them so self-evident that the special case of the asymmetrical animal moving in a spiral has not usually been considered.<sup>14</sup> To Jennings and Mast, on the other hand differences such as these have appeared so important that they appear to have prevented these two investigators from recognizing the fundamental similarity of the reactions of *Euglena* and the amphipods.

#### 6. THE NATURE OF THE STIMULUS

In this section we have to consider whether the stimulus which causes the heliotropism of *Euglena* consists in temporal changes of light intensity or in some continuous action of the light which is independent of changes in intensity. The difference between

<sup>14</sup>Torrey, however, in a forthcoming paper in *Science* does consider this question and shows that the orienting mechanism in *Euglena* is identical with that producing the direct orientation in bilateral organisms.

these two types of stimulation and between the reactions which depend upon them has long ago been pointed out by Loeb ('93, pp. 100-103). The power to react to differences he calls *Unterschiedsempfindlichkeit* (differential sensibility). In the tropisms, on the other hand, he thinks the stimulus is some constant or continuous action of the light<sup>15</sup> ('97, p. 400).

It is not always easy to determine whether a given reaction is produced by changes in the light or by its continuous action. It may even be possible that future work will show that we have here only one, and not two fundamental reactions. But, in the present state of our knowledge, these two types of reactions appear to be fairly distinct.

*A. Criteria for distinguishing reactions due to differential and constant light reaction*

Loeb ('10, p. 465) has pointed out that if it can be shown that the Bunsen and Roscoe law holds for any reaction then we have the best evidence that we are at present capable of furnishing that the reaction depends upon the continuous action of the light. According to this law the photochemical effect depends upon the quantity of light. If the light intensity is constant its effect depends upon the duration. If the duration is constant the effect is proportional to the intensity. If both intensity and duration of the light vary the photochemical effect is a function of the product of the two. Unfortunately it is not easy to apply this law to heliotropism and so far it has only been found to hold for the heliotropism of several plants (Blaauw '08; Fröschel '08).<sup>16</sup>

<sup>15</sup> But Loeb does not think, as has been assumed by some writers, that if a tropism is shown to be due to differential sensibility that by definition it ceases to be a tropism.

<sup>16</sup> But so little is the importance of this law realized by Mast that he says ('11, p. 258): "It should however be emphasized again that in no case has it been demonstrated that orientation is 'a function of the constant intensity' as maintained by Loeb." The truth is that in all those cases where it has been found possible to demonstrate which of these two light effects has been responsible for the orientation it has been found that the orientation is "a function of the constant intensity." And of these cases the one which has been most conclusively demonstrated and which (granting the correctness of the facts) cannot possibly be disproved in our

The best criterion that we have for determining that changes of intensity constitute the stimulus is that the reaction shall be produced when the change of intensity is rapid but shall not be produced when the change is made gradually. This is the usual criterion employed in electrical stimulation. Its validity has been pointed out for light stimulation by Loeb ('97, p. 439-441) and it has been used by Jennings ('04, p. 52) and by Mast ('11, p. 94) for the shock-movements of *Euglena*. While this criterion is perfectly satisfactory and conclusive wherever it is applicable there are reactions to which it cannot be applied. Accordingly, in these cases, other less satisfactory criteria have to be employed, and we may now pass to an examination of some of these.

Mast says ('11, p. 215) "In *Euglena* . . . it was demonstrated that the orienting stimulus is due to a change of light intensity." But so far as I can see all the evidence adduced is: (1) Orientation takes place by shock-movements for which the stimulus is acknowledged to be change of intensity; (2) As the *Euglena* revolves on its long axis some of its organs are subjected to changes of illumination. The sudden swerving which is responsible for orientation bears a definite relation to these changes and hence must be due to them (Mast '11, p. 104). The first argument has already been disproved in the previous chapter. The second is of no value in this connection because the changes in illumination also subject the photosensitive organs to light of different absolute intensities. It may well be that the continuous action of strong light<sup>17</sup> causes the sudden swerving while neither the action of the weaker light nor the change of intensity is capable of producing the reaction. The light may exert its continuous action even when the intensity is not constant. The law of Bunsen and Roscoe may still hold even when the light intensity varies.

present state of knowledge is contained in the paper of Blaauw to which Mast refers in his book in another connection. The fact that Mast could make such a statement as that quoted shows the need for the consideration of the criteria for distinguishing these two reactions.

<sup>17</sup>Lasting of course only for the fraction of a second during which the photosensitive organ is turned towards the light.

This may perhaps be made clearer by a mechanical analogy. Let us imagine a spring which holds a vessel into which water is flowing. Now we may say that the water acts on the spring in two ways: (1) It produces oscillations in the tension of the spring if the changes in the amount of water in the vessel are made with sufficient rapidity. (2) It exerts a constant pull due to the total amount of water in the vessel; and it continues to exert this continuous pull, which is different from the effect produced by the changes, even when the amount of water in the vessel is changing and the vessel may be oscillating in response to these changes.

Other criteria for deciding the nature of the stimulus are employed by Mast in his paper on the firefly ('12, p. 271, 270). He says: "The males do not orient when exposed to continuous illumination." "These results, together with the fact that orientation in the male ordinarily does not begin until after the flash of light which induces it has vanished, demonstrate conclusively that the process of orientation and the direction of locomotion after orientation are not regulated by the continuous action of the light, and that these reactions are consequently not in accord with Loeb's theories of tropisms according to which orientation is, as he puts it ('06, p. 135) a function of the constant intensity.' Stimulation which results in orientation in the fireflies studied is unquestionably due to changes in light intensity much as it is in *Stentor*, *Euglena* and many other similar organisms." Here the criteria that were apparently employed in deciding the question are:

1. Lack of orientation to continuous illumination.
2. Short duration of orienting light.
3. Orientation occurs after the orienting illumination has ceased.

Of these criteria the last two are entirely without value in deciding this question for Blaauw has shown that for *Avena* the Bunsen-Roscoe law holds for light duration of  $\frac{1}{4000}$ ,  $\frac{1}{8000}$  and even  $\frac{1}{10000}$  of a second. Moreover, in many of his experiments the orientation regularly took place after the light which caused the orientation had ceased to act. And yet Blaauw proved by means of the Bunsen-Roscoe law that in this case the orientation was a

function of the constant intensity of the light. The first criterion *may* be of value in deciding this question, but it is not necessarily conclusive. A discussion of the possibilities involved would not be advisable here. But it may be well to point out that Mast's conception of how it is that the stimulation is unquestionably due to changes in light intensity is somewhat difficult to understand in the light of his additional statement that the factor which is primarily involved in allowing the male to recognize the flash of the female to which he becomes oriented is probably the duration of the flash.

### *B. Experimental data*

*a. Continuous widening of the spiral.* In the experiments mentioned on page 30 in which the mechanism for the shock-movements was fatigued without fatiguing the heliotropic mechanism, another phenomenon was observed which is of interest in this connection. After the Euglenae had been subjected to the light for half an hour or more some were noticed to be moving away from the light in a wider spiral than usual. If these individuals were watched as the arc light was shaded the wide spiral was at once seen to change to the usual narrow type. But it reappeared with the bright light and was seen to persist as long as the light did. No attempt was made to observe this wide spiral for longer than one or two minutes. During that period the width of the spiral was frequently seen to diminish gradually; but its width was still sufficient so that there was no doubt that the spiral was suddenly narrowed when the strong light was shaded. When these selected Euglenae were first subjected to the arc light the usual response was a weak shock-movement, manifesting itself as a very wide spiral, which rapidly merged into the moderately wide spiral which lasted as long as the bright light did. In a few individuals, however, in which the mechanism for the shock-movements was still more fatigued, it was found possible by slowly removing the screen from in front of the arc light to bring about a *gradual* increase in the width of the spiral. In these cases there was no sign of shock-movements, and the wide spiral persisted during the intense illumination.

Here, then, we have a widening of the spiral which is undoubtedly due to the continuous action of the light; for during the whole reaction the *Euglenae* remained accurately oriented<sup>18</sup> to the arc light and consequently there was no opportunity for any part of the organism to be subjected to changes in light intensity. Furthermore this continuous light affects the locomotor mechanism in the same way that it is affected during the gradual heliotropic turning. There is a slight widening of the spiral which stops instantly when the light is reduced and does not continue for an appreciable time after the stimulus as is usually the case with the motor reactions. The only difference is that during the heliotropic turning the plants are not oriented and so they swerve more during one portion of the spiral than during the other.

But while this reaction does prove that the continuous light action may cause a widening of the spiral, and makes it very probable that it also causes the gradual heliotropic turning, it does not prove that the heliotropism is caused by the continuous action of the light. For, during the heliotropic orientation, the lateral illumination gives an opportunity for the sensitive region to be subjected to changes of intensity resulting from the revolution on the long axis; and it might be urged that these changes furnish the orienting stimulus.

It is not contended that the mechanism which underlies this reaction is identical with the heliotropic mechanism. For, heliotropism ordinarily occurs without this widening of the spiral during illumination, and conversely some of the exceptional individuals which are not heliotropic even in a bright light occasionally show the widened spiral during illumination.

However, it may be that this mechanism is identical with that which controls the gradual heliotropic orientation. We know that the stronger the light and the longer it lasts the more pronounced the negative heliotropism becomes. It may be that this continuous widening of the spiral is merely the last step in the increase of the strength of the negative heliotropism. But what this

<sup>18</sup>These experiments were performed with horizontal parallel rays and the *Euglenae* were placed in a trough with parallel glass sides so as to reduce the lateral light to a minimum.

reaction does prove is that the continuous action of the light may affect the locomotor mechanism of *Euglena* in the same way as it is affected during the gradual heliotropic turning.

*b. Orientation maintained by continuous light action.* On page 411 it was shown that in bright light the *Euglenae* are kept oriented in spite of stimuli tending to destroy that orientation. Here it should be emphasized that since the organisms remain oriented, and there is consequently no opportunity for any photosensitive region to be subjected to changes in light intensity, that the orientation must be maintained by the continuous action of the light. In this case it seems probable that the mechanism which maintains the orientation is identical with that which produces the orientation, for in strong light all the heliotropic individuals remain oriented, and as the light intensity is increased the prompter turning which results is always accompanied by the maintenance of the more accurate orientation. Accordingly, since these two phenomena always vary together, and since the maintaining of the orientation is undoubtedly a function of the continuous light action, we have very strong evidence that in *Euglena* the heliotropic turning is also a function of the continuous action of the light as has been maintained by Loeb for many other organisms.

*c. Heliotropism and variations in the rapidity of the change in illumination.* The more rapidly *Euglena* revolves upon its long axis, the more rapid will be the changes in light intensity. If it is these changes in intensity which are responsible for the orientation, then it might be possible by decreasing the rapidity of the revolution on the long axis to abolish the heliotropism without preventing locomotion. A positive result would tell strongly in favor of Jennings's theory, but a negative result would not be decisive either way. My results were negative but are given here because the method seemed to be the most promising one for settling the question.

Hanging drop preparations were made up of mixtures of culture fluid and Irish moss jelly containing one-half, three-quarters and seven-eighths of the jelly. These mixtures reduced the forward locomotion more than they did the revolution on the long

axis. But in all of them a certain amount of forward swimming was possible and in all of them negative heliotropism occurred in spite of the fact that in the drops containing seven-eighths of the jelly the speed of revolution about the long axis was reduced from once in half a second to once in  $2\frac{1}{2}$  or 3 seconds. When the jelly was made so stiff that no swimming was possible the changes in the shape of the body were so extensive that nothing about the effect of the light on the flagellum could be made out.

### *C. Conclusions*

So far as I know the only evidence that Jennings and Mast have presented to show that the stimulus which is responsible for the gradual heliotropic orientation of *Euglena* is a change in light intensity is:

1. The stimulus for the motor reactions is a change in the light intensity.
2. The gradual heliotropic orientation is essentially a series of motor reactions.
3. The sudden swerving which produces the gradual heliotropic orientation is accompanied by changes in the light intensity, and therefore may conceivably be produced by these changes.

The main evidence and the main argument is included under 2. Consequently with the demonstration in the previous chapter that the gradual heliotropic orientation depends upon quite a different mechanism from the motor reactions all of the real evidence presented by these authors falls to the ground.

On the other hand it has just been shown that in favor of the view that the gradual heliotropic orientation is a function of the continuous action of the light we have the direct evidence that:

1. The continuous action of the light is capable of producing a continuous widening of the spiral which lasts as long as the light.
2. The continuous action of the light keeps the *Euglenae* oriented in the face of influences which tend to destroy the orientation.



Since no one has yet been able to show that the Bunsen and Roscoe law holds for the gradual heliotropic orientation of *Euglena*, or that this reaction is abolished when the changes in illumination which accompany it are made more gradual, it cannot be said that the nature of the stimulus has been conclusively demonstrated. It may be said, however, that at present we have no evidence against the view that in *Euglena* the gradual orientation is a function of the continuous action of the light, and that we have strong evidence in favor of this view.

In *Euglena* we are not familiar with the anatomical relations that allow light coming from one direction to stimulate more effectively than light coming from another. But most eyes, including our own, are so arranged that the light produces its maximum effect when coming from some one particular direction. As a result of this fact, in both ourselves and *Euglena*, revolution about the long axis in light coming from one side will subject the photosensitive substances to changes in light intensity (Jennings, '09, p. 318). But it does not follow from this that it is the changes in light intensity which constitute the stimulus for light perception or for reactions to light. In ourselves we know from the researches of Bloch and Charpentier that the law of Bunsen and Roscoe holds good for the threshold of the perception of light of very short duration. Each case must be independently investigated; and in the case of *Euglena* we have seen that what evidence we have is all in favor of the view advocated by Loeb that the heliotropism is a function of the continuous action of the light.

## 7. SUMMARY

*A. Direct orientation vs. 'Trial and error'*

According to Jennings's theory positive heliotropism is conditioned by motor reactions produced by sudden shading; and negative heliotropism by motor reactions to sudden illumination. He considers that the gradual heliotropic orientation is a series of motor reactions. It has been found, however, that:

1. There is no necessary relation between the sign of the heliotropism and the character of the motor reactions. Positive heliotropism may be accompanied by motor reactions to either sudden shading or sudden illumination; and the same is true of negative heliotropism.

2. The ability to react to sudden changes in illumination by means of motor reactions is possessed by *Euglenae* which are not heliotropic. Conversely there are heliotropic *Euglenae* that cannot be made to respond to changes in illumination.

3. The mechanisms for heliotropism and for the motor reactions in response to light behave quite differently as regards sensitization, fatigue and recovery.

4. If sudden changes of intensity and direction of the light are avoided, orientation may be brought about without any evidence of motor reactions, and this under circumstances which are perfectly capable of producing these motor reactions if the changes are sudden.

5. In a weak arc light which produced good heliotropism and motor reactions the time required for sudden illumination to bring about motor reactions was three times the time required for half a revolution of the organism on its long axis. Hence it is impossible for the gradual heliotropic orientation to be produced by a series of motor reactions.

6. According to Jennings's theory the heliotropic swerving must be considered weak motor reactions. But as the intensity of the light is diminished there is no tendency for the character of the gradual orientation and motor reactions to resemble each other, they remain perfectly distinct.

7. Jennings and Mast hold that *Euglena* keeps oriented because "there is no longer any cause for turning." It was shown, however, that in an uneven environment the orientation disappears within a few seconds after the light is cut off. As during illumination the orientation in the same environment is precise, the orientation must be maintained by the light.

8. Jennings thinks that heliotropic orientation in *Euglena* is not direct because among the ciliate infusoria galvanotropic orientation is more direct than the heliotropic orientation. In *Euglena*, however, it was found that the mechanism of galvanotropic orientation is identical with that of heliotropic orientation.

9. Hence it must be concluded that:

a. The mechanism for producing heliotropic orientation is different from the mechanism for producing motor reactions, and one does not depend upon the other.

b. The gradual heliotropic orientation of *Euglena* does not take place by 'trial and error' but is as direct as the locomotor mechanism of the organism will allow.

### *B. The nature of the stimulus*

Jennings and Mast have come to the conclusion that changes in the intensity of illumination are the stimulus that is responsible for the heliotropic orientation of *Euglena*. It was shown, however, that:

1. The criteria employed by Jennings and Mast are of no value in deciding whether the stimulus consists in the changes or the continuous action of the light.

2. On the other hand, in favor of the view that the heliotropism is due to the continuous action of the light there is the evidence that:

a. Continuous light may cause a gradual widening of the spiral which lasts as long as the light does.

b. The continuous action of the light is responsible for *maintaining* the orientation of *Euglena* in an uneven environment.

3. It is thus evident that there is no valid evidence against the view that heliotropism in *Euglena* is due to the continuous light action; and there is strong evidence in favor of it.

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## DILUTION EFFECTS AND BICOLORISM IN CERTAIN EYE COLORS OF DROSOPHILA

T. H. MORGAN AND C. B. BRIDGES

*The Zoölogical Laboratory, Columbia University*

As shown in a colored plate in an earlier paper,<sup>1</sup> there is striking bicolorism in the eye color called eosin. In pure cultures the eye color of the female is a yellowish pink while that of the male is a pinkish yellow, more translucent, and less intense. It was shown further, that when an eosin female is crossed to a white male, the sons are of the ordinary color for eosin males, but the daughters instead of being red or even dark eosin, are of the same color as the sons. The sons are eosin, because eosin is a sex-linked character, that is, the factor which differentiates between eosin and red is carried by the sex chromosome, unpaired in the male of *Drosophila*. The one sex chromosome which the son has, came directly from his mother, both of whose sex chromosomes bore the factor for eosin.

The explanation at that time offered was that the dark eye color of the mother was due to the fact that she was duplex with regard to the 'color producer' (i.e., the allelomorph dominant to white) while the light color of the son was explained as the result of his being haploid for that same factor. It was assumed that the white eyed flies 'lacked the color producer,' consequently the daughters from the cross of colored females by white males should be haploid with regard to the color producer. Their composition should then be the same as that of the brothers, that is, both should show the color of the eosin male. But if we assume that

<sup>1</sup> Further experiments with mutations in eye-color of *Drosophila*. Jour. Acad. Nat. Sciences, Philadelphia, vol. 15, Second Series, November, 1912.

it is the presence of the color producer in simplex in the male, and in duplex in the female, that is responsible for bicolorisms in the eosin why then are not other sex linked mutations such as vermilion, also bicolored, for the same relation obtains there?

To account for the fact that the daughters were eosin and not red in this cross of eosin female by white male, we assumed that all white flies 'lacked' not only the color producer but a color determiner also, i.e., white was assumed to be a double recessive. We further assumed that the linkage between these two factors was well nigh absolute for the following reason: If a white male is mated to a red female there should appear in  $F_2$ , unless the linkage is complete, four types of males—red, white, *eosin* and a *new* type which should be the single recessive, or simple white. We have records of approximately 150,000 flies from such a cross and its reciprocal and yet only two classes of males appeared—red and white. On the assumption that white represents a double recessive, we were therefore justified in concluding that the linkage was complete.

An alternative interpretation for this set of phenomena and the one we now believe to be the better one, is the treatment of red, white, and eosin as a set of *triple allelomorphs*. We assume that in a certain locus in the sex chromosome a determiner mutated to produce white. In another fly a separate mutation in the same locus produced eosin. Each is a simple sex-linked recessive with respect to the normal determiner carried by the red fly. White and eosin are mutually exclusive with respect to their common locus in any one sex chromosome. For example, in the female where two sex chromosomes occur, either chromosome can carry in the common locus eosin *or* white *or* the dominant form of these determiners, i.e., red. Since in the male there is only one sex chromosome, he exhibits directly his composition as red, *or* white, *or* eosin.

We now think of the white as a simple primary mutation and of eosin as another primary mutation which is, however, characterized by bicolorism. When an eosin female is mated to a white male two normally recessive determiners enter the same

zygote in an allelomorphous relation. In this case the sex chromosome, derived from the mother, contains the eosin allelomorph ( $w^e$ ), while in the paternally derived chromosome of the homologous pair, the determiner is in the form white ( $w$ ).

The formula for the daughters of the above cross becomes then  $w w^e$ . We suggest calling this individual a 'white-eosin compound,' and the use of the term 'compound' as a name for such zygotes as are formed by the union of the mutant factors of a multiple allelomorph system. In the special case of sex chromosomal characters in *Drosophila*, compounding can occur only in the female.

Eosin is our only eye-color which shows bicolorism and breeds true for that condition. However, another case which we interpret as analogous to the lightening of the color of the white-eosin compound is the difference observable between the female of double recessive stock vermilion pink (the 'orange' of previous papers) and the vermilion pink female that is heterozygous for eosin or white. The vermilion pink female, heterozygous for eosin, is lighter in color than the vermilion pink female of stock. This lightening is more marked still in vermilion pink heterozygous for white. This case is, as we now interpret it, simply the effect of the heterozygosis in eosin or white, under the favorable condition of the low pigmentation of the vermilion pink eye.

It soon became apparent that certain factors when heterozygous produced dilution effects in some combinations and not in others. Most of the following experiments have as their object the analysis of the *conditions* of dilution and the definition of the *limits* to which dilution effects may be observed at either end of the dark-light series.

Several other problems are dealt with in these experiments, notably the determination of the coupling strength between vermilion and the white-eosin locus.

In a few crosses certain flies appeared which are inexplicable under any current Mendelian hypothesis. These have been mentioned in footnotes to the present paper, and will be dealt with in a separate paper by Mr. Bridges.

For the sake of simplicity, in this paper we have restricted our studies to four eye color factors, although we have confirmatory data on important points from experiments with five other primary eye colors. The four eye colors used here have been described and figured in former papers, but a short comparative description may assist in following the experiments.

The eye of the wild fly is a deep *red*, dense, and with a dark metallic fleck which changes its position according to the angle of light and the viewpoint of the observer.

The *white* eye (sex-linked) looks as though it were due to a white pigment. A transparent fleck indicates the facet turned directly to the observer.

The *eosin* eye color (sex-linked) has been already described. We may add that the color is slightly cloudy in appearance except at the margin, where a rim of greater translucency gives the impression that the pigment has retreated from the periphery to the deeper portion of the eye. The fleck is yellowish.

The *vermilion* eye (sex-linked) is a very bright, clean, true vermilion, not transparent, and with the fleck very inconspicuous. This eye was formerly called 'coral' or 'bright red.'

The *pink* eye color (third chromosome group) is a transparent, deep, pink, which uniformly fills the eye, and shows no fleck.

The various 'permutants' or double and triple recessives possible with these primary eye mutations will be described as we have occasion to use them. Since, however, white is invariable in color it is impossible to distinguish between simple whites, white vermilions, white pinks, and white vermilion pinks. In objective classifications these will all be referred to as 'white.'

A recent paper by Morgan<sup>2</sup> gives our reasons for the use here of a new system of nomenclature. We shall use as the symbol for the factor which differentiates any mutations from the normal, the initial letter of the descriptive name of the mutation. Since we now have over one hundred primary mutations we shall often have to use two letters to avoid confusion. For example, we use for the factor concerned in the recessive muta-

<sup>2</sup> Factors and unit characters in Mendelian heredity. Am. Nat., January 1913.



tion 'vestigial wing,' the initial letter (*v*) and a second convenient letter (*g*), writing the (*g*) as a sub-letter in smaller type thus: (*v<sub>g</sub>*). The dominant form of the determiner concerned in a recessive mutation we designate by the corresponding capital letter, thus, *V<sub>g</sub>* = normal allelomorph, and *p* = pink factor, *P* = its normal allelomorph. In the case of multiple allelomorphs, the first modification of the series will usually supply the initial symbol for the whole series, and the third and other allelomorphs will receive as modifications of that symbol a suggestive letter written as an *exponent*. Thus white is represented by (*w*), normal by (*W*), and eosin by (*w<sup>e</sup>*).

For simplification we put in the formula for an eye color only such symbols as directly concern the cross we intend to make. Thus the full formula for vermilion is *X W v P* etc. *X W v P* etc. and of pink *X W V p* etc. *X W V p* etc. but if in the analysis of the cross vermilion by pink we discard from both formulae all factors not under comparison except the sex differentiator, thus, *X v P* — *X v P* (vermilion ♀) and *X V p* — — *p* (pink ♂).

In our tables of analysis, there appears in the formula for the male a long dash to represent the fact that half the gametes formed by the male carry no sex chromosome, for example, the vermilion pink male forms the two gametes *X v p* and ——*p*. The vermilion pink female forms the gametes *X v p* and *X v p*. The formulas for *F*<sub>1</sub> and *F*<sub>2</sub> zygotes are generally written in this paper with the letters for the allelomorphs in vertical alignment.

In the course of our former work various heterozygous reds have been examined, namely, red heterozygous for pink, vermilion, white, and for both vermilion and pink, but no difference was observed between these and the red eye color of the wild fly. All of these combinations appear in "An attempt to analyze the constitution of the chromosomes on the basis of sex-linked inheritance in *Drosophila*." In order to see whether differences would appear if the reds were heterozygous for other factors, or combinations of factors, the following series of experiments was made.

## RED

*Red heterozygous for eosin*

When a red female is mated to an eosin male the red-eyed female offspring are heterozygous for eosin. The male has the composition and color of the wild male. He is haploid for all sex-linked characters and can be used as a standard for comparison with his sisters reared under the same conditions:

P <sub>1</sub>	red ♀	XW — XW
	eosin ♂	Xw <sup>e</sup> — —
	F <sub>1</sub> female	F <sub>1</sub> male
	XW	XW
	Xw <sup>e</sup>	—
	red ♀	red ♀
	317	346
	203	230
	<u>189</u>	<u>171</u>
	679	747

The eye color of these females heterozygous for eosin is the same as that of their red-eyed brothers. No effect of the heterozygous condition can be seen.

*Red heterozygous for white*

In former papers many records of such flies have appeared, but to make a more critical examination we repeated the cross:

P <sub>1</sub>	white ♀	Xw — Xw
	red ♂	XW — —
	F <sub>1</sub> females	F <sub>1</sub> males
	red ♀	white ♂
	Xw	<del>Xw</del>
	XW	—
	225	203

The eye color of the red females heterozygous for white was like that of the wild stock females.

*Red heterozygous for vermilion and pink*

A careful examination of the  $F_1$  females from vermilion female by pink male failed to show any change produced by the heterozygosity of the two factors for vermilion and pink. The results have been given in "*Further experiments with mutations in eye color of Drosophila*,"<sup>3</sup> but under a different system of nomenclature from the one here adopted.

$P_1$	vermilion ♀ pink ♂	$X v P$ $X \dot{v} p$	—	$X v P$ —	$P$ $p$
	F <sub>1</sub> female			F <sub>1</sub> male	
	$X v p$			$X v P$	
	$X V p$			—	$p$
	red ♀			vermilion ♂	
	721			706	

*Red heterozygous for eosin and pink*

When the eosin female is mated to a pink male, the daughters are red heterozygous for both eosin and pink but in color are like the normal red. The sons are eosin, as explained in the introduction in the cross of eosin female to white male, because of sex-linkage:

$P_1$	eosin ♀ pink ♂	$Xw^e P$ $XW p$	—	$Xw^e P$ —	$P$ $p$
	F <sub>1</sub> females			F <sub>1</sub> males	
	$X w^e P$			$X w^e P$	
	$X W p$			—	$p$
	red ♀			eosin ♂	
	190			138*	

\* In addition there appeared one red male

The converse cross produced normal red sons, and females heterozygous for eosin and pink:

$P_1$	pink ♀ eosin ♂	$XW p$ $Ww^e P$	—	$XW p$ —	$P$ $P$
	F <sub>1</sub> female			F <sub>1</sub> Male	
	$XW p$			$XW p$	
	$Xw^e P$			—	$P$
	red ♀			red ♂	
	254			235	

<sup>3</sup> Jour. Acad. Nat. Sciences, Philadelphia, November, 1912, p. 338.

*Red heterozygous for white and pink*

Data for crosses involving pink and white have appeared in *Science*, vol. 33, 1911, and the *Journal of Experimental Zoology*, 1911. We obtained the following additional data in order to study the eye color more critically.

These results given below are from single-pair cultures except those marked (m) which are in mass. In the F<sub>1</sub> culture IX (mass) there appeared in addition to the 159 red males, two white males. In F<sub>1</sub> culture X (pair) there appeared in addition to the twelve red males, four white males, two of which were tested but proved sterile:

P <sub>1</sub>		pink ♀	X W p	—	X W p		
		white ♂	X w P	—	—	P	
		F <sub>1</sub> females				F <sub>1</sub> males	
		X W p				X W p	
		X w P				—P	
		red ♀				red ♂	
I		37				38	
II		39				45	
III		75				80	
IV		46				49	
V		68				54	
VI		37				37	
VII		94				93	
VIII		94				90	
IX		168 (m)				159	
X		17				12	
		712				665	
		Gametes of F <sub>1</sub> ♀	X W P	—	X W p	—	X w p
		Gametes of F <sub>1</sub> ♂	X W P	—	X W p	—	—P — —p
F <sup>2</sup>	red ♀ (6)	pink ♀ (2)	white ♂ (4)		red ♂ (3)		pink ♂ (1)
I	78	16	37		40		9
II	64	11	39		34		5
III	77	15	49		42		10
IV	42	11	25		22		5
V	24	16	29		23		14
VI	79	15	42		39		10
VII	76	21	44		44		12
VIII	66	10	22		48		6
IX	190(m)	55	116		99		24
X	58(m)	27	25		23		8
XI	65	18	36		27		14
	819	215	464		441		117

The reciprocal cross was also made as follows:

$P_1$	white ♀	X w P —	X w P		
	pink ♂	X W p —	— p		
		F <sub>1</sub> female		F <sub>1</sub> male	
		X w P		X w P	
		X W p		— p	
		red ♀		white ♂	
	I	152		152	
	II	13		18	
	III	45		47	
	IV	59		56	
	V	125		101	
	IV	228(m)		208	
	VII	139		136	
		761		718	
		Gametes of F <sub>1</sub> ♀ X W P — X w P		— X W p — X w p	
		Gametes of F <sub>1</sub> ♂ X w p — X W P		— — p — — P	

white ♀ (4)	white ♂ (4)	red ♀ (3)	red ♂ (3)	pink ♀ (1)	pink ♂ (1)
I 120(m)	112	70	73	26	18
II 25	27	24	13	13	11
III 21	54	41	33	12	7
IV 73(m)	114	67	78	21	20
V 17	16	11	23	7	2
VI 7	7	10	10	2	2
VII 33	28	21	29	6	8
VIII 48	45	30	42	14	8
IX 54	43	32	43	12	13
X 51	44	41	39	14	15
XI 67(m)	86	84	74	25	16
XII 56	37	32	29	15	19
XIII 65	56	41	37	11	9
XIV 21	16	11	15	5	1
678	685	515	538	183	151

The above results are from single pairs, except those cultures marked (m).

Still other females (910) heterozygous for white and pink were produced in the following cross:

P <sub>1</sub>	white pink ♀	X w p — X w p
	red ♂	X W P ——— P
	F <sub>1</sub> female	F <sub>1</sub> male
	X w p	X w p
	X W P	——p
	red ♀	white ♂
	I 212	207
	II 187	186
	III 291	247
	IV 220	205
	910*	845

\*Culture I gave in addition seven white females and six red males. Culture II, gave one white female and two red males, Culture III, one white female and three red males, and Culture IV gave two white females and eight red males. Each of these cultures was obtained by mating five virgin females of pure white pink stock to several wild males.

All the red females heterozygous for white and pink which have been observed fail to show any dilution.

*Red heterozygous for eosin and vermilion*

When red females are mated to eosin vermilion males the daughters are red heterozygous for eosin and vermilion. The sons are normal red in composition and color:

P <sub>1</sub>	red ♀	X W V — X W V
	eosin vermilion ♂	X w <sup>e</sup> v ———
	F <sub>1</sub> female	F <sub>1</sub> male
	X W V	X W V
	X w <sup>e</sup> v	———
	red ♀	red ♂
	200	189

These F<sub>1</sub> red-eyed females have the same eye color as the normal F<sub>1</sub> males, although heterozygous for two factors. In this case the F<sub>1</sub> females were mated back to the eosin vermilion males and gave in the next generation 1279 red of exactly the same composition as F<sub>2</sub>. Here again no difference from the normal could be discovered.

The gametes of the F<sub>1</sub> female and of the P<sub>1</sub> male used in the back cross are as follows:

	Gametes of F <sub>1</sub> ♀		Gametes of P <sub>1</sub> ♂	
	X w <sup>e</sup> V	X W V	X w <sup>e</sup> v	X W v
Females	X w <sup>e</sup> v	X w <sup>e</sup> v	X w <sup>e</sup> v	X W v
Males	eosin ♀	red ♀	eosin ♀	vermilion ♀
	X w <sup>e</sup> v	X W V	X w <sup>e</sup> v	X W v
	eosin ♂	red ♂	eosin- verm. ♂	vermilion ♂
	93	181	130	83
	59	148	118	66
	82	141	130	70
	64	120	107	55
	119	205	169	94
	45	113	79	49
	147	246	240	128
	77	125	139	89
	886	1279	1132	636

The numerical results give a measure of the linkage (coupling) between eosin and vermilion.

*Red heterozygous for white and vermilion*

When vermilion females were mated to white males the red daughters were normal in eye color, although heterozygous for the two factors in question:

P <sub>1</sub>	vermilion ♀	X W v — X W v
	white ♂	X w V — — —
	F <sub>1</sub> female	F <sub>1</sub> male
	X W v	X W v
	X w V	— — —
	red ♀	vermilion ♂
	25	24
	98	74
	123	98

The converse cross gave 94 red females and 83 white males.

Thus far the red females heterozygous in not more than two factors have been considered. We may now take up two cases in which three factors are involved.

*Red heterozygous for eosin, vermilion and pink*

When a vermilion pink female is mated to an eosin male, all the red-eyed daughters are heterozygous for eosin, vermilion and pink. The vermilion eyed sons are heterozygous for the non-sex-linked factor, pink. The vermilion pink permutation has been described under the name 'orange' and figured in "Further experiments, etc." cited. The color is a deep true orange, darker and brighter than the color of the eosin male. There is a light cloudy yellow fleck. The color of the male is identical with that of the female.

P <sub>1</sub>	vermilion pink ♀ eosin ♂	X W v p — X W v p X w <sup>e</sup> V P — —P
	F <sub>1</sub> female X W v p X w <sup>e</sup> V P red ♀ 202	F <sub>1</sub> male X W v p ———P vermilion ♂ 189

These F<sub>1</sub> females although heterozygous for these three factors are indistinguishable from the wild red-eyed females. The heterozygous vermilion male will be considered later, with the other vermilions.

A suspicion having arisen as to whether a difference in color might exist when the factors came in from the egg rather than from the sperm, red females heterozygous for these same three factors, namely, eosin, vermilion and pink, were produced by mating, to a wild female, males of the eosin, vermilion, pink stock. The method by which this stock was obtained has been given and the eye color figures, in "Further experiments with mutations in eye color of *Drosophila*," cited before. The colored figure (5) shows at about the center of the left eye, the true color of the eosin vermilion pink female. The race is bicolored, the corresponding male being a trifle lighter than the female, although this bicolorism is not strong enough so that a separation made on color alone is found to be a complete sex separation also.



P <sub>1</sub>	red ♀	X W V P	—	X W V P
	eosin vermilion	X w <sup>e</sup> v p	—	—p
	pink ♂			

---

F <sub>1</sub> females	F <sub>1</sub> males
X W V P	X W V P
X w <sup>e</sup> v p	—p
red ♀	red ♂
208	150

The F<sub>1</sub> females and males were exactly alike in color and like the wild flies. An eosin female gave by a vermilion pink male 55 red daughters heterozygous for eosin, vermilion and pink and 45 eosin males.

*Red heterozygous for white, vermilion, and pink*

When wild red females were crossed to white vermilion pink males the daughters were heterozygous for the desired three factors:

P <sub>1</sub>	red ♀	X W V P	—	X W V P
	white verm. pink ♂	X w v p	—	—p

---

F <sub>1</sub> females	F <sub>1</sub> males
X W V P	X W V P
X w v p	—p
red ♀	red ♂
104	96
38	33
<hr/> 142	<hr/> 129

The red eyed females are like the wild flies.

The preceding crosses show that the color of the red eye is not diluted, as far as can be seen, by being heterozygous in one, two or three factors. Whether dilution can be produced by the introduction of heterozygosity for still more eye colors we cannot state, since we have not made up red-eyed flies heterozygous for these eye color factors plus the other eye color factors known to us at present. The evidence seems to indicate that the red eye is too densely pigmented for differences produced by heterozygosity to become evident.

## PINK

The next series of experiments test the ability of various factors to produce dilution in the pink.

*Pink heterozygous for vermilion*

When pink females are mated to vermilion pink males, the heterozygous  $F_1$  daughters are indistinguishable from pink stock females:

$P_1$	pink ♀	X V p — X V p
	verm. pink ♂	X v p — —p
	$F_1$ female	$F_1$ male
	X V p	X V p
	X v p	—p
	pink ♀	pink ♂
	1264	976

The reciprocal cross likewise gives pink females heterozygous for vermilion and these are standard pink as before:

$P_1$	verm. pink ♀	X v p — X v p
	pink ♂	X V p — —p
	$F_1$ female	$F_1$ male
	X v p	X v p
	X V p	—p
	pink ♀	vermilion pink ♂
	969	941

*Pink heterozygous for eosin*

When pink females are mated to eosin pink males the daughters are pink heterozygous for eosin. The eosin pink stock was obtained by mating eosin females to pink males and extracting in  $F_2$ :

$P_1$	pink ♀	X W p — X W p
	eosin pink ♂	X w <sup>e</sup> p — —p
	$F_1$ female	$F_1$ male
	X W p	X W p
	X W <sup>e</sup> p	—p
	pink ♀	pink ♂
	108	110
	127	111
	235	221

The daughters were indistinguishable from the sons in eye color.

*Pink heterozygous for white*

Pink daughters heterozygous for white were obtained in F<sub>1</sub> by mating white pink females to pink males. The F<sub>1</sub> females were white. In order to get pink males for comparison in the same cross which gave the desired females we continued the experiment to the F<sub>2</sub> generation. The eye color of the pink was not lighter, although heterozygous for white.

P <sub>1</sub>	white pink ♀	X w p — X w p		
	pink ♂	X W p — —p		
	F <sub>1</sub> female		F <sub>1</sub> male	
	X w p		X w p	
	X W p		—p	
	pink ♀		white (pink) ♂	
	24		20	
	39		32	
	36		45	
	23		11	
	26		12	
	148		120	
	Gametes of F <sub>1</sub> ♀ X w p — X W p		Gametes of F <sub>1</sub> ♂ X w p — —p	
	F <sub>2</sub> females		F <sub>2</sub> males	
	X w p	X W p	X w p	X W p
	X w p	X W p	—p	—p
	white pink ♀	pink ♀	white pink ♂	pink ♂
	27	29	24	27
	51	53	37	34
	53	36	51	89
	131	118	112	120

*Pink heterozygous for eosin and vermilion*

When pink females are mated to eosin vermilion pink males the daughters are like the sons and like stock pink females. A back cross between the F<sub>1</sub> females and eosin vermilion pink males was made to get material for further study. The results show the degree of linkage between eosin and vermilion.

P <sub>1</sub>	pink ♀		X W V p — X W V p				
	eosin verm. pink ♂		X w <sup>e</sup> v p ———— p				
	pink ♀		pink ♂				
	184		205				
	419		358				
	603		563				
Gametes of F <sub>1</sub> ♀ X w <sup>e</sup> V p — X W V p — X w <sup>e</sup> v p — X W v p							
Gametes of F <sub>1</sub> ♂				X w <sup>e</sup> v p — ———— p			
Females				Males			
Xw <sup>e</sup> Vp	XWVp	Xw <sup>e</sup> v	pXWvp	Xw <sup>e</sup> Vp	XWVp	Xw <sup>e</sup> vp	XWvp
Xw <sup>e</sup> vp	XW <sup>e</sup> vp	Xw <sup>e</sup> v	pXw <sup>e</sup> vp	— p	— p	— p	— p
eosin	pink ♀	eosin	verm.	eosin	pink ♀	eosin	verm.
pink ♀		verm.	pink ♀	pink ♀		verm.	pink ♂
		pink ♀				pink ♂	
39	77	95	37	37	98	110	36
73	169	161	50	88	165	184	67
63	144	117	61	56	143	137	72
91	158	139	81	94	164	157	97
63	135	140	74	55	122	140	68
72	155	132	61	48	95	136	54
56	115	108	65	34	100	113	50
52	110	113	36	43	106	137	55
53	102	111	54	34	77	107	52
562	1165	1116	539	471	1070	1221	511

One of the preceding experiments (page 439) furnishes data for the amount of association of the factors eosin and vermilion. In both the former cross and in this cross the gametic ratio is shown directly both in the male and female classes. The classes of the preceding experiment, added to those here give as totals:

w <sup>e</sup> V	WV	w <sup>e</sup> v	Wv
1717	3424	3469	1729

The sum of the classes showing the original combinations is 6893 and the crossover combinations 3446, which gives a percentage of crossing-over (or chromosomal distance) of 33.3.

*Pink heterozygous for white and vermilion*

The pink daughters from the cross pink female by white vermilion pink male were identical with stock pinks, although heterozygous for white and vermilion:

P <sub>1</sub>	pink ♀	X W V p	—	X W V p
	white verm. pink ♂	X w v p	—	—————p
<hr/>				
	F <sub>1</sub> females			F <sub>1</sub> males
	X W V p			X W V p
	X w v p			—————p
	pink ♀			pink ♂
	260			231
	133			102
	—			—
	393			333

VERMILION

So far no dilution effects have been observed in the red or pink. However, we have found that in some of the following series of experiments vermilion shows a slight dilution.

*Vermilion heterozygous for pink*

When vermilion females were mated to vermilion pink males the daughters (75) were vermilion heterozygous for pink. The color of the eye was indistinguishable from that of stock vermilion. The sons (78) likewise were indistinguishable from stock

*Vermilion heterozygous for eosin*

The heterozygous females were observed in a cross (page 439) of eosin vermilion males to F<sub>1</sub> females out of wild females by eosin vermilion males. The eye color was indistinguishable from ordinary vermilion.

*Vermilion heterozygous for white*

Vermilion females heterozygous for white constitute the F<sub>1</sub> female class of the cross vermilion females by white vermilion males. They impress one as being slightly brighter than stock vermilion, or their vermilion brothers:

P <sub>1</sub>	vermilion ♀	X W v	—	X W v
	white vermilion ♂	X w v	—	—————
<hr/>				
	F <sub>1</sub> females			F <sub>1</sub> males
	X W v			X W v
	X w v			—————
	vermilion ♀			vermilion ♂
	111			82

*Vermilion heterozygous for eosin and pink*

When vermilion females are mated to eosin vermilion pink males, the daughters are vermilion heterozygous for eosin and pink. There seems to be some very slight difference in eye color as compared with vermilion, but the difference is not always evident. This last experiment was carried further by back crossing the  $F_1$  female with eosin vermilion pink males in order to get in the next generation material for the study of the lighter eye colors. The results show the non-linkage of eosin and pink. The complete data are as follows:

$P_1$	vermilion ♀ eosin verm. pink ♂	$XWvp - XWvP$ $Xw^evp - \text{————}p$					
	F <sub>1</sub> females	F <sub>1</sub> males					
	$XWvp$	$XWvp$					
	$Xw^evp$	————p					
	vermilion ♀	vermilion ♂					
	293	337					
	326	325					
	619	662					
	Gametes of F <sub>1</sub> ♀	Gametes of F <sub>1</sub> ♂					
	$XWvp - Xw^evp$	$XWvP - Xw^evp$					
	Gametes of P <sub>1</sub> ♂	$Xw^evp - \text{————}p$					
	F <sub>2</sub> females	F <sub>2</sub> males					
$XWvp$	$XWvp$	$Xw^evp$	$Xw^evP$	$XWvp$	$XwvP$	$XWvp$	$XWvP$
$Xw^evp$	$Xw^evp$	$Xw^evp$	$Xw^evp$	————p	————p	————p	————p
verm. pink ♀	verm. ♀	eosin verm. pink ♀	eosin verm. ♀	verm. pink ♂	verm. ♂	eosin verm. ♂	eosin verm. ♂
75	93	83	65	91	83	134	
61	61	53	65	66	47	99	
101	98	111	93	83	110	189	
58	50	98	55	47	70	100	
87	101	100	95	84	100	194	
382	403	397	373	371	420	716	

*Vermilion heterozygous for white and pink*

When vermilion females are mated to white vermilion pink males the daughters are vermilion heterozygous for white and pink. There is a distinct slight difference between these females and stock vermilion females or males. The difference is best

seen by comparing the F<sub>1</sub> females above with their young brothers of the same age; for, as we have seen before, the vermilion male heterozygous for pink is exactly similar to the stock vermilion male:

P <sub>1</sub>	vermilion ♀	X W v P	—	X W v P
	white verm. pink ♂	X w v p	—	—————p
	F <sub>1</sub> females			F <sub>1</sub> males
	X W v P			X W v P
	X w v p			————— p
	vermilion ♀			vermilion ♂
	200			194

EOSIN

In the following series of experiments involving eosin direct comparison of the daughters was made with stock eosin females, because of the lighter color of the eosin males.

*Eosin heterozygous for vermilion*

In the experiment on page 439 the eosin female class is heterozygous for vermilion and is indistinguishable from the stock eosin. The corresponding eosin male has a constitution identical with that of the stock eosin male and is identical with him in color.

*Eosin heterozygous for vermilion and pink*

Eosin females mated to eosin vermilion pink males gave eosin females of the desired composition and eosin males heterozygous for pink. The analysis is as follows:

P <sub>1</sub>	eosin ♀	X w <sup>e</sup> V P	—	X w <sup>e</sup> V P
	eosin verm. pink ♂	X w <sup>e</sup> v p	—	—————p
	F <sub>1</sub> females			F <sub>1</sub> males
	X w <sup>e</sup> V P			X w <sup>e</sup> V P
	X w <sup>e</sup> v p			————— p
	eosin ♀			eosin ♂
	30			35
	37			28
	7			6
	190			211
	264			280

Females of the same composition were obtained by mating eosin vermilion females to eosin pink males. The daughters, 81 in number, were like the 264 above. Both were like the dark eosin females, except that the range of variation seemed to be somewhat extended in the direction of dilution.

#### WHITE-EOSIN COMPOUND

When an eosin female is crossed to a white male the daughters are white eosin compounds and the sons are regular eosin males. The eye colors of the sons and daughters are apparently identical. The eye color of the female drops from dark to light eosin:

$P_1$	eosin ♀ X w <sup>e</sup> — X w <sup>e</sup> white ♂ X w — —		
	F <sub>1</sub> females X w <sup>e</sup> X w white-eosin compound ♀ 205 489 <hr style="width: 50%; margin: 0 auto;"/> 694	F <sub>1</sub> males X w <sup>e</sup> — eosin ♂ 149 430 <hr style="width: 50%; margin: 0 auto;"/> 579	
	Gametes of F <sub>1</sub> ♀ X w <sup>e</sup> — X w Gametes of F <sub>1</sub> ♂ X w <sup>e</sup> — —		
	F <sub>2</sub> females X w <sup>e</sup> X w X w <sup>e</sup> X w <sup>e</sup> eosin ♀ white- eosin comp. ♀ 420 357	F <sub>2</sub> males Z w <sup>e</sup> X w eosin ♂ white ♂ 370 374	

In both these cases the eosin female and the white-eosin compound were produced in the same cross and could be easily compared, and the difference noted above observed.

The reciprocal cross furnished more data, as follows:

$P_1$	white ♀ X w — X w eosin ♂ X w <sup>e</sup> — —		
-------	---	--	--



F <sub>1</sub> females		F <sub>1</sub> males	
X w		X w	
X w <sup>e</sup>		—	
white-eosin compound ♀		white ♂	
365		332	
Gametes of F <sub>1</sub> ♀ X w — X w <sup>e</sup>			
Gametes of F <sub>1</sub> ♂ X w — —			
F <sub>2</sub> females		F <sub>2</sub> males	
X w	X w <sup>e</sup>	X w	X w <sup>e</sup>
X w	X w	—	—
white ♀	white-eosin compound ♀	white ♂	eosin ♂
370	357	344	357

*White-eosin compound heterozygous for pink*

The color of the white-eosin compound female was not reduced by heterozygosity for pink:

P <sub>1</sub>	eosin pink ♀	X w <sup>e</sup> p — X w <sup>e</sup> p
	white ♂	X w P — —P
	F <sub>1</sub> female	F <sub>1</sub> male
	X w <sup>e</sup> p	X w <sup>e</sup> p
	X w P	—P
	white-eosin compound ♀	eosin ♂
	179	178

*White-eosin compound heterozygous for vermilion*

Daughters of the above composition were obtained from the cross of eosin female by white vermilion males. Both sons (102) and daughters (87) exhibited the regular color for the eosin male.

*White-eosin compound heterozygous for vermilion and pink*

To find out whether the color of the white-eosin compound could be diluted by being made heterozygous for both vermilion and pink, such females were prepared by mating eosin females to white vermilion pink males as below:

P <sub>1</sub>	eosin ♀	X w <sup>e</sup> V P — X w <sup>e</sup> V P
	white pink verm. ♂	X w v p ————— p
	F <sub>1</sub> females	F <sub>1</sub> males
	X w <sup>e</sup> V P	X w <sup>e</sup> V P
	X w v p	————— p
	white-eosin compound ♀	eosin ♂
	273	260
	298	227
	<u>571</u>	<u>487</u>

We have shown before that the color of an eosin male is the same whether it is pure for non-pink (PP) or heterozygous for pink (Pp). Direct comparison of the F<sub>1</sub> males and females above showed no appreciable difference in color between them, so that probably the color of the white-eosin compound is not diluted by heterozygosity for both vermilion and pink.

In addition to the above we have examined 2,402 more of the white-eosin compounds heterozygous for vermilion and pink, which appeared in the cross below:

P <sub>1</sub>	white ♀	X w V P — X w V P
	eosin verm. pink ♂	X w <sup>e</sup> v p ————— p
	F <sub>1</sub> females	F <sub>1</sub> males
	X w V P	X w V P
	X w <sup>e</sup> v p	————— p
	white-eosin compound ♀	white ♂
	I 168	168
	II 353	339
	III 344	316
	IV 193	162
	V 94	85
	VI 72	82
	VII 206	227
	VIII 365	350
	IX 261	233
	X 96	91
	XI 151	142
	<u>2402*</u>	<u>2303</u>

\* In Culture I there appeared a female having one eye the color of the white-eosin compound and the other eye white. Upon testing her with a vermilion pink male she produced 39 red ♀♀, 31 vermilion ♀♀, 43 pink ♀♀, 37 verm. pink ♀♀, 61 white ♂♂, 8 eosin ♂♂, 33 ♂♂ in the double class eos. verm. and eos. pink, and 21 ♂♂ eos. verm. pink. On the assumption that although one eye

*Vermilion pink heterozygous for eosin*

Vermilion pink females mated to eosin vermilion pink males gave vermilion pink daughters heterozygous for eosin. As stated in the introduction, these females are lighter than the vermilion pink brothers or the stock vermilion pink females. They are of about the intensity of the eosin male, perhaps darker, but with no light rim and different in opacity.

P <sub>1</sub>	vermilion pink ♀	X W v p — X W v p
	eosin verm. pink ♂	X w <sup>e</sup> v p — ————p
	F <sub>1</sub> females	F <sub>1</sub> males
	X W v p	X W v p
	X w <sup>e</sup> v p	————p
	vermilion pink	vermilion pink ♂
	heterozygous for	
	eosin ♀	
	59	55
	245	221
	144	121
	307	290
	755	691
	Gametes of F <sub>1</sub> ♀	X W v p — X w <sup>e</sup> v p
	Gametes of P <sub>1</sub> ♂	X w <sup>e</sup> v p — ————p

was white the female behaved as would any of her fully colored sisters, and allowing for the known strength of association between vermilion and the white-eosin locus, the expectation is as follows: red, vermilion, pink and vermilion pink classes, each 34; white ♂♂ 68; eosin ♂♂ 11; eos. verm. + eos. pink ♂♂ 34; eos. verm. pink ♂♂ 23. The observed numbers approach as closely as is probable for so few individuals. In Culture II, appeared a white female, and she also behaved as would her fully colored sisters. When mated to a wild male, she gave 117 red ♀♀, 51 white ♂♂, 39 eos. verm. ♂♂ and 14 eosin ♂♂, where the expectations called for 111 red ♀♀, 55 white ♂♂, 37 eos. verm. and 18 eosin ♂♂. *Here we have then a case of a fly which somatically was colorless, yet genetically was colored.* In Culture III appeared three white females which, however, were not tested. The mother in Culture V was miniature (sex-linked) as well as white. There appeared one long eosin male and one long white male which were not tested. In Culture VIII appeared one white female which was not tested and one female much lighter than the sisters which proved sterile. There were also two males which were not white like their brothers, but were light in color. Both were sterile. In Culture IX appeared a male in color about eosin, but when crossed to vermilion this color did not reappear in F<sub>2</sub>. In Culture XI appeared another light male which also was sterile.

X W v p	X w <sup>e</sup> v p	X W v p	X w <sup>e</sup> v p
X w <sup>e</sup> v p	X w <sup>e</sup> v p	—————p	—————p
verm. pink	eosin verm.	verm. pink ♂	eosin verm.
heterozygous	pink ♀		pink ♂
for eosin ♀			
139	115	100	127
217	206	200	199
144	125	115	128
166	159	150	181
163	153	144	161
224	119	208	190
<u>1050</u>	<u>947</u>	<u>926</u>	<u>986</u>

In these results the vermilion pink males (926) and females (1050) differed as did the F<sub>1</sub> males and females above.

A pair of the F<sub>1</sub> flies of the first half of this experiment (755 ♀♀ 691 ♂♂) were bred from and gave in F<sub>2</sub>:

verm. pink ♀	verm. pink ♀	vermilion ♂	eosin verm.
	heterozygous		pink ♂
	for eosin		
19	14	14	16

This last test produced in the same culture females of the ordinary vermilion pink type and females vermilion pink heterozygous for eosin. The comparison confirmed the preceding statement.

#### *Vermilion pink heterozygous for white*

Vermilion pink females mated to white vermilion pink males gave daughters heterozygous for white. These females were lighter than those heterozygous for eosin. The color was in fact nearly a simple yellow.

P <sub>1</sub>	vermilion pink ♀	X W v p — X W v p
	white verm. pink ♂	X w v p — —————p
	F <sub>1</sub> females	F <sub>1</sub> males
	X W v p	X W v p
	X w v p	—————p
	verm. pink ♀	verm. pink ♂
	heterozygous for white	
		152
	218	152
	237	231
	<u>455</u>	<u>283</u>

Gametes of F<sub>1</sub> ♀ X W v p — X w v p  
 Gametes of F<sub>1</sub> ♂ X W v p — ———p

F <sub>2</sub> females		F <sub>2</sub> males	
X W v p	X w v p	X W v p	X w v p
X W v p	X W v p	————p	————p
verm. pink ♀	verm. pink ♀	verm.pink ♂	white ♂
	heterozygous for white		
69	50	63	81
<u>180</u>	<u>181</u>	<u>182</u>	<u>187</u>
259	231	245	268

In the last F<sub>2</sub> classes the pure vermilion pink females are considerably darker than those heterozygous for white. This is the same difference spoken of above as shown between F<sub>1</sub> females compared with the stock females or any vermilion pink males.

A few vermilion pink females heterozygous for white (like F<sub>1</sub> females above) were mated to white vermilion pink males and gave as already published (1912, page 343), the following:

vermilion pink ♀ heterozygous for white	vermilion pink ♂	white ♀	white ♂
80	82	26	41

In another cross some vermilion pink females heterozygous for white were mated to pink males and gave:

pink ♀	vermilion pink ♂	white ♂
117	61	70

*Eosin pink heterozygous for vermilion*

On page 444 among the F<sub>2</sub> classes are found 562 eosin pink females heterozygous for vermilion and 471 eosin pink males (Xw<sup>e</sup>Vp ———p) which correspond to the eosin pink females. These eosin pink females are lighter than the stock females homozygous for V.

The comparison is more clearly brought out in the following cross because both classes of females appear under the same conditions. Some of the F<sub>2</sub> eosin pink females and males derived from the experiment referred to above were inbred as follows:

Gametes of F <sub>2</sub> ♀ X w <sup>e</sup> V p — X w <sup>e</sup> v p		Gametes of F <sub>2</sub> ♂ X w <sup>e</sup> V p — ———p	
F <sub>3</sub> females		F <sub>3</sub> males	
X w <sup>e</sup> V p	X w <sup>e</sup> v p	X w <sup>e</sup> V p	X w <sup>e</sup> v p
X w <sup>e</sup> V p	X w <sup>e</sup> V p	————p	————p
eosin pink ♀	eosin pink ♀ heterozygous for verm.	eosin pink ♂	eos. verm pink ♂
51	56	70	71
80	72	72	72
16	18	21	16
153	146	163	159

*White-eosin compound pink*

This female is best compared to the eosin-pink male, for according to the result obtained with the simple white-eosin compound and eosin male, the expectation here is, that these two combinations (X w p — X w<sup>e</sup> p and X e p — ———p) should be identical in color. Direct comparison is possible if the cross is made by mating an eosin pink female to a white pink male. The results confirmed our belief that the sons and daughters of this cross should be of the same color, namely, that of the stock eosin pink male:

P <sub>1</sub>	eosin pink ♀ X w <sup>e</sup> p — X w <sup>e</sup> p	
	white pink ♂ X w p — ———p	
	F <sub>1</sub> female	F <sub>1</sub> male
	X w <sup>e</sup> p	X w <sup>e</sup> p
	X w p	————p
	white-eosin compound pink ♀	eosin pink ♂
	184	174

The converse cross of white pink female to eosin pink male gave daughters like those of the last cross, but white sons:

P <sub>1</sub>	white pink ♀ X w p — X w p	
	eosin pink ♂ X w <sup>e</sup> p — ———p	
	F <sub>1</sub> females	F <sub>1</sub> males
	X w p	X w p
	X w <sup>e</sup> p	————p
	white-eosin compound pink ♀	white ♂
	161	160

*White-eosin compound pink heterozygous for vermilion*

When white pink females are mated to eosin vermilion pink males the daughters are white-eosin compound pink heterozygous for vermilion. These females are slightly lighter than the white-eosin compound pink females or the eosin pink males, because of further dilution due to heterozygosity for vermilion. No male can be made to correspond to this color since the male has but one chromosome and cannot be made heterozygous, in the same sense, i.e., he must always be haploid for V, and as we have seen, a haploid V in the male is equivalent in color to VV in the female. The analysis is as follows:

P <sub>1</sub>	white pink ♀	X w V p — X w V p
	eosin verm. pink ♂	X w <sup>e</sup> v p — —p
	F <sub>1</sub> female	F <sub>1</sub> male
	X w V p	X w V p
	X w <sup>e</sup> v p	—p
	white eosin comp. pink ♀	white pink ♂
	heterozygous for vermilion	
	I 246	269
	II 228	225
	III 490	414
	IV 265	281
	V 173	192
	VI 110	83
	VII 195	170
	VIII 366	403
	<u>2073*</u>	<u>2037</u>

\* In addition there appeared in Culture I one light male (sterile), in Culture III one white female, in Culture IV one white female and one light male (sterile), in Culture V one white female, in VII two white females, and in VIII four white females.

The dilution of the white-eosin compound pink heterozygous for vermilion can be directly observed in the offspring of an eosin pink female by white vermilion pink male, namely eosin pink sons and the above daughters:

P <sub>1</sub>	eosin pink ♀	X w <sup>e</sup> V p — X w <sup>e</sup> V p
	white verm. pink ♂	X w v p — —p
	F <sub>1</sub> female	F <sub>1</sub> male
	W w <sup>e</sup> V p	X w <sup>e</sup> V p
	X w v p	—p
	white-eosin comp. pink ♀	eosin pink ♂
	heterozygous for verm.	
	194	187

*Eosin vermilion heterozygous for pink*

In the experiment on page 446, 373 eosin vermilion females heterozygous for pink are recorded. Similar males heterozygous in pink were also obtained. These flies inasmuch as they are eosin derivatives show the peculiarity of eosin, namely, sexual bicolorism, and because they are heterozygous in pink, both sexes are lighter than the corresponding simple eosin vermilion.

The dilution of the color due to heterozygosity was more readily observed in the following cases, by direct comparisons. Some of the 373 eosin vermilion females were mated in pairs to some of the eosin vermilion males (picked out from the 716 double class, page 446) and gave the following results:

F <sub>2</sub> eosin vermilion ♀			X w <sup>e</sup> v P — X w <sup>e</sup> v p		
F <sub>2</sub> eosin vermilion ♂			X w <sup>e</sup> v p — X w <sup>e</sup> v P — —p — —P		
F <sub>3</sub> females			F <sub>3</sub> males		
X w <sup>e</sup> v P	X w <sup>e</sup> v p	X w <sup>e</sup> v P	X w <sup>e</sup> v P	X w <sup>e</sup> v p	X w <sup>e</sup> v P
X w <sup>e</sup> v p	X w <sup>e</sup> v p	X w <sup>e</sup> v P	—————p	—————p	—————p
and			and		
X w <sup>e</sup> v p	X w <sup>e</sup> v p	X w <sup>e</sup> v P	X w <sup>e</sup> v P	X w <sup>e</sup> v p	X w <sup>e</sup> v P
X w <sup>e</sup> v P	X w <sup>e</sup> v p	X w <sup>e</sup> v P	—————p	—————p	—————p
57	32	48	65	35	32
56	24	32	71	34	26
74	23	46	83	33	27
35	16	16	49	22	11
70	26	49	54	36	27
292	121	191	322	160	123

In both the males and females the class eosin vermilion is separably darker than the class eosin vermilion heterozygous for pink. The eosin vermilion pink classes are still lighter. Each class of females is darker than the corresponding class of males. To compare the eosin vermilion heterozygous for pink more closely to the eosin vermilion pink an experiment was devised to produce only these desired classes in males and in females. One of the females of the 373 (page 446) was mated to an eosin vermilion pink male as follows:



Gametes of F <sub>2</sub> ♀		X w <sup>e</sup> v P	—	X w <sup>e</sup> v p
Gametes of eosin verm. pink ♂		X w <sup>e</sup> v p	—	——p

---

F <sub>3</sub> females		F <sub>3</sub> males		
X w <sup>e</sup> v P	X w <sup>e</sup> v p	X w <sup>e</sup> v P	X w <sup>e</sup> v p	
X w <sup>e</sup> v p	X w <sup>e</sup> v p	——p	——p	
eosin verm. ♀	eosin verm.	eosin verm. ♂	eosin verm.	
heterozygous	pink ♀	heterozygous	pink ♂	
for pink		for pink		
31	25	23	24	

*White-eosin compound vermilion*

These females were produced in the cross of eosin vermilion females to white vermilion males in order that the sons might be eosin vermilion males for comparison. As we had suspected, the colors of the daughters and sons were identical:

P <sub>1</sub>	eosin vermilion ♀	X w <sup>e</sup> v	—	X w <sup>e</sup> v
	white vermilion ♂	X w v	—	——

---

F <sub>1</sub> females		F <sub>1</sub> males	
X w <sup>e</sup> v		X w <sup>e</sup> v	
X w v		——	
white-eosin compound		eosin vermilion ♂	
vermilion ♀			
260		248	

*White-eosin compound vermilion heterozygous for pink*

When eosin vermilion females are mated to white vermilion pink males the daughters are white-eosin compounds vermilion heterozygous for pink, and the males are eosin vermilion heterozygous for pink. As in the experiment (page 456) where eosin vermilion males heterozygous for pink were produced, these sons were lighter than the stock eosin vermilion males. We have seen in the last experiment that the white-eosin compound vermilion female is exactly the color of the eosin vermilion male. In this cross the daughters and sons are likewise exactly alike in color, but are lighter than in the last cross because here both are diluted, and equally diluted in being heterozygous for pink. They are, then, the color of the males of the Experiment of page 456:

P <sub>1</sub>	eosin vermilion ♀	X w <sup>e</sup> v P	—	X w <sup>e</sup> v P
	white verm. pink ♂	X w v p	—	————p
F <sub>1</sub> female		F <sub>1</sub> male		
X w <sup>e</sup> v P		X w <sup>e</sup> v P		
X w v p		————p		
white-eosin compound		eosin vermilion ♂		
vermilion ♀ heterozygous		heterozygous for pink		
for pink				
247		186		

The converse experiment, white vermilion females mated to eosin vermilion pink males, gave white-eosin compounds vermilion heterozygous for pink, and white males. The F<sub>1</sub> here does not offer as direct a comparison as the preceding, but in F<sub>2</sub> below some very interesting combinations occur:

P <sub>1</sub>	white vermilion ♀	X w v P	—	X w v P				
	eosin verm. pink ♂	X w <sup>e</sup> v p	—	————p				
F <sub>1</sub> females		F <sub>1</sub> males						
X w v p		X w v P						
X w <sup>e</sup> v P		————p						
eosin-eosin comp.		white (verm.) ♂						
vermilion ♀								
86		60						
33		43						
212		192						
331		295						
Gametes of F <sub>1</sub> ♀		X w v p	—	X w <sup>e</sup> v p	—	X w <sup>e</sup> v P	—	X w <sup>e</sup> v p
Gametes of F <sub>1</sub> ♂		X w v p	—	X w v P	—	————p	—	————P
F <sub>2</sub> females				F <sub>2</sub> males				
Xwvp	XwvP	Xw <sup>e</sup> vp	Xw <sup>e</sup> vP	Xwvp	XwvP	Xw <sup>e</sup> vp	Xw <sup>e</sup> vP	
Xwvp	Xwvp	Xwvp	Xwvp	——p	——p	——p	——p	
Xwvp	XwvP	Xw <sup>e</sup> vp	Xw <sup>e</sup> vP	Xwvp	Xwvp	Xw <sup>e</sup> p	Xw <sup>e</sup> vP	
XwvP	XwvP	XwvP	XwvP	——P	——P	——P	——P	

As the zygotic formulae above show, half of all the flies (eight classes) should be white. The colored females are of three kinds, white-eosin compound vermilion, white-eosin compound vermilion heterozygous for pink, and white-eosin compound vermilion pink. Likewise the colored males are of three colors, eosin-vermilion, eosin-vermilion heterozygous for pink, and eosin-vermilion pink. The separation of the males is exactly the same

as on page 456 and the separation of the females is an analogous one differing only *in that the colors of the females correspond exactly class by class to that of the males* instead of being darker in every case as before:

Females	Males
white (4 classes)	white (4 classes)
55	37
white-eosin comp. verm. (1 class)	eosin-verm. (1 class)
$X w v$	$X w^e v$
$X w^e v$	<hr style="width: 50%; margin: 0 auto;"/>
10	6
white-eosin comp. verm. heterozygous for pink (2 classes)	eosin-vermilion heterozygous for pink (2 classes)
$X w v P$	$X w^e v P$
$X w^e v p$	<hr style="width: 50%; margin: 0 auto;"/> p
22	18
white-eosin compound vermilion pink (1 class)	eosin vermilion pink (1 class)
14	14

The comparisons of males and females of like color are here observable in the same experiment, though they have all been studied intensively elsewhere.

### *Eosin vermilion pink*

This eye color, which has been fully described on page 440, is the lightest eye color we have tried to synthesize. Without introducing other factors into this paper, the only dilution we can subject it to is to make the female a white-eosin compound vermilion pink.

### *White-eosin compound vermilion pink*

Females of this kind were obtained in reciprocal crosses. The most advantageous cross is to mate eosin vermilion pink females to white vermilion pink males, since this gives in  $F_1$  eosin vermilion pink males to compare directly with the compounds. As expected the daughters and sons were exactly alike in color. The

color is that of the stock eosin vermilion pink male and as described is so close in color to white that one not familiar with the colors would pass it over as white.

P <sub>1</sub>	eosin vermilion pink ♀	X w <sup>e</sup> v p	—	X w <sup>e</sup> v p
	white vermilion pink ♂	X w v p	—	————p
-----				
	F <sub>1</sub> female			F <sub>1</sub> male
	X w <sup>e</sup> v p			X w <sup>e</sup> v p
	X w v p			————p
	white-eosin comp.			eosin verm. pink ♂
	verm. pink ♀			
	78			44

The reciprocal cross gave daughters like the above and white sons for comparison:

P <sub>1</sub>	white vermilion pink ♀	X w v p	—	X w v p
	eosin verm. pink ♂	X w <sup>e</sup> v p	—	————p
-----				
	F <sub>1</sub> female			F <sub>1</sub> male
	X w v p			X w v p
	X w <sup>e</sup> v p			————p
	white-eosin comp.			white ♂
	verm. pink ♀			
	276			248
	From the above F <sub>2</sub> a pair produced:			
	X w v p	X w <sup>e</sup> v p	X w v p	X w <sup>e</sup> v p
	X w v p	X w v p	————p	————p
white ♀	white-eosin		white ♂	eosin-verm. pink ♂
	comp. verm. pink ♀			
24	13		21	15

#### SUMMARY OF OBSERVED EFFECTS

The preceding results establish that the red eyed fly, heterozygous for pink, vermilion, eosin, or white, or any combination of these factors, shows no dilution of the red eye color.

The *pink eyed female*, heterozygous for vermilion, eosin, or white or any combination of these factors, shows no dilution of the pink eye color.

The *vermilion eyed fly*, heterozygous for pink or eosin (in the female), shows no dilution effect, but when heterozygous for both pink and eosin there is a suggestion of a dilution effect.

Vermilion heterozygous for white seems to be slightly diluted. Vermilion heterozygous for white and pink together shows a distinct though slight dilution.

The *eosin eyed fly*, heterozygous for pink or vermillion (in the female) shows no dilution. When heterozygous for both, the eye color is somewhat more variable than eosin.

The double recessive eye colors heterozygous for other factors may next be considered.

Thus, *eosin pink female* heterozygous for vermillion shows distinct and marked dilution. This can only be studied in the female since the male cannot be made heterozygous in vermillion which is sex-linked.

The *eosin vermillion fly* heterozygous for pink shows a dilution like that in the last case but both in the male and female.

The white-eosin female is the same in color as the eosin male.

The *white-eosin female*, heterozygous for pink or for vermillion, shows no dilution. When heterozygous both for pink and vermillion it seems to have an extended lower range of variation in color.

The *vermillion pink female*, heterozygous for eosin, is strongly diluted below the color of the vermillion-pink male. The vermillion-pink female heterozygous for white is still lighter than the female in the last case.

The *white-eosin pink female* is the same in color as the eosin-pink male, that is, the eosin pink female, which is darker than the male, is diluted to the level of the male by the substitution of one white for one eosin factor in the female.

The *white-eosin pink female*, heterozygous for vermillion, is diluted in the same proportions as the eosin pink heterozygous for vermillion is diluted. The *white-eosin vermillion female*, heterozygous for pink, is diluted in the same proportions as eosin vermillion heterozygous for pink.

The *white-eosin vermillion pink female* has the same eye color as the eosin vermillion pink male.

## CONCLUSIONS REGARDING SPECIFIC DILUTION EFFECTS

The most important relations that these detailed comparisons have brought to light are the following: It appears that the four factors involved may be arranged in the order of their *specific effectiveness in dilution*. First comes pink; second, vermilion; third, eosin; fourth, white.

Vermilion and pink eye colors cannot with advantage be directly compared because of differences in the quality of the eye colors. Their relative effect in dilution may, however, be compared in the following way. Starting with eosin as a base, and substituting the factors vv (vermilion) for VV, we obtain a specific dilution effect as the double recessive eosin vermilion. If we start with eosin and substitute pp for PP a specific dilution occurs giving eosin pink. A comparison of eosin vermilion color with eosin pink color shows that there is little difference between them—if either is lighter it is the eosin vermilion. The only other way to determine the relative effectiveness of these two factors in dilution is to observe which of the two becomes more diluted by heterozygosity for the same combination of other factors. If the end results in the two cases are unequal in color, the difference must be due to initial differences in the bases, for the diluters added in each case are the same. If then vermilion is diluted by a certain combination and pink is not, we may conclude that vermilion is a greater help to dilution than is pink. For instance, pink heterozygous for white shows no dilution effect, while vermilion heterozygous for white does show a slight dilution effect. Again, pink heterozygous for vermilion and white shows no effect, while vermilion heterozygous for white and pink shows a distinct dilution. It follows if the validity of the argument is admitted that vermilion is a slightly stronger diluter than pink.

If vermilion is used as a base and pp is substituted for PP a strong effect is produced. If vermilion is used as a base and w<sup>c</sup>w<sup>c</sup> is substituted for WW a still stronger dilution is produced, which shows that eosin is a stronger diluter than pink. Starting with pink as a base and adding vermilion we observe a certain

dilution effect (vermilion pink). Starting again with pink and adding eosin we obtain an eye color (eosin pink) much lighter than the last, showing that eosin is a stronger diluter than vermilion.

The white eye is obviously lighter than eosin. Its influence as a diluter in heterozygosis may next be shown. Vermilion heterozygous for white and pink is lighter than vermilion heterozygous for eosin and pink. Again, vermilion pink heterozygous for white is markedly lighter than vermilion pink heterozygous for eosin.

We have shown that the white-eosin compound female ( $w-w^e$ ) has the same eye color as the eosin male ( $w^e$ ). The substitution of  $pp$  for  $PP$  in the white-eosin female and the eosin male dilutes the eye color of each sex to a lower common stage. If one starts with the two equal stages of color the white-eosin compound female and the eosin male, and adds to each the same diluter or combination of diluters, then the final stages of each are equivalent in color. Thus, white-eosin compound vermilion female heterozygous for pink is the same in color as the eosin male heterozygous for pink, but both are much lighter than the eosin-white compound female or the eosin male. On the other hand, if one starts with the unequal stages, eosin female and eosin male and adds the same diluter to each, the two colors are proportionally reduced so that the end results have the same relative differences as the eosin female and male, as seen in eosin pink female and eosin pink male.

In certain combinations it is possible to lower the eye color of the female by half a step which is not possible in the male because of the diploid nature of the female in sex-linked characters and of the haploid condition of the male. For instance, the vermilion female heterozygous for white and pink is lighter than the male heterozygous for pink. A better case is that of vermilion pink female heterozygous for eosin or white being lighter than the vermilion pink male. Again white-eosin pink female heterozygous for vermilion ( $X w V p / X w^e v p$ ) is lighter than the eosin pink male ( $X w^e V p / -p$ ). She is also lighter than the

eosin pink female heterozygous for vermilion. White-eosin vermilion female heterozygous for pink ( $X w V P / X w^e v p$ ) is of the same color as the male eosin vermilion heterozygous for pink ( $X w^e v P / \text{---} p$ ) but both are lighter than the eosin vermilion female heterozygous for pink ( $X w^e v P / X w^e v p$ ). This is the only opportunity to dilute the eye of the male by heterozygosity because pink is the only factor employed that may exist in diploid in the male, and may therefore be halved, while the eosin vermilion is the only eye color of the right degree of pigmentation to be sensitive to such a dilution.

Accepting the eye color of the wild fly as the standard, we can, as we have seen in the preceding pages, describe all results obtained in terms of dilution, that is, as darker or lighter. The factors differ in their effectiveness as diluters. The dilution effect of each factor is specific, and having once determined the order of effectiveness of a series of factors (diluters) we can predict the relative effect of the same factors starting with any other stages of the same series. There are obviously several possible physical conditions which could give such a series of effects, for example, dilution might be accomplished by a decrease in the number or size of the colored granules, or by the addition of white granules, or the colors might be stages in a chemical process. Again, there might be several series of distinct pigments which would give different effects according as one or the other is changed in amount, and so forth.

The color of the eye might be compared to stature in man, which is the sum of the length of the legs, back, neck, head, etc. A shortening in any one of these parts would decrease the height. Obviously height is here only a common physical measure without intrinsic significance. Thus it may be with eye color, and, if so, any particular eye color may be the summation of several internal effects and 'dilution' refers only to the actually observed results.

In Mendelian literature certain factors are spoken of as diluters or intensifiers, as though their peculiar action were only to modify, up or down, certain other characters which by implication



are more fundamental. Miss Durham speaks for instance, of a factor in mice that dilutes black to blue and chocolate to silver fawn, and in cats Doncaster speaks of a diluter that changes black to blue and orange to cream. We feel that too much of a distinction has been made between so-called 'diluters' and other factors which cause changes in color. We mean here by dilution any effect that gives a lighter color, and the factors that produce such effects we speak of as diluters. The factors pink, vermilion, eosin, and white we put in the general class, diluters, because the colors produced by them are lighter than that of the wild fly. However, other eye color factors, that we know of, give colors darker than the wild red, and these we speak of as 'intensifiers.' Likewise in body color, yellow is lighter than wild gray, and black is darker. This statement applies to the pure recessive forms, pp (pink) yy (yellow) etc., and the lightening in heterozygous forms (whenever the effect shows) is essentially the same. In the former case the two diluters (pp) act as it were against the whole cell, in the latter, one diluter acts against the same cell as well as against its normal allelomorph. In the double recessive, such as vermilion pink, there are two pairs of diluters acting together against the rest of the cell and the dilution effect is roughly a summation of the effects of each.

Whether pink, vermilion, eosin, and white eye colors are due to losses from the original germ-plasm (presence and absence theory) or whether each is due to the presence of another active factor substituted for the original allelomorph, cannot be answered from the data here presented. The occurrence of multiple allelomorphism in the red-white-eosin case suggests however that the latter view is correct, and from other grounds (e.g., reversible mutations) we have adopted it as our working hypothesis. In connection with sex-linked factors there is perhaps a question of real absence from the point of view that the female has two sex chromosomes and two sets of sex-linked factors, while the male has only one sex chromosome and therefore only one set of sex-linked factors. For instance, the vermilion male differs from the vermilion female in that his single sex chromosome carries only

one vermilion factor while her two sex chromosomes carry two vermilion factors. The effects produced by the two sets of conditions are the same, and we have found that generally in sex linked characters the effects are equivalent in the two sexes, that is, one sex chromosome produces an effect in the male equivalent to that produced by two in the female.

Columbia University  
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# VARIATIONS IN THE PROGENY OF A SINGLE EX-CONJUGANT OF PARAMECIUM CAUDATUM

GARY N. CALKINS AND LOUISE H. GREGORY

*Department of Zoölogy, Columbia University*

EIGHT DIAGRAM FIGURES

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## 1. NATURE OF THE PROBLEM

The traditional view, advocated by Weismann, 1881, that all protozoa are potential germ cells, has held the stage for so long that it seems almost iconoclastic to doubt the statement. Recent writings, however, seem to foreshadow a change of opinion. Woodruff writes('09): "I believe these results indicate that the phenomenon (i.e., conjugation) is not so frequent in the life history as is generally believed" (p. 304). Nor was Woodruff able to induce the individuals in his long culture to conjugate: "There has been no tendency to conjugate among the paramecia of this culture and a large series of experiments, which have been made with the individuals left over from the four lines of the culture after the daily isolations, have, so far, yielded not a single pair of conjugants" (1911, p. 264). In striking contrast to this result is that obtained by Jennings with a race of the same species as that used by Woodruff (*Param. aurelia*). The individuals conjugate every two or three weeks and can be depended upon to conjugate whenever the conditions are provided (race k). But along with this conjugating race Jennings has cultivated other strains of *P.* which have never been observed to conjugate (e.g., race D, Jennings '10), while other races possessed the power in varying degrees. Furthermore, it is a matter of common experience to find wild cultures in the laboratory which yield epidemics of conjugation while other cultures equally rich give none at all.

The results of previous careful experimental work therefore shows that some races of *Paramecium* will conjugate, some will not, under the conditions usually employed to induce conjuga-

tion. *Paramecium* may be accepted as a typical form of infusoria with which to test the value of Weismann's generalization, and with the various observations and results of experimental work on *Paramecium* in mind, a series of experiments was planned to find out, if possible, what the conditions are which underlie conjugation. In the course of the investigation many other points showing variations in the progeny of a single ex-conjugant have been obtained. These indicate a phenomenon of the nature of a developmental process in the life history of *Paramecium* and throw new light as well upon the old problems of age and death in protozoa. The experiments were started in July, 1912, Miss Gregory sharing the work from November on.

## 2. EXPERIMENTAL METHODS

All experiments here described were each started with a single-celled ex-conjugant of *Paramecium caudatum*. The most striking results were obtained in connection with a race known in the laboratory records as the J series and this may serve to illustrate the methods employed in all cases, since the methods have been the same in all.

A single pair, I-J of conjugating *Paramecium caudatum* was isolated from a 'wild' pond culture on July 21, 1912; on the 22nd the two cells, I and J, had separated and each was isolated in a small watch glass with four drops of twenty-four hour hay infusion as culture medium. On the 25th each of them had divided twice, forming four cells each and from that time division was fairly regular for the J series, while the I series became irregular in division and weak in vitality, the entire race finally dying in August. The first four cells formed after conjugation and referred to in the following pages as quadrants A, B, C, and D, have particular importance for the reasons given in the preceding section. This stage of development is important because if there is any differentiation it is reasonable to suppose that it should take place in this early period of development when by division and metamorphosis the normal condition of the organism

is attained for the first time. In order to see if further division of these four would lead to other variations, each was carefully followed and the progeny isolated after each of the next three divisions, eight lines finally resulting from each of the four original cells.

#### *A. Permanent cultures*

These original thirty-two cells raised to this stage by daily isolation in watch glasses, were placed in separate vials and allowed to multiply, the culture fluid (twenty-four hours hay) being changed every two or three days at first, later about once a week. These cultures of pure lines may be designated the 'permanent cultures.' In adding new food medium the contents of the vial amounting to about 2 cc. are poured off, leaving about 0.25 cc. on the bottom, fresh medium then is added to make up 2 cc. By this process the *Paramecia* are kept in good condition and are not over-fed and conjugation rarely occurs. (In April and March two pairs were found in the J 1 line). In order to safeguard the cultures against accidents, three complete sets of permanent cultures are carried along at the same time. Many of the pure lines have been saved by this precaution, for notwithstanding the utmost care with them the entire population of a vial is found dead from time to time, the causes of such mortality being some slight difference in density or of bacterial flora. In all tests made from permanent cultures all three sets are used.

#### *B. Petri dish cultures*

In addition to the permanent cultures exposed to daylight in corked vials, other cultures of the pure lines have been kept in a dark cabinet in Petri dishes. Such cultures, however, do not flourish as well as those in the vials—multiplication is less rapid and the organisms require much more frequent change of medium. These cultures have not been used in making the various tests.

*C. Vitality tests*

Vitality of the different pure lines is tested from time to time by the division rate method. Two representative individuals of each pure line are isolated in small flat-bottomed watch glasses, containing four to five drops of twenty-four hour hay infusion, a single individual in each glass. These are examined, the number of divisions noted, and one individual is selected and isolated daily in fresh twenty-four hours hay. Each test extends over a period of thirty days, the division rate is then averaged for both individuals in all the lines and for consecutive periods of five days each. This gives a fairly accurate basis for comparison of the relative vitality of progeny from different quadrants (p. 476).

*D. Conjugation tests*

It is a well-recognized fact at the present time that a sudden change from conditions inducing rich feeding and rapid multiplication to conditions involving a cessation of growth and multiplication will cause *Paramecium* to conjugate provided the power to conjugate is in them. Jennings, Zweibaum, Woodruff, and others have employed this method with success, each working it out in his own way. For years at Columbia we have been in the habit of obtaining conjugations when desired by transferring *Paramecium* from rich laboratory cultures to small Syracuse dishes 65 mm. in diameter and 5 mm. deep with a capacity of 16 cc. The bacteria transferred with the animals are soon exhausted and if the *Paramecia* will conjugate at all they will do so within forty-eight hours. If the bacteria are not exhausted a state of equilibrium is established and the ciliates may live for weeks without conjugating, multiplying slowly. The same method is employed in making the conjugation tests in these experiments, but several steps are necessary. The tests are made once a month and the first step is the preparation of a rich culture. For this the contents of the permanent cultures are distributed in three or four vials where the organisms are allowed to multiply for two days. They are then used for seeding rich food media

in ordinary cylindrical staining pots having a capacity of 175 cc, the medium consisting of twenty-four to forty-eight hour hay infusion plus a small quantity of sterilized chopped hay. This is the second step. In these staining pots the organisms are allowed to multiply rapidly for a period of from two to three days giving rise to what we call 'rich cultures.' The first conjugation test is then made. This, the third step in the process, consists in transferring with a pipette used exclusively for one pure line, a thousand or more Paramecium from the staining pot to a Syracuse dish and to this collection a little fresh hay infusion is also added which seems to give a stimulus for further, but limited, cell division. The Syracuse dishes are numbered to correspond with the pure line used, and stacked away for daily observations. On the second day there are from 2500 to 3500 Paramecium in each Syracuse dish. All of the pure lines are tested simultaneously and the fourth step in the process consists in the daily search amongst these thousands of Paramecium for conjugating pairs. For these examinations we use a Zeiss binocular microscope with lens combination of no. 2 oculars, and an A<sub>2</sub> double objective, giving a magnification of about 24 diameters. In making the examinations the numbers on the Syracuse dish are kept hidden and are looked at only when the record of the examinations is made; in this way all of the pure lines are subjected to the same careful scrutiny and if conjugating pairs are observed, the number counted is recorded. The condition of the organism is also recorded by symbols and the minimum and maximum temperatures for 24 hours are taken daily. These daily examinations last for a period of from four or five, to ten days, or until the minus sign appears on two or more consecutive records (p. 502).

Three such tests at least are made for each rich staining pot culture, the first one after two to three days in the rich culture, the second after from five to eight days, the third after eight to ten days, and finally the staining pots themselves are examined for at least four different periods, to see if conjugations are in progress there. Approximately 12,000 different individuals from each pure line are thus watched for a period of several days and if conjugations occur there is little chance of their being over-



looked. After each test the organisms examined are thrown away, the permanent cultures alone being maintained. Each conjugation test therefore, involves organisms which have not conjugated since the initial isolation of the ancestral cell.

### 3. HISTORIES OF THE DIFFERENT SERIES STUDIED

In addition to the J series started in July, ten additional series have been studied for comparison; these, described under their laboratory names, are as follows:

1. The J series described above.
2. The B series, started December 14, 1912, from a conjugating pair from a wild culture,
3. The E' series started December 14 from another pair from the same wild culture,
4. The G series started on the same date from the same culture,
5. The H' series from the same,
6. The L series, also from the same culture,
7. The M series,
8. The Q series, and
9. The T' series,

were all started on the same date from different pairs in the same culture.

10. The O series was started December 4, 1912, from a conjugating pair obtained from one of the conjugation tests of pure line no. 8 of the J series and

11. The P series was started December 3, 1912, from a conjugating pair from the same test. The history of these series will be described separately to avoid confusion.

#### *A. The J series*

It is not a simple matter to get 32 pure lines started from the first four cells from an ex-conjugant. In the July set, no less than 16 ex-conjugants were isolated and the progeny carried through the preliminary divisions until some one or more individuals died, thus blocking the experiments for that set, for it

is absolutely necessary to have representative lines from each of the first four cells. The J series was the 16th set, starting as an ex-conjugant July 22, and dividing for the first time on the 24th. The second division occurred on the 25th, thus establishing the four quadrants. These, and the following divisions are shown graphically in diagram 3 and the origin of the different pure lines given. These were numbered in the order of their appearance and without regard to sequence in the different quadrants (see p. 487).

The five cell divisions, necessary to start the 32 lines, although lagging in quadrant marked B, were finally completed on July 27 with only one mishap. This was a pathological division of no. 19 which resulted in two deformed specimens no. 19 and no. 32, and both of which finally died on August 3 without further division. Two of the 32 lines were thus lost at the outset. The single individuals representing these 30 lines were allowed to divide without further isolations after the 5th division and the progeny of each were transferred to vials for the permanent cultures. These were examined every four or five days, the physical condition recorded, and the culture medium changed each time. The record shows that the organisms in certain lines were weak from the start, thus lines no. 3, no. 22, and no. 23 were weak at each period of inspection and finally died, no. 3 and no. 22 on August 22, no. 23 on August 26. Two other lines, no. 5 and no. 6, also were dead on the 22nd, although they had been strong and normal up to that time. Line no. 4 died out on October 7 after a period of weakness. The death of these eight lines left 24 pure lines distributed as follows: Four from quadrant A, five from quadrant B, eight from quadrant C, and seven from quadrant D. One other line, no. 14, from quadrant C, has died since October after the conjugation tests had been running for three months, so that only 23 lines are now alive (diagram 3, p. 487).

*B. The B series*

The pair B B' was isolated on December 13 from a wild culture. The progeny of B' died after three divisions, those of B were not strong but enough of them continued to live through the early division stages to give three pure lines from quadrant A, two from quadrant B, five from quadrant C, and four from quadrant D, fourteen lines in all representing all four quadrants. The history up to the present is shown in diagram 4 (p. 490).

*C. The E' series*

This series also started on December 13 with a conjugating line from the same wild culture as the B series, gave more satisfactory results. The progeny from individual E soon died, while E' continued to live. The history is shown in diagram 5. Twenty-four lines continued to live, 3 from quadrant A, 7 from quadrant B, 7 from quadrant C, and 7 from quadrant D (p. 492).

*D. The G, H', L, M, Q and T' series*

These series are all from conjugating pairs isolated from the same wild culture December 13. They differ from the other series described above in comprising only four lines each, these lines coming from the first four cells of the ex-conjugant in each case. Each line thus corresponds to an entire progeny from a quadrant of the preceding series. The history of these series is shown in diagram 6 (p. 494).

*E. The O and P series*

These series came from conjugating pairs in line 8 of the J series isolated on December 3. Although many attempts were made to get perfect sets, none was successful, the O series and the P series being the nearest approach to success, and in these only 11 pure lines were obtained in the O series and only 10 in the P series, representing in both cases only two of the four quadrants. Their particular interest lies in the fact that they represent the F<sub>2</sub> generation and have been derived from a pure line whose history is known.

## 4. THE RELATIVE VITALITY OF PURE LINES

Jennings ('13) has clearly shown that some races of *Paramecium* derived from ex-conjugants continue to live in the particular environment of the experimental laboratory conditions, while other races die. This fact indicates an initial difference in vitality in different races and justifies the conclusion that conjugation does not result in all cases in such a reorganization of cellular elements as will enable the ex-conjugant to live as well as either of the parents had lived with its organization prior to conjugation. Our experience with many ex-conjugants confirms these observations in general and leads to the further problem of the relative vitality of different pure lines derived from a single ex-conjugant. We will first consider the vitality of different ex-conjugants.

We have examined more than 360 ex-conjugants in the course of these experiments. Of these 18 per cent continued to live and multiply normally for more than two months after conjugation; 20 per cent died within twenty-four hours after conjugation; 6.5 per cent died in forty-eight hours; 8.5 per cent in seventy-two hours; 4 per cent in ninety-six hours; 7 per cent in one hundred and twenty hours; 18 per cent in ten days; 7 per cent in twenty days; 5.5 per cent in one month and 2.5 per cent in two months. The chance of life, therefore, is very good if the organisms live through the first ten days during which the causes of death are due in the main to faulty reorganization. The results show in general that about 18 per cent of the ex-conjugants develop progeny capable of living under the conditions of the experiments a much lower percentage than that obtained by Miss Cull (70 per cent) which may be explained in part by our use of part wild, part cultured *Paramecium*.

Our particular interest at present centers in the relative vitality of the different pure lines derived from a single ex-conjugant progeny of one of the 18 per cent that continued to live. Of these the J series is the oldest and most important.

From the outset it has been evident that the progeny from the different quadrants vary in respect to both vitality and size. The size variations will be considered in another section. The vitality variations are indicated, in general, by the history of the

permanent cultures given above (p. 474), in which it is shown that four lines out of eight of quadrant A died out entirely whereas three lines (two of these due to a pathological division which may be considered an indication of weakness) died from quadrant B, and one each from quadrants C and D. In order to test the relative vitality more directly, however, the division rate of representative specimens from each line was carefully kept for thirty days in October and November, and again for forty-two days in February and March. The rates were averaged for five-day periods, the results of both tests being shown in the accompanying graphs. These are based upon the averages for five-day periods of all the lines of each quadrant.

The division rate is generally accepted as an index of vitality since it represents the rapidity with which the organisms reach the limit of growth or the rapidity of metabolism. It is evident from this test, together with the evidence from the permanent cultures, that the pure lines derived from quadrant A were physiologically weaker than those from the other quadrants for a period of at least five months, a result shown in the first test by the averages of the division rates of all lines of each quadrant—0.65 for quadrant A, 0.908 for quadrant B, 0.81 for quadrant C, and 0.95 for quadrant D. Or, in one hundred days a typical representative of A would have divided 65 times, of B, 90 times, of C, 81 times and of D, 95 times.

The March vitality test (diagram 1) is interesting as showing a much closer agreement amongst the pure lines from different quadrants. For this test we are indebted to Mrs. Binkley who carried it on for more than six weeks under our direction and with our methods. Her results, worked out biometrically, will be published independently. It must be noted that a period of four months intervened between the first and second tests all lines in this interval being maintained as nearly as possible under identical conditions, the cultures being replenished at the same times and with the same media. The graphs show that the four lines of quadrant A are now almost if not quite as vigorous as those of the other quadrants, all lines indicating greater vigor than at the period of the first test.

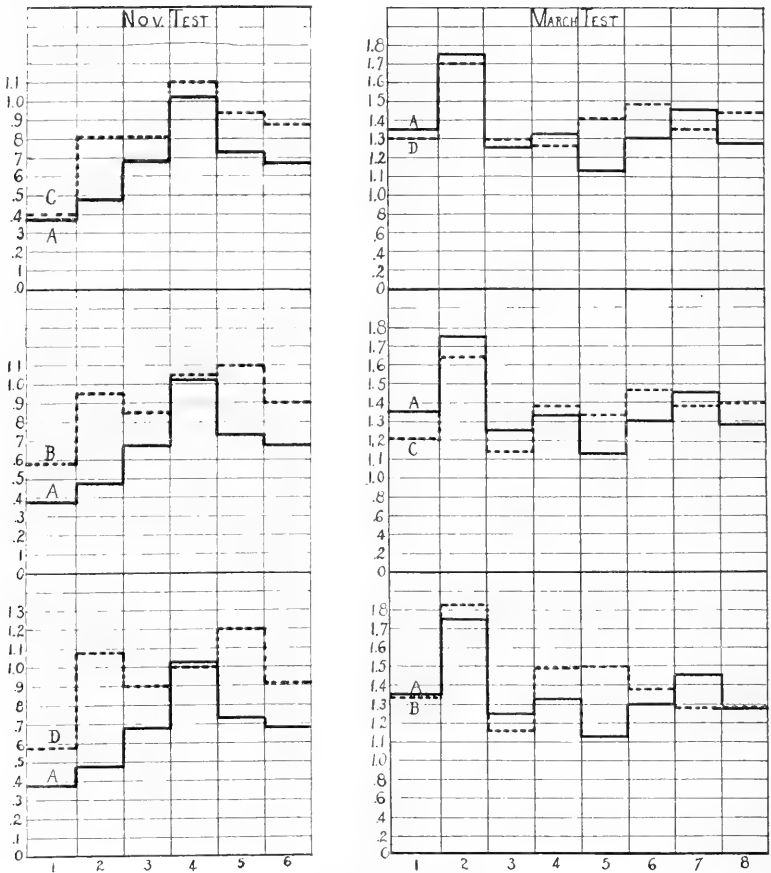


Diagram 1

Similar variations in vitality are shown in the histories of series B, E', O and P, and of the 4-line series G, H', L, M, Q and T', although the daily division rate has not been tested for these other cases. Of the 14 pure lines of the B series, 3 only are left from quadrant A, 1 from B, 5 from C and 4 from D, the greatest mortality occurring in A and B. Of the 15 lines now living of the E' series only 2 are in quadrant A, 4 are in quadrant B, 6 in C, and 3 in D. Again quadrant A is the weakest. In series O

and P only two quadrants are represented, the other two dying out before reaching the 32-cell stage.

The histories of the 4-line series are interesting because they indicate physiological differences in the first four cells derived from an ex-conjugant. Twenty-two conjugating pairs were isolated December 14, from a wild culture in an old hay infusion and the 44 ex-conjugants were followed in culture and the descendants isolated until four pure lines from each were obtained or until death of one of the early descendants made the series worthless for these experiments when the series was discarded. Of these 44 individuals 15 died on the day following isolation without dividing; 3 died on the second day without dividing; 5 were discarded on the fifth day because of death of one or more quadrants; 5 were discarded on the sixth day because of death of all the progeny of one quadrant; 1 was discarded on the seventh day for the same reason; 2 on the twelfth day because of death of two quadrants; 3 gave four pure lines from the four quadrants, all of which lived for three weeks when they were discarded because of death of one or two quadrants; 4 were discarded for the same reason at the end of one month, the remainder (6) continued to live and form the basis of the experimental conjugation tests of the 4-line series. In all of these cases of discard it must be borne in mind that the entire race in each case had not died, only the progeny of one or two of the original quadrants showing that mortality is not evidence of hereditary weakness but of individual weakness. The remaining three or two quadrants were healthy and normal and would undoubtedly have continued to live. The extinction shows a relative weakness in some of the progeny from an ex-conjugant while others are strong. In other words, the same physiological variations that are found between the progenies resulting from different ex-conjugants, are to be found in the progeny from the same ex-conjugant.

## 5. SIZE VARIATIONS IN DIFFERENT PURE LINES FROM ONE ANCESTRAL CONJUGANT

The pure lines of *Paramecium caudatum* which we have been working with have given an excellent opportunity for the study of size variations in different lines from one ancestral cell. This is especially true of the J series which has been more extensively studied than any other set. The daily observation of individuals in different lines of this series have shown clearly marked and constant variations in size amongst them. Line 20 for example is made up of individuals which always appear smaller than those of any other line, while line 21 is always made up of relatively enormous forms.

In order to test the matter of size variations more accurately two sets of measurements were taken at an interval of about six weeks. The material was obtained at each period from rich cultures which had been prepared for the monthly conjugation tests. These cultures were seeded on March 6 and April 17 with material from the permanent cultures of the 23 lines of the J series. For each set of measurements random samples from top and bottom of each rich culture were killed in Worcester's fluid four days after the rich cultures were seeded, for example, on March 10 and April 21. Measurements were made immediately and without removing the organisms from the killing fluid. For these an ocular micrometer was used in which each division has the value of 13.75 microns. The measurements were made by one of us only, 100 individuals from each line being measured at the first period and 50 from each line at the second. As there were only four lines remaining in quadrant A the number of lines measured was limited to 16, or 4 from each quadrant, the lines being so chosen as to give material for correlation studies of pure lines from later sister cells in the first two quadrants and from earlier ancestral cells in the last two. Thus lines 1 and 7, 16 and 20, 17 and 41, are from sister cells in the 32-cell stage while 2 and 10, 13 and 15, 26 and 29, 28 and 30 came from sister cells in the 16-cell stage after conjugation. We are deeply indebted to Prof. H. E. Crampton for assistance in working up the material according to biometrical methods.



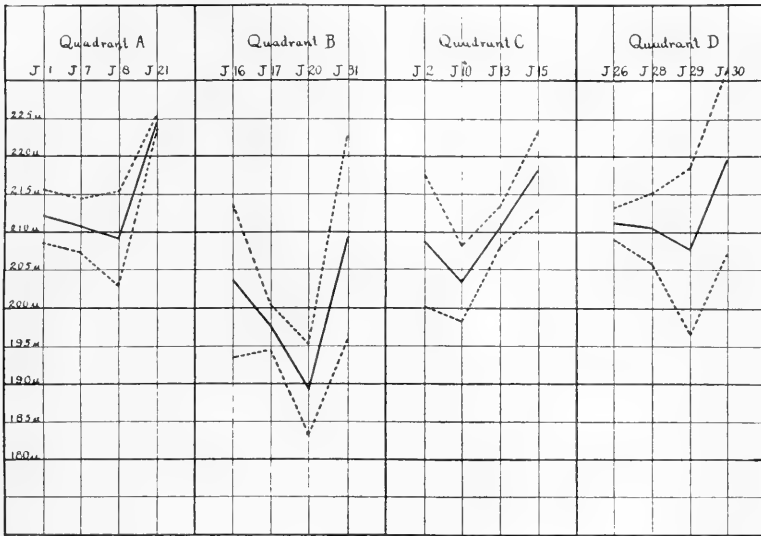


Diagram 2

The mean length and breadth and the standard deviation are given for each measurement and for each of the 16 lines in tables 1 and 2 arranged in quadrants. The means for length alone are given in table 3 arranged in serial order for both periods. From the latter table (column 3) it is evident that three distinct groups separated from one another by at least 10 microns were present in the first series of measurements. The means of the second series of measurements were greater in all cases than those of the first, the actual differences in microns for lengths only are graphically shown in diagram 2.

The tables show that certain lines are highly variable both as to length and breadth. Some, notably 31, increased in all dimensions; others, notably 16, 15, 13 and 21, increased in length at the expense of breadth. Some lines, notably 21, 26, 13, 20, 7 and 1 are more stable. Diagram 2 shows that quadrant A, consisting of J 1, 7, 8, and 21, is less variable as a group than any of the other quadrants. As the sequel will show the quadrant A lines are all conjugating lines while the others are not. This

TABLE I  
*Constants of variations in lengths of 16 pure lines from ex-conjugant J*

LINES	RANDOM SAMPLE; 100 INDIVIDUALS EACH, MARCH 10				RANDOM SAMPLE; 50 INDIVIDUALS EACH, APRIL 21			
	Mean length in microns	Standard deviation in microns	Coefficient of variation	Mean length in microns	Standard deviation in microns	Coefficient of variation		
Quadrant A J	1	208.65 ± 1.047	19.373 ± 0.798	7.511 ± 0.358	215.60 ± 1.566	16.428 ± 1.106	7.619 ± 0.513	
	7	207.35 ± 0.966	14.345 ± 0.683	6.918 ± 0.329	214.36 ± 1.469	15.415 ± 1.039	7.195 ± 0.484	
	8	202.74 ± 0.962	14.272 ± 0.680	7.039 ± 0.335	215.32 ± 1.307	13.710 ± 0.924	6.367 ± 0.428	
	21	223.85 ± 0.990	14.689 ± 0.699	6.560 ± 0.312	225.36 ± 1.740	18.255 ± 1.230	8.100 ± 0.545	
Quadrant B J	16	193.60 ± 1.216	18.051 ± 0.860	9.323 ± 0.444	213.67 ± 1.562	16.374 ± 1.104	7.663 ± 0.516	
	17	194.56 ± 0.914	13.563 ± 0.646	6.971 ± 0.332	200.33 ± 1.277	13.400 ± 0.903	6.689 ± 0.450	
	20	183.15 ± 0.985	14.632 ± 0.697	7.989 ± 0.380	195.11 ± 1.343	14.081 ± 0.948	7.215 ± 0.485	
	31	195.93 ± 0.926	13.750 ± 0.655	7.017 ± 0.333	222.75 ± 1.556	16.226 ± 1.110	7.267 ± 0.489	
Quadrant C J	2	200.23 ± 1.080	16.039 ± 0.764	7.629 ± 0.363	217.52 ± 1.824	19.138 ± 1.289	8.798 ± 0.592	
	10	198.27 ± 0.952	14.146 ± 0.673	7.134 ± 0.340	208.17 ± 1.304	13.682 ± 0.922	6.572 ± 0.442	
	13	208.03 ± 1.072	15.911 ± 0.757	7.645 ± 0.364	213.26 ± 1.526	16.005 ± 1.079	7.504 ± 0.505	
	15	212.85 ± 0.918	13.626 ± 0.649	6.399 ± 0.304	223.26 ± 1.311	13.762 ± 0.928	6.156 ± 0.414	
Quadrant D J	26	209.00 ± 0.913	13.545 ± 0.644	6.478 ± 0.308	213.26 ± 1.520	13.944 ± 1.075	7.476 ± 0.503	
	28	205.85 ± 0.994	14.756 ± 0.702	7.166 ± 0.341	215.18 ± 1.923	20.172 ± 1.359	9.374 ± 0.631	
	29	196.62 ± 1.354	20.090 ± 0.957	10.217 ± 0.486	218.48 ± 1.332	13.974 ± 0.941	6.395 ± 0.430	
	30	207.21 ± 0.814	12.489 ± 0.594	6.027 ± 0.287	231.82 ± 1.484	16.615 ± 1.112	7.167 ± 0.482	

TABLE 2  
*Constants of variation in breadths of 16 pure lines from ex-conjugant J*

LINES	RANDOM SAMPLE; 100 INDIVIDUALS EACH, MARCH 10				RANDOM SAMPLE; 50 INDIVIDUALS EACH, APRIL 21			
	Mean breadth in microns	Standard deviation in microns	Coefficient of variation		Mean breadth in microns	Standard deviation in microns	Coefficient of variation	
Quadrant A	1	54.381 ± 0.387	5.7585 ± 0.176	10.589 ± 0.505	54.892 ± 0.563	5.9125 ± 0.398	10.776 ± 0.726	
	7	52.456 ± 0.484	7.1871 ± 0.409	13.701 ± 0.653	50.737 ± 0.613	6.4332 ± 0.433	12.677 ± 0.855	
	8	50.462 ± 0.449	6.6811 ± 0.314	13.239 ± 0.631	52.593 ± 0.570	5.9826 ± 0.402	11.390 ± 0.768	
	21	56.512 ± 0.371	5.7172 ± 0.262	10.116 ± 0.482	55.137 ± 0.558	6.0692 ± 0.408	11.007 ± 0.742	
Quadrant B	16	64.900 ± 0.525	7.796 ± 0.371	12.012 ± 0.572	56.100 ± 0.591	6.2040 ± 0.418	11.052 ± 0.745	
	17	55.687 ± 0.401	5.9551 ± 0.290	10.693 ± 0.510	54.450 ± 0.611	6.4253 ± 0.433	11.800 ± 0.795	
	20	56.237 ± 0.435	6.4762 ± 0.308	11.515 ± 0.549	55.137 ± 0.528	5.7502 ± 0.374	10.428 ± 0.703	
Quadrant C	31	51.837 ± 0.451	6.6948 ± 0.309	12.915 ± 0.616	61.462 ± 0.686	7.1981 ± 0.485	11.711 ± 0.789	
	2	56.100 ± 0.391	5.8107 ± 0.279	10.357 ± 0.494	52.112 ± 0.541	5.6842 ± 0.382	10.907 ± 0.735	
Quadrant D	10	51.218 ± 0.416	6.1778 ± 0.294	12.061 ± 0.575	51.287 ± 0.572	6.0252 ± 0.405	11.747 ± 0.792	
	13	55.343 ± 0.460	6.8310 ± 0.324	12.347 ± 0.588	52.112 ± 0.435	4.5787 ± 0.308	8.786 ± 0.592	
	15	58.025 ± 0.411	6.0953 ± 0.290	10.504 ± 0.511	52.800 ± 0.462	4.8496 ± 0.325	9.184 ± 0.619	
Quadrant E	26	53.025 ± 0.402	5.9922 ± 0.284	11.174 ± 0.522	54.450 ± 0.583	6.1242 ± 0.413	11.247 ± 0.758	
	28	52.937 ± 0.345	5.0985 ± 0.242	9.631 ± 0.459	52.250 ± 0.540	5.6691 ± 0.382	10.850 ± 0.731	
	29	52.868 ± 0.365	5.4381 ± 0.258	10.286 ± 0.490	53.625 ± 0.368	3.885 ± 0.261	7.251 ± 0.489	
	30	54.175 ± 0.347	5.1700 ± 0.246	9.543 ± 0.455	53.908 ± 0.433	4.5471 ± 0.306	8.435 ± 0.568	

TABLE 3

*Mean lengths in microns of 16 pure lines from ex-conjugant J, arranged in groups*

MARCH 10			APRIL 21		
1	2	3	4	5	6
No.	Line	Mean	No.	Line	Mean
100	20	183.150	50	20	195.112
100	16	193.600	50	17	200.337
100	17	194.560	50	10	208.175
100	31	195.937	50	13	213.262
100	29	196.625	50	26	213.262
100	10	198.275	50	16	213.675
100	2	200.237	50	7	214.362
100	8	202.743	50	28	215.187
100	28	205.850	50	8	215.325
100	30	207.212	50	1	215.600
100	7	207.350	50	2	217.520
100	13	208.037	50	29	218.487
100	1	208.656	50	31	222.750
100	26	209.000	50	15	223.575
100	15	212.85	50	21	225.365
100	21	223.85	50	30	231.825

may indicate a correlation between stability and conjugating power.

The same correlation is shown by the coefficients of variation for the two measurements in respect to proportions of length to breadth given in table 4.

#### 6. VARIATIONS IN CONJUGATING POWER OF PURE LINES

We now come to the most interesting and suggestive results of these experiments, namely, the differences shown by different pure lines from the same ancestral ex-conjugant in respect to the ability to conjugate. The methods employed in the conjugation tests have been described (p. 471) but attention may be called again to the fact that conjugations do not occur in the permanent cultures and that conjugations unless otherwise stated, are always strictly paedogamous.

TABLE 4  
*Constants of variation in proportions of lengths and breadths of 16 pure lines from ex-conjugant J*

LINES	RANDOM SAMPLE; 100 INDIVIDUALS EACH, MARCH 10				RANDOM SAMPLE; 50 INDIVIDUALS EACH, APRIL 21			
	Mean proportion	Standard deviation	Coefficient of variation		Mean proportion	Standard deviation	Coefficient of variation	
Quadrant A	1	25.68 ± 0.1624	2.4078 ± 0.1148	9.376 ± 0.447	25.06 ± 0.2300	2.4111 ± 0.1626	9.621 ± 0.648	
	7	24.99 ± 0.2013	2.9847 ± 0.1423	11.943 ± 0.5696	23.44 ± 0.2425	2.5425 ± 0.1714	10.824 ± 0.731	
	8	24.68 ± 0.1956	2.9000 ± 0.1383	11.750 ± 0.560	24.00 ± 0.2351	2.4657 ± 0.1663	10.273 ± 0.692	
	21	24.90 ± 0.1645	2.4392 ± 0.1163	9.795 ± 0.467	24.06 ± 0.1911	2.0049 ± 0.1351	8.332 ± 0.561	
Quadrant B	16	33.35 ± 0.2922	4.3322 ± 0.2066	12.990 ± 0.619	25.92 ± 0.2497	2.6177 ± 0.1765	10.099 ± 0.681	
	17	28.15 ± 0.1699	2.5194 ± 0.1201	8.949 ± 0.426	26.74 ± 0.2841	2.9786 ± 0.2009	11.137 ± 0.751	
	20	30.42 ± 0.2437	3.6143 ± 0.1724	11.881 ± 0.566	27.80 ± 0.2257	2.3664 ± 0.1596	8.512 ± 0.574	
	31	26.05 ± 0.1871	2.7744 ± 0.1324	10.650 ± 0.508	27.22 ± 0.2323	2.4354 ± 0.1642	8.947 ± 0.603	
Quadrant C	2	26.42 ± 0.1812	2.6876 ± 0.1280	10.172 ± 0.485	23.64 ± 0.2558	2.6814 ± 0.1808	11.342 ± 0.765	
	10	25.58 ± 0.2139	3.1723 ± 0.1513	12.401 ± 0.591	24.30 ± 0.2092	2.1931 ± 0.1478	9.020 ± 0.608	
	13	26.50 ± 0.2062	3.0577 ± 0.1458	11.534 ± 0.540	24.14 ± 0.1561	1.6371 ± 0.1104	6.781 ± 0.457	
	15	26.90 ± 0.1694	2.5119 ± 0.1198	9.337 ± 0.445	23.18 ± 0.1634	1.8618 ± 0.1255	8.031 ± 0.541	
Quadrant D	26	25.26 ± 0.1281	1.9006 ± 0.0906	7.524 ± 0.358	25.24 ± 0.3038	3.1847 ± 0.2148	12.611 ± 0.850	
	28	25.20 ± 0.1632	2.4207 ± 0.1159	9.605 ± 0.458	23.92 ± 0.2537	2.6593 ± 0.1793	11.117 ± 0.749	
	29	26.80 ± 0.2653	3.9344 ± 0.1876	14.680 ± 0.700	24.42 ± 0.1749	1.8340 ± 0.1237	7.510 ± 0.506	
	30	25.76 ± 0.1349	2.0005 ± 0.0954	7.765 ± 0.370	23.12 ± 0.1836	1.9249 ± 0.1298	8.325 ± 0.561	

In the J, B, and E' series the pure lines dealt with represent the first 32 cells of an ex-conjugant; the O and P series deal with pure lines from only two quadrants in the 32 cell stage the progeny from the other two quadrants having died, the series G, H', L, M, Q, and T' deal with pure lines from the 4-cell stage on. As the conditions thus are somewhat different in the different series we shall consider them separately.

#### *A. Conjugations in the J series*

The first conjugation tests made with the 24 pure lines of this series were exogamous tests made with 50 individuals from the pure lines, and 50 individuals from a pure ex-conjugant culture, known as no. 34'. The mixtures were made October 22. No conjugations resulted, probably because of the small numbers used. Similar mixtures with pure culture no. 74', likewise from one ex-conjugant, were made on October 28. No conjugations resulted. Another set was made with pure culture no. 41 on November 1. No conjugations resulted. These mixtures were watched in each case for from ten to twenty days. A fourth set of mixtures was made with pure culture no. 29 on November 6 and a small number of pairs (4) were found in the mixture containing J 8. It was evident from these initial tests that the numbers employed were not large enough to furnish evidence of the conjugating power of the different lines. Furthermore, it was seen that when conjugations did occur it was impossible to determine whether the unions were paedogamous or exogamous—the size differences furnishing no clue—although control tests were made at the same time with individuals from the pure cultures in each case. From this time on therefore we confined our observations to paedogamous tests alone, Calkins ('02) and Jennings ('10) having shown that paedogamous conjugations are just as effective as exogamous.

The first paedogamous test of the 24 lines was made November 19, an equal number (as nearly as possible where thousands are dealt with) were taken from the rich cultures and placed in Syracuse dishes. Thirty pairs were counted in the J 1 line and 100 pairs in the J 21 line and not a single pair in the other twenty-two

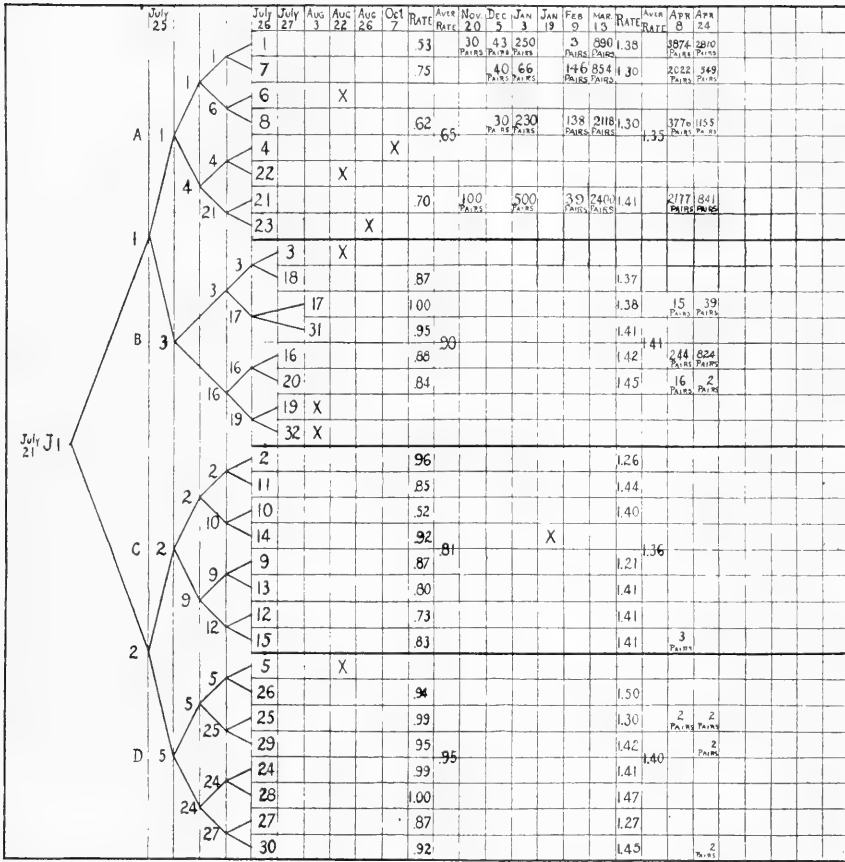


Diagram 3

lines. The test was repeated November 22 with the same result but this time only 4 pairs appeared in J 1 and only 63 in J 21, none in the others. A third test from the same rich cultures was made November 27 and gave negative results for all 24 lines. The complete record by months of the J series and the division rates in October and March are shown in diagram 3.

New rich cultures of all lines of the J series were seeded during the last week in November and the first test was made on December 1, a second on December 3, and 4. The result of these

tests was 48 pairs in J 1, 49 in J 7, 34 in J 8, and, 9 in J 21, all from quadrant A while not one pair was found in the other twenty pure lines. A mixture of J 1, J 8 and J 21 gave more than 500 pairs. The result of this test for one date is shown in diagram 3, December 5.

For the January test the rich cultures were seeded on the 26th of December and tests were made at different times, the aggregate result being 388 pairs in J 1; 135 pairs in J 7; 528 pairs in J 8; 816 in J 21, all in quadrant A and not one pair in the remaining twenty lines.

For the February test the rich cultures were seeded February 1 and the tests were made on the 3rd, 5th and 9th, resulting in 3 pairs in J 1; 136 in J 7; 138 in J 8; 39 in J 21, again all in quadrant A, and not one pair in the remaining nineteen lines (no. J 14 died out January 19).

For the March test the rich cultures were seeded March 6 and the tests were made on the 9th, 13th and 15th. The earlier history was again repeated but larger numbers of pairs were found in the conjugating lines of quadrant A: 890 pairs in J; 1854 pairs in J 7; 2118 in J 8, and 1400 in J 21. Again not a single pair was found in the remaining nineteen lines. The five monthly tests from permanent culture material which had not conjugated since isolation of the ex-conjugant in July, thus show a remarkable physiological variation in the progeny from that ex-conjugant. The variations furthermore were remarkably consistent during this period, the lines that had conjugated at the first test, continued to give conjugations at each subsequent test and would have given conjugations at any time the test might have been made. During all of this period the non-conjugating lines were equally consistent, not a single pair occurring in all of the non-conjugating lines. The interest of this finding is heightened by the fact that the conjugating lines all come from one quadrant of the ex-conjugant which was separated from the other three quadrants at the second division after conjugation.

The April test brought a surprise. The rich cultures were seeded March 31 and tests were made on April 5, 6 and 9, resulting in epidemics of conjugation in the usual lines, J 1 with 3874



pairs, J7, with 2022 pairs, J8, with 3776 pairs, and J21, with 2177 pairs. In addition to these usual findings 3 conjugating pairs were found in line 15 (from quadrant C); 244 pairs in line 16, 15 pairs from line 17, and 16 pairs from line 20 (all three from quadrant B), and 2 pairs were found in line 25 (from quadrant D). Thus five of the former nineteen non-conjugating lines have begun to conjugate after nine months of sterility. The conjugating power is apparently weak however, as shown by the small numbers of pairs contrasted with the epidemics in lines, 1, 7, 8 and 21. The remaining fourteen lines gave no evidence at all of conjugating.

A second April test of the J series was made two weeks later to see if the above result would be confirmed. The rich cultures were seeded April 17 and conjugation tests were made on the 21st and the 24th resulting in 2810 pairs in J1, 549 pairs in J7, 1155 pairs in J8, and 841 pairs in J21, the usual numbers (two tests only) for quadrant A. In quadrant B there were 824 pairs in line 16, 39 pairs in line 17, and 2 pairs in line 20. In quadrant D there were two pairs from line 25, 2 from line 29, and 2 from line 30. In quadrant C there were no pairs at this time. The first April test was thus confirmed with the exceptions that no conjugations occurred in line 15 (quadrant C) and 2 pairs were found in each of lines 29 and 30 (quadrant D) in which no pairs had previously been found.

With these tests the number of non-conjugating lines is reduced from 19 to 12 and the number of conjugating lines raised from 4 to 11 although the conjugating power of the latter seven lines is much weaker than that of the original four. All four quadrants are now represented in the conjugating lines indicating that the power to conjugate is not a matter of nuclear factors which can be shuffled about like specific Mendelian characteristics.

Evidences of physiological weakness are now (May) beginning to manifest themselves in the conjugating lines. This is shown relatively, by the number of pathological divisions observed in the Syracuse dishes. In April 420 pathological divisions were counted in one Syracuse dish containing representatives of J1 in which 1349 pairs had been counted. In J21 during the same

test 12 pathological divisions were counted in one dish, and so forth. It is interesting and possibly significant that these abnormal divisions should appear now, whereas there has been no evidence of them heretofore.

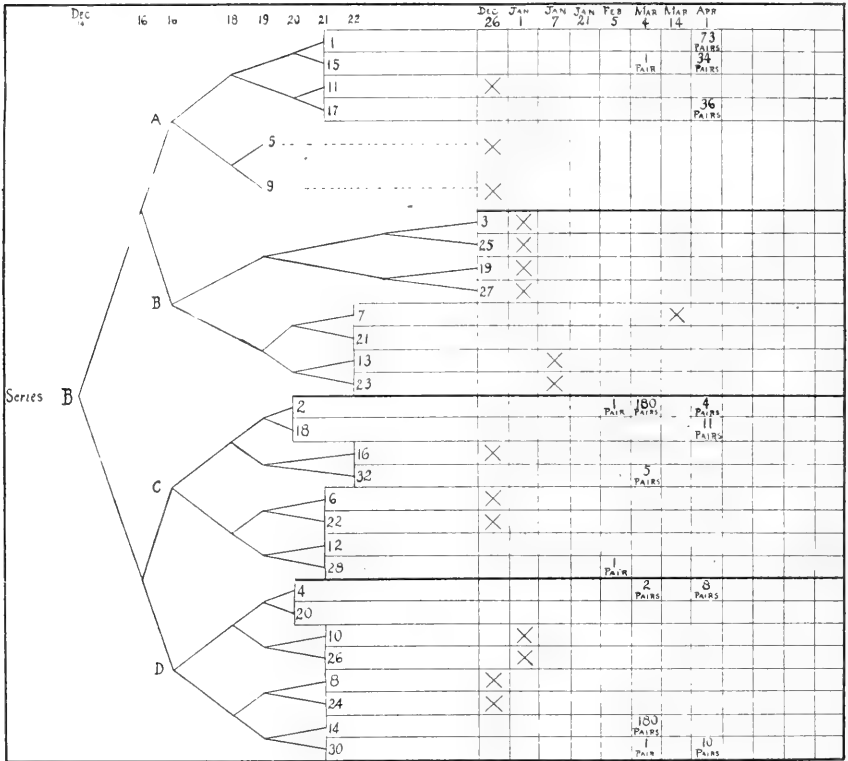


Diagram 4

*B. Conjugations in the B series*

The history of the B series is given on page 475, and condensed in diagram 4. The first rich cultures were made January 25, six weeks after isolation of the ex-conjugant. Tests were made on the 28th of January, 2d and 6th of February. One pair of conjugants appeared in B2 and one pair in B28, both lines in quadrant C.

A second set of rich cultures was made February 21 and tests were started February 24, 26, March 1 and March 3; 180 pairs were obtained from B2; 2 pairs from B4; 1 from B15; 1 pair from B17; one from B30; and 5 pairs from B32. B15 and 17 belong to quadrant A; B 2 and B 32 belong to quadrant C; and B4 and B30 to quadrant D.

A third set of rich cultures was started March 25 and conjugation tests were made March 27, 29 and 30, and 31; 73 pairs appeared in B1; 34 in B15; 38 in B17, all in quadrant A. Four pairs appeared in B 2; 11 in B 18, both from quadrant B. Eight pairs appeared in B4; and 10 in B30, both from quadrant D.

A fourth test in May gave no pairs at all, the entire series remaining sterile. Series B therefore is a relatively weak race in respect to conjugation, epidemics never occurring.

### *C. Conjugations in the E' series*

The E' series was started from an ex-conjugant from a wild culture December 14, 1912, and 28 pure lines out of 32 were kept in permanent cultures (diagram 5, p. 492). Thirteen of these have since died leaving 15 lines distributed as follows: 2 from quadrant A, 4 from quadrant B, 6 from quadrant C, and 3 from quadrant D.

Rich cultures were made January 9, twenty-seven days after isolation of the ex-conjugant and three tests on January 15, 20 and 27 gave negative results in all 24 lines save one, alive at that time. In one line, E'9, four pairs were found in the final test.

Rich cultures were again made February 23 and tests started on the 28th of April, 4th and 8th of March. These tests resulted in 221 pairs in E'17, and 23 pairs in E'24 and none at all in any of the other lines. A glance at diagram 5 shows that E'9 and E'17 are from quadrant A, and E'24 is from quadrant C.

A third series of tests was started with rich cultures on March 27, one month later. Tests were made March 30, April 1 and April 4, resulting in 18 pairs in E'17; and none in any of the other 18 lines alive at that time.

Here again, the conjugation power is feebly developed epidemics never occurring in the pure lines.

*D. Conjugations in the T' (4-line) series*

The T' series was started with an ex-conjugant from a wild pair isolated December 13, 1912. The first two divisions took place on the 16th and the 17th, and the four lines were successfully reared in permanent cultures. The first rich cultures were made on December 26 and the first conjugation tests on December

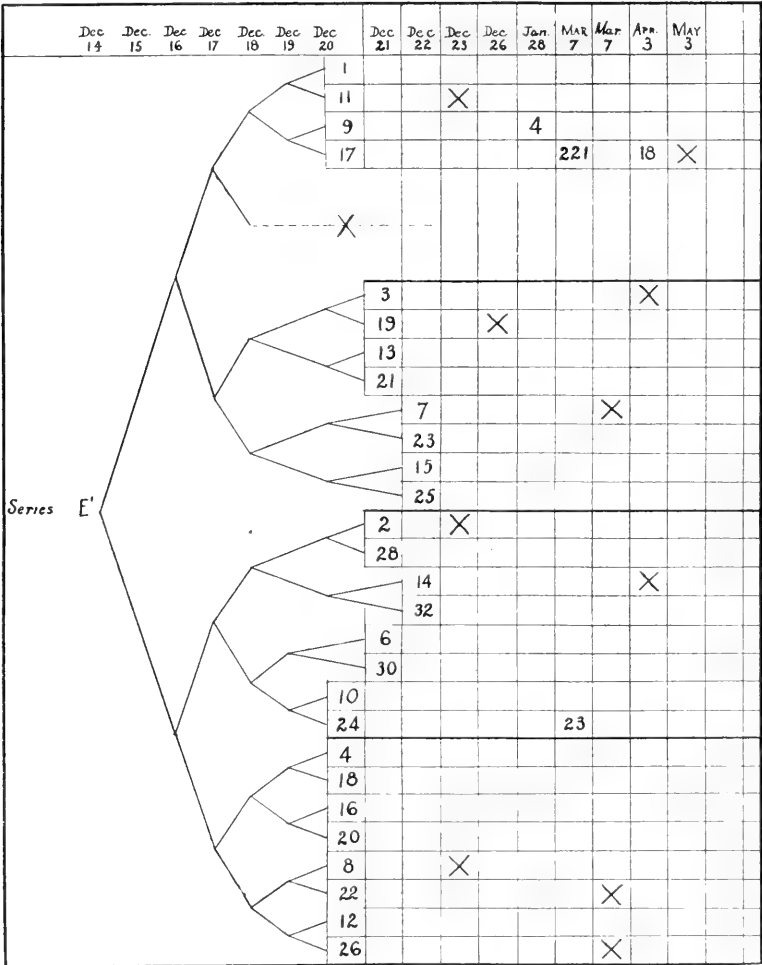


Diagram 5

30 two weeks after isolation, and again on January 4, and January 8, and 35 pairs were obtained from quadrant B, none from the others. The second rich cultures were made February 6 with conjugation tests on the 9th and 14th, giving 343 conjugating pairs from quadrant B again and not one from the three other quadrants (see diagram 6).

A third set of rich cultures was made March 15 and conjugation tests were made March 19, 22 and 26. 841 pairs were obtained from quadrant A, 2606 pairs from quadrant B, 798 pairs from quadrant C, and none from quadrant D.

Similarly in April, 1281 pairs were obtained from quadrant A, 227 from quadrant C and again none at all from quadrant D. Quadrant B, the most prolific of this series, died out April 12 one week before the tests were made.

In this series therefore, progeny from one of the four quadrants appear to lack the power to conjugate while those of another (B) possess it to a high degree.

#### *E. Conjugations in the H series (diagram 6)*

This series was also started December 13 and the first four sets were isolated in permanent cultures on the 16th. Rich cultures were made on January 7 and conjugation tests were made January 9, January 11, and January 16, giving 23 pairs in quadrant A, but none in the three other quadrants.

A second set of rich cultures was made February 13 and tests started February 17, 20, and 22. These yielded only 2 pairs, again, in quadrant A, none in the others.

A third set of rich cultures was started March 15. Conjugation tests made on March 19, 22, and 26 gave only 2 pairs in quadrant D, none in the others.

A fourth set started April 19 gave 151 pairs in quadrant D after three tests, and no pairs in any of the other lines.

Here therefore, the conjugating power is slightly developed in two quadrants, not at all in the other two.

*F. Conjugations in the L series (diagram 6)*

The four lines from an ex-conjugant of a wild pair of December 13 were isolated in permanent cultures on December 17. Rich cultures were made on January 7, and conjugation tests on January 19, January 23, 13 pairs being found in quadrant B, and 6 pairs in quadrant C. A second set of rich cultures started

	DEC 14	DEC 15	DEC 16	DEC 17	JAN 7	JAN 12	JAN 18	JAN 24	JAN 30	FEB 15	FEB 22	MAR 21	APR 26
Series G				1							49	1610	D
				3		X							
				2							1044	1500	D
				4	16						1916	2540	D
Series H				1		23				2			
				3									
				2									
				4								2	151
Series L				1								192	15
				3			13						20
				2			6					517	90
				4							X		
Series M				1							1444	X	
				3			57				99	218	D
				2			4				105		D
				4			4				2	802	D
Series Q				1					5	24		1656	D
				3					93	1303		640	D
				2					4	20	X		
				4								306	D
Series T'				1								840	1281
				3								2806	X
				2								788	227
				4									

Diagram 6

February 6 and tested for conjugations on February 8, 9th, 14th and 17th failed to confirm the results of the first tests, yielding no pairs in any quadrant.

A third set of rich cultures was made March 15 with three quadrants only, one, quadrant D, having died immediately after the February test. Conjugation tests were made March 19, 22,

and 26 and 192 pairs were obtained from quadrant A, 110 pairs from quadrant B, and 507 pairs from quadrant C, a result that was confirmed in the April test when 15, 90 and 20 pairs were found in quadrants, A, B, and C, respectively.

*G. Conjugations in the Q series (diagram 6)*

The four permanent cultures were started as above on December 17 and the first rich cultures were made January 12, and conjugation tests on the 15th, the 20th, and the 27th. Conjugating pairs were found in three quadrants, 5 in quadrant A, 4 in quadrant B, and 93 in quadrant C, none being found in quadrant D. A second set of rich cultures were made on February 6 and conjugation tests on February 8, 9th, 14th, and February 20th, confirmed the findings of the previous month. Quadrant A gave 24 pairs; quadrant B, 20 pairs; quadrant C gave 1303 pairs while quadrant D gave none at all.

In this series the power to conjugate is distributed between three of the four original cells, but it is quite apparent that quadrant C is the chief conjugating line, about 10 per cent of them conjugating under the conditions of the test (on February 14 this percentage rose to 33).

A third set of rich cultures was made March 15, from which only one conjugation test was taken on March 19. This single test yielded 1656 pairs in quadrant A, 640 pairs in quadrant B, and 306 pairs in quadrant D. (Quadrant C died between February 15 and 22.)

This series was abandoned after the March test since all lines possess high conjugating power.

*H. Conjugations in the G series (diagram 6)*

Again the four permanent cultures were started on December 17 and the first rich culture tests were made on December 26, the first set of conjugation tests on December 28, and January 4, yielding 16 pairs in quadrant D, none in the three others. The second set of rich cultures was made February 13 and conjugation tests were made on February 17 and 20. In the meantime,

however, the progeny of one quadrant (B) died out on January 12, leaving only three pure lines. In these the February tests showed conjugations in all three; 49 pairs from quadrant A; 1016 pairs from quadrant C, and 1890 pairs from quadrant D. Here, therefore, two lines of the four are strong conjugating lines and these were derived from the same parent cell in the 2-cell stage.

A third set of rich cultures was started March 15 and conjugation tests were made on the 19th and 22nd. These two tests gave 4610 pairs in quadrant A, 2760 pairs in quadrant C and 2540 pairs in quadrant D. The series was then discarded since one quadrant had died out and the others were all capable of giving high numbers of pairs. The progeny from this ex-conjugant were particularly fertile yielding conjugations in less than two weeks after isolation of the ancestral ex-conjugant, and the epidemics of conjugation at each succeeding test.

#### *I. Conjugations in the M series (diagram 6)*

In this series all four cells of the original 4-cell stage gave rise to conjugating lines, in which however, the power to conjugate is quite different. The series was started as before on December 13, and the first rich cultures were made on January 7. The first conjugation tests on January 9, 11, and 24, yielded 4 pairs from quadrant B; 57 pairs from quadrant C; 4 from quadrant D, but none from quadrant A. The second set of rich cultures were made on February 13 and conjugation tests on February 17 and 20. These tests yielded 1420 pairs in quadrant A; 105 pairs in quadrant C; 99 in quadrant B; and 2 in quadrant D.

These tests show that if the conjugating ability is present it may be weak at one time (e.g., quadrant A in January) but may blossom out in an epidemic at another time.

A third set of rich cultures was started March 15th and one test was started on the 19th. This gave no conjugations in quadrant C, 218 pairs in quadrant B, and 1802 pairs in quadrant D, showing that the earlier sterility in the last was only temporary. Quadrant A died out before this third test was started. The entire series was then discarded.



*J. Conjugations in the G' series*

This series is from the conjugating mate of the ancestral cell of the G series above. It is interesting as being the only case in which four pure lines from both conjugants continued to live as long as the first conjugation test. Unfortunately the vitality did not keep up long enough to make the conjugation records of any value. The first rich cultures on December 28 were tested for conjugations on January 1, 3, 8, and 13, but not one pair was found. The entire series died between January 21 and 30 so we cannot draw conclusions from this one series.

*K. Conjugations in the O and P series*

The O and P series were derived from ex-conjugants of the J 8 line which were paired December 3, 1912. Rich cultures were seeded in both series January 24, February 27, and April 14 and tests were made from each in the usual manner. In the O series (January tests) one pair occurred in O 13 and none in the P series. In the February tests, there was one pair in O 12, none in the P series. In the April tests every line of both the O and P series gave conjugations (9 pure lines of the O series and 9 of the P series representing only two quadrants in each case). O 1 gave 1300 pairs; O 3, 1377; O 4, 1259; O 5, 458; O 7, 964; O 11, 18, O 12 507; O 13, 1, O 17, 606, pairs. P 1, gave 200 pairs; P 3, 739; P 4, 8; P 9, 938; P 10, 95; P 11, 7; P 12, 1072; P 15, 1; and P 17, 253 pairs.

It is unfortunate that only two quadrants are represented in these series, but enough lines are given to show that wide variations occur in the strength of the conjugating power (e.g., O 13 and P 15 give 1 only as against O 3 and P 12 with 1377 and 1072 pairs respectively).

## 7. NOTES ON THE CONJUGATION TESTS

*A. So-called 'maturity' of Paramecium in rich cultures*

Some interesting data have accumulated in connection with the conjugation tests on a phenomenon which for lack of a better term we may call 'maturity.' This does not mean maturity in the sense used by Maupas, namely, that a certain number of generations must elapse before the progeny of an ex-conjugant can again conjugate. The experience of numerous observers has shown that if a line has the ability to conjugate at all, such conjugations may occur at any time. Jennings, for example, has shown that closely related individuals of *Paramecium* in the 16-cell stage after conjugation may conjugate again. In our G series abundant conjugations appeared in the progeny three weeks after conjugation of the parent cell; in the H' series, five weeks after; and in the L and Q series six weeks after conjugation. 'Maturity' in the Maupas sense, therefore, is not a necessary condition for conjugation. Our experiments show that the organism must remain a certain length of time in the rich cultures where rapid multiplication takes place under certain conditions, one of which is abundant nourishment. When ready for conjugation under these conditions we may say that the organisms are mature. Table 5 and the frequency polygon based upon it show this very clearly (diagram 7). Of the 129 observations, 40 fall in the period from five to nine days, that is, in the curve of the region of the mode; 52 in the period from one to four days; and 37 in the period from ten to twenty-one days. The curve has little value statistically because different races are included and because observations are so unevenly distributed. These 4 observations on the fifth day gave over 1000 pairs, whereas on the sixth day, 3 observations gave only 17 pairs. This discrepancy is due to the fact that different series are grouped together. Nevertheless, the differences are so clearly marked that the conclusions are perfectly obvious. Thus 52 observations made on *Paramecium* from one to four days in the rich cultures gave only 242 pairs, while 38 observations made on material from five to eight days in rich cultures gave 8578 pairs. From this it appears that ma-

TABLE 5

TEST		NUMBER OF DAYS IN RICH CULTURE PRIOR TO CONJUGATION TESTS, AND NUMBERS OF PAIRS FOUND ON SUCH DAYS											Total	
		2	3	4	5	6	7	8	9	10	12	14		21
J 1	November.....							37		4			0	41
	December.....	0						58				24		82
	January.....		0					300		86				386
	February.....	0		0				3				0		3
J 7	November.....							0		0		0	0	0
	December.....	0						0		49		0		49
	January.....		40		91		4							135
	February.....	0		0				136				10		146
J 8	November.....							0		0		0		0
	December.....	0						34				8		42
	January.....		40		302		186							528
	February.....	0		0				138				0		138
J 21	November.....				200			63				0	0	263
	December.....	0		9										9
	January.....		58		560		186							804
	February.....	0		31				8				0		39
G 1	January.....		0	0		0						0		0
	February.....			0			49							49
G 2	January.....		0	0		0						0		0
	February.....			0			1044					0		1044
G 4	January.....		0			16						0		16
	February.....			0			1914							1914
H '1	January.....	0							23					23
	February.....						0		2					2
M 1	January.....	0		0								0		0
	February.....			0			1420					24		1444
M 2	January.....	0		0								4		4
	February.....			7			98					0		105
M 3	January.....	0		57								0		57
	February.....			0			99					0		99
M 4	January.....	0		0								4		4
	February.....			0			2					0		2
Q 1	January.....		0					0				5		5
	February.....	0	0					8				16		24
Q 2	January.....		0					0				4		4
	February.....	0	0					20				0		20
Q 3	January.....		0					0				93		93
	February.....	0	0					1303				0		1303
T'3	January.....		0					0			35			35
	February.....	0	0					323				20		343
Total.....		0	138	104	1153	16	5002	2431	25	139	35	212	0	9255

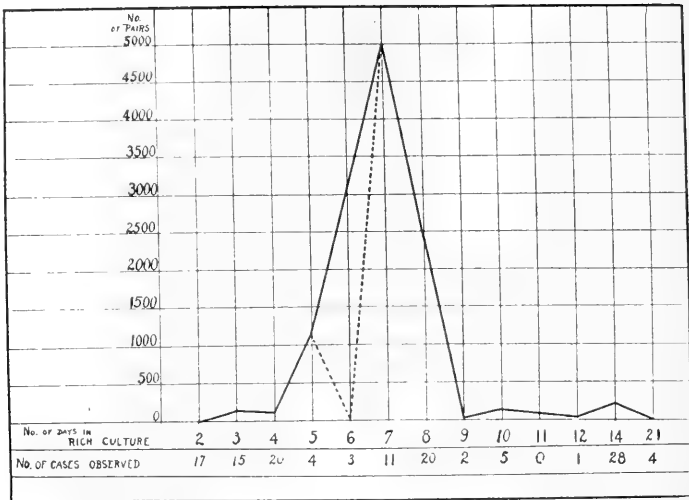


Diagram 7

turity in a rich culture is reached after from five to seven days. After this period, the conjugation power decreases. Thus eleven observations on the seventh day gave 5002 pairs, whereas *twenty* observations on the eighth day gave only 2407 pairs. There is evidently a gradual lessening of the power to conjugate after the optimum on the seventh day until it is practically gone by the fourteenth day and entirely gone in three weeks, provided the rich cultures are not re-filled. This does not mean that more ex-conjugants are taken from the rich cultures at the later periods for conjugations do not appear in the rich cultures until after the seven day period in these particular tests. During the later periods a few pairs may appear in the rich culture jars, but they are not abundant. This is probably due to the fact that the change in medium is so gradual in the rich cultures that conjugations are not brought about, whereas transference to a Syracuse dish involves a relatively rapid change in medium and conjugation follows, provided, of course, that it is a fertile race. In the later periods of a rich culture a physiological balance is established and the *Paramecia* then live normally for months and without conjugating.

These considerations lead us to a further test of maturity as shown by the rapidity of response to the changed condition. This is shown by:

*B. History of the Syracuse dish tests*

A typical conjugation test for the J series is shown in table 6 taken from the protocols. The symbols used are as follows: S = seeding of rich cultures with progeny from the permanent cultures, S<sup>1</sup> S<sup>2</sup> S<sup>3</sup> = first, second, and third, and so forth, tests, that is, transference from rich cultures to Syracuse dishes; + = symbol for physical condition of the organisms, and means that they are actively dividing. ± = symbol to indicate good physical condition but absence of dividing forms; it marks the beginning of decline in vigor; - = symbol to indicate physical deterioration, loss of size, etc. When this condition is reached conjugation never occurs. X = death of individual Paramecia or all. (36), (2), and so forth = symbols to indicate conjugations and the number of pairs counted. D = discard of the test. K = killed for microscopical examination. [400] = number of pathological divisions observed. The maximum and minimum temperatures are given for each twenty-four hours.

In order to test the rapidity of response of conjugating lines to changes from rich cultures to Syracuse dishes we have combined all of the protocols up to March 1, similar to that given in the above table. The general results may be seen in the following table 7 in which the pairs found at different periods of maturity are recorded for all the conjugating lines save H' and T'. This table may be summarized as follows: After two days in rich cultures none of the lines conjugate. After three days, conjugations appear on the third and fourth days after transferring. After four days, conjugations appear on the first, second, third, and fourth days after transferring. After five days, conjugations appear on the first, second, and third days but are most abundant on the second, while none appear on the fourth. After seven days in rich cultures conjugations appear on the first, second, third, and fourth days, but are most abundant on the sec-

TABLE 6

A typical Syracuse-dish conjugation record for the J Series. April.

Date	3/31	4/5	6	7	8	6	7	8	9	10	9	10	11	12	13
J 1	S	S <sup>1</sup> <sub>(2)</sub>	(7)	(7)	+	S <sup>2</sup> <sub>(+)</sub>	(12)	(18)	(10)	(12)	S <sup>3</sup> <sub>(18)</sub>	(7)	(11)	(1)	(2)
J 2	S	S <sup>1</sup>	+	+	-	S <sup>2</sup> <sub>(+)</sub>	+	+	+	-	S <sup>3</sup> <sub>(+)</sub>	+	-	-	-
J 7	S	S <sup>1</sup> <sub>(2)</sub>	(10)	(2)	-	S <sup>2</sup> <sub>(60)</sub>	(11)	(8)	+	-	S <sup>3</sup> <sub>(7)</sub>	(11)	(2)	-	-
J 8	S	S <sup>1</sup> <sub>(16)</sub>	(6)	(5)	-	S <sup>2</sup> <sub>(30)</sub>	(3)	(1)	(10)	-	S <sup>3</sup> <sub>(10)</sub>	+	(4)	(1)	(2)
J 9	S	S <sup>1</sup>	+	-	-	S <sup>2</sup> <sub>(+)</sub>	+	+	-	-	S <sup>3</sup> <sub>(+)</sub>	+	+	-	-
J 10	S	S <sup>1</sup>	+	+	-	S <sup>2</sup> <sub>(+)</sub>	+	+	+	-	S <sup>3</sup> <sub>(+)</sub>	+	+	-	-
J 11	S	S <sup>1</sup>	+	-	-	S <sup>2</sup> <sub>(+)</sub>	+	+	+	-	S <sup>3</sup> <sub>(+)</sub>	+	+	-	-
J 12	S	S <sup>1</sup>	+	+	-	S <sup>2</sup> <sub>(+)</sub>	+	-	-	-	S <sup>3</sup> <sub>(+)</sub>	+	-	-	-
J 13	S	S <sup>1</sup>	+	+	+	S <sup>2</sup> <sub>(+)</sub>	+	+	+	-	S <sup>3</sup> <sub>(+)</sub>	+	+	-	-
J 15	S	S <sup>1</sup> <sub>(6)</sub>	+	+	-	S <sup>2</sup> <sub>(+)</sub>	+	-	-	-	S <sup>3</sup> <sub>(+)</sub>	+	+	-	-
J 16	S	S <sup>1</sup>	(8)	+	-	S <sup>2</sup> <sub>(16)</sub>	(4)	(12)	(2)	-	S <sup>3</sup> <sub>(8)</sub>	(12)	(2)	-	(3)
J 17	S	S <sup>1</sup>	+	+	+	S <sup>2</sup> <sub>(14)</sub>	(1)	+	+	-	S <sup>3</sup> <sub>(+)</sub>	+	+	-	-
J 18	S	S <sup>1</sup>	+	+	+	S <sup>2</sup> <sub>(+)</sub>	+	+	+	-	S <sup>3</sup> <sub>(+)</sub>	+	+	-	-
J 20	S	S <sup>1</sup> <sub>(9)</sub>	+	+	-	S <sup>2</sup> <sub>(1)</sub>	(1)	+	-	-	S <sup>3</sup> <sub>(+)</sub>	+	+	-	-
J 21	S	S <sup>1</sup> <sub>(10)</sub>	(9)	(12)	(18)	S <sup>2</sup> <sub>(6)</sub>	(10)	(1)	(4)	-	S <sup>3</sup> <sub>(2)</sub>	(6)	(1)	(5)	(12)
J 24	S	S <sup>1</sup>	+	+	-	S <sup>2</sup> <sub>(+)</sub>	+	+	-	-	S <sup>3</sup> <sub>(+)</sub>	+	-	-	-
J 25	S	S <sup>1</sup>	+	+	-	S <sup>2</sup> <sub>(2)</sub>	+	+	+	-	S <sup>3</sup> <sub>(+)</sub>	+	-	-	-
J 26	S	S <sup>1</sup>	+	+	-	S <sup>2</sup> <sub>(+)</sub>	+	+	+	-	S <sup>3</sup> <sub>(+)</sub>	+	-	-	-
J 27	S	S <sup>1</sup>	+	+	-	S <sup>2</sup> <sub>(+)</sub>	+	+	+	-	S <sup>3</sup> <sub>(+)</sub>	+	+	-	-
J 28	S	S <sup>1</sup>	+	+	-	S <sup>2</sup> <sub>(+)</sub>	+	+	+	-	S <sup>3</sup> <sub>(+)</sub>	+	+	-	-
J 29	S	S <sup>1</sup>	+	+	-	S <sup>2</sup> <sub>(+)</sub>	+	+	+	-	S <sup>3</sup> <sub>(+)</sub>	+	+	-	-
J 30	S	S <sup>1</sup>	+	-	-	S <sup>2</sup> <sub>(+)</sub>	+	+	+	-	S <sup>3</sup> <sub>(+)</sub>	+	+	-	-
J 31	S	S <sup>1</sup>	+	-	-	S <sup>2</sup> <sub>(+)</sub>	+	+	+	-	S <sup>3</sup> <sub>(+)</sub>	+	-	-	-
Temperature	65°-72°	64°-72°	68°-72°	66°-70°	68°-64°	68°-72°	66°-70°	64°-68°	54°-70°	64°-68°	54°-70°	64°-68°	68°-68°	69°-70°	68°-71°



ond and third. After eight days the same is true. After ten or fourteen days conjugations appear chiefly after two or three days subsequent to transference. We have seen (table 5) that the period of maximum maturity is reached on the seventh day of rich feeding, and from this present table the conclusion may be drawn that the response to the changed environment is more rapid and more intense the nearer the period of maturity is approached. Or, the results may be summarized in another way as follows: of 8464 pairs observed in the Syracuse dishes 540 appeared on the first day after transferring; 5109 pairs appeared on the second day; 2270 on the third day; 545 on the fourth day and none at all on the subsequent days. The maximum conjugation power therefore is found on the second day after transference to Syracuse dishes of *Paramecium* which have been for seven days in a rich culture.

#### 8. DISCUSSION

These experiments demonstrated clearly that there are well marked physiological variations in different 'pure lines' of *Paramecium caudatum* all derived from the same ancestral ex-conjugant, and in the light of these results it is necessary to re-open some of the old questions in general biology. These questions are:

- A. Is there a limited potential of vitality common to the fertilized metazoan egg, and the ex-conjugant *Paramecium*?
- B. Are all protozoa potential germ cells?
- C. Do some protozoa die a natural death from old age?
- D. Are variations which arise through amphimixis different from those which arise by differentiation of any ex-conjugant?
- E. Is rejuvenescence or is amphimixis the most important effect of conjugation?

##### *A. The potential of vitality*

The experiments and observations of Maupas ('88), Joukowsky ('98) and Simpson ('01) all indicated a limited metabolic activity for *Paramecium* and allied forms in cultures, the general result being that the race dies after some three months under these conditions. Calkins' experiments ('02-'04) were made with



more carefully guarded methods, the individual cells being daily transferred to fresh twenty-four-hour hay infusion. Under these conditions the race continued to live for twenty-three months. During this period, however the entire race would have died at any one of three successive periods had not precautionary methods been used. These periods, marked by sluggish metabolism and by a greatly diminished division rate, were termed 'depressions' and they occurred with surprising regularity once in six months. Minor fluctuations in vitality which Woodruff ('05) called 'rhythms' were self-regulated by the organisms. The depressions were overcome by the use of beef extract, or by simple salts such as potassium phosphate or potassium chloride. At each depression period save the first one when this condition of the organisms was novel, half of the race were continued on the hay diet and half were treated with beef extract and salts for a short period. In all cases the first half died within three weeks, the other half continued to live with renewed metabolic power and with high division rates. In the final period of depression, however, the use of salts or extracts was not followed by renewal of vitality and the entire race died in the 742d generation.

Further observations and experiments of similar nature confirmed the earlier conclusion of Maupas. Woodruff ('05) carried *Oxytricha fallax* through 860 generations when the race died; *Pleurotricha lanceolata* through 440 generations and *Gastrostyla* through 288 generations; Gregory ('09) carried *Tillina magna* through 548 generations when the race died; Moody ('12) carried *Spathidium spatula* through 218 generations until it died; Calkins (unpublished) carried *Actinobolus radians* through 8 months of culture when the race died in the 446th generation, and also carried *Blepharisma undulans* through eleven months of culture when this race died in the 224th generation.

Several different observers with several different infusoria have thus reached the conclusion that a race of protozoa has a certain potential of vitality which under the conditions of culture weakens until the organisms die.

These conclusions have been criticized by Enriques, Jennings and others on the ground that the conditions under culture are

abnormal and that environmental influences bring about the fatal result. Enriques, ('07) for example, kept *Glaucoma scintillans* under culture for 683 generations (from Jennings '12). He found that if the organisms are left in the same small culture fluid for some time they weaken and die whereas if the medium is changed every day, they continue to live and he kept different organisms for long periods under these conditions without apparent effect. He concludes, therefore, that Calkins' results with *Paramecium* were due to the continued use of bacterial culture media until the *Paramecia* finally succumbed to bacterial poisons, but ignored the important technical point that Calkins' method was the only one which he himself had found favorable for *Glaucoma* and other ciliates, namely, changing the medium every day. This argument is further contradicted by the results obtained with other infusoria which do not live on bacteria, for example, *Spathidium spathula* and *Actinobolus radians*. The former eats *Colpidium colpoda*; the latter *Halteria grandinella*. In these cases the organisms are kept in pure water which is changed daily and a certain number of *Colpidium* or *Halteria* added to it. Here no question of bacterial poisoning can enter into the problem, but the infusoria died nevertheless.

Criticisms based on methods, however, were apparently supported by the splendid and arduous experiments of Woodruff who for more than five years and a half followed the life history of *Paramecium aurelia* through more than 3500 generations. The method employed is practically the same as that used by his predecessors with this difference—the infusions were made by boiling different vegetable matters from time to time, as well as beef, so that the race of *Paramecium* had a variable substratum in the food medium. It was argued that this change reproduced more nearly the normal conditions of *Paramecium* than does a constant medium of hay infusion, and that the race has continued to live because of this change.

There is reason for accepting this argument in part, for the salt content of infusions from different sources undoubtedly varies and the changes may act somewhat as did the salts and change of medium in Calkins' experiments. Nevertheless, we do not

believe this to be the sole reason for the longevity of Woodruff's cultures. In the first place, Woodruff's are the first observations that have been made on isolated *Paramecium aurelia* and its life history is unknown. In the second place, the food of his race has probably remained the same despite the varied medium. Not only are the infusions open to the air of the laboratory but with each transference the new medium is seeded with bacteria from the old. His long continued culture, therefore has been subject to the same chance of bacterial poisoning that Calkins' cultures were, but his race is still flourishing and the explanation must lie in the makeup of the organisms themselves rather than in the method employed. This conclusion is reached by Woodruff ('09, p. 03) who believed there are "strong and weak strains among infusoria" and it is strengthened by his later experiences ('11) with *Paramecium aurelia* which he maintained for several months without apparent loss of vitality on a constant medium of beef extract. Woodruff is confident that his race will live indefinitely: "I believe this culture shows clearly that *Paramecium aurelia* when subjected to suitable culture conditions, has the power of unlimited reproduction by division without conjugation or artificial stimulation" (Woodruff, '11 p. 266), and again ('11, p. 141) "The conclusion seems justified that this culture of *Paramecium (aurelia)* can, in all probability, be continued indefinitely on this 'constant' medium (beef extract)." We do not share his optimism, however, and can only say that while his results are remarkable, his race is not yet dead. Is there any clue to the particular makeup of this race of *Paramecium aurelia*?

*B. Are all Paramecia potential germ cells?*

Woodruff ('09) states that he may have been fortunate in obtaining a particularly strong race in his long culture. "I would not suggest that the protoplasm of every wild *Paramecium* has the potential to attain over twelve hundred generations or more." (p. 303). This statement appears to strike the right pitch in the disharmony of results achieved in these various observations on the life history of the Infusoria. Differences in individuals se-

lected have the greatest importance on the extent of vitality in organisms under culture. This was hinted by Simpson (p. 410, '01) when he spoke of the 'individuality' of different *Paramecia*, and by Woodruff ('05) "My cultures lead me to believe with Simpson, that the personal equation, if I may use that term, of the individual selected to start a culture, has the most influence in determining the number of generations attained before the 'initial potential of vitality' is exhausted" (p. 604) and again by Gregory ('09) "Each individual of the same species as well as of different species, has its own peculiar protoplasm reactions" (p. 414). Finally, Woodruff ('09) re-states the point as follows: "Again, it is possible that the different races of *Paramecium* which Jennings has been able to isolate may have a physiological as well as a morphological basis of distinction" (p. 303).

It is on the basis of these individual differences that an explanation is to be sought of the long-continued vitality of Woodruff's cultures. The results of our present investigation show convincingly that a single ex-conjugant gives rise to varied progeny in the form of pure lines, each line remaining true to its type for many months at least. Some of these are conjugating lines (all the progeny of one quadrant in the J series), while others are non-conjugating or weak-conjugating lines. The old tradition, clearly formulated by Weismann, that every protozoan is a potential germ cell, must therefore be modified. Now, Woodruff's *P. aurelia* is a non-conjugating pure line. He has repeatedly tried to induce conjugation, using every device that has proved successful in other cases, but to no avail. "Up to the present time, however, there has been no tendency to conjugate among the *Paramecia* of this culture and a large series of experiments, which have been made with the individuals left over from the four lines of the culture after the daily isolations have, so far, yielded not a single pair of conjugants" ('11, p. 264). Jennings' cultures of *P. aurelia* on the other hand conjugate regularly: "It is thus clear that the race *k* (*aurelia*) has a remarkable tendency to conjugate readily, even though the progeny of only one individual are involved ('10, p. 285).

The difference in conjugating power of different races of *Paramecium* has been clearly shown by Jennings: "The conditions determining conjugation differ greatly in different races of *Paramecium* (*aurelia* or *caudatum*). Some races conjugate frequently and under conditions readily supplied in experimentation. Others under the same conditions, conjugate very rarely or not at all." ('10, p. 298). The latter statement is an expression of the result of careful experimentation and should be contrasted with an earlier statement based on a traditional belief; "Unicellular animals are essentially free germ cells," (Jennings '09, p. 322), a statement that certainly cannot embrace the rhizopods or the sporozoa where germ cells are formed and the parent cells die.

What Jennings finds true of races we find to be true of different lines within the same race. Some lines will conjugate whenever the conditions favorable to conjugation are prepared; other lines have never conjugated under such conditions. We have found no race (we have called them 'series') as yet in which the conjugation power of some one line is not much more developed than in the others (for example, J21, G4, M1, Q3, H' 1, T' 3, etc.) (See diagrams 3-6.)

It is possible, of course, that under different conditions the remaining non-conjugating lines might have been induced to conjugate. Under the usual laboratory conditions however, it is evident that the progeny of an ex-conjugant is not a homogeneous race, but consists of differentiated individuals which give rise to pure lines some of which conjugate, others do not. In other words, some *Paramecia* are potential germ cells; others apparently are not.

### *C. Do some Protozoa die a natural death from old age?*

To return to Woodruff's race of *P. aurelia*, we find this important difference between his material and that which Calkins worked with. The *Paramecium caudatum* which formed the material for the earlier observations was a conjugating line as shown by conjugation tests made from time to time (cf. Calkins '02, '04). In this line, therefore, a normal function—conjugation—

tion—was inhibited; metabolism weakened steadily until physiological death carried off all of the race not artificially stimulated. Finally, 'germinal death' preceded by the degeneration of the micronucleus and the cortical plasm, carried off the last individual. These last individuals "were of full size and were filled with gastric vacuoles and partly digested food, while the body form was normal" ('04, p. 446). It has since been shown that the micronucleus is the center of divisional activity (Calkins '10), hence its degeneration would account for the decrease in division rate and the ultimate cessation of division.

In Woodruff's material on the other hand, the race is non-conjugating, hence no normal function has been suppressed by keeping the individuals apart. So far as known the life history of such non-conjugating forms has never before been undertaken and Woodruff has shown that they will live for at least five and one-half years. As Gregory pointed out these organisms cannot be compared with those of the earlier experiments because of essential differences in individual vigor. Conjugation furthermore has been prevented in one case and not in the other since the power to conjugate is absent in the latter. Experimentation has shown with some probability therefore, that we must distinguish between conjugating and non-conjugating lines of *Paramecium* in dealing with the questions of old age and natural death. That these phenomena occur in the former has been demonstrated for different types by Maupas, Jennings and Calkins; that they do not occur in non-conjugating lines is still in the experimenting stage. Five and a half years is indeed a long period for *Paramecium*, but immortality is quite a different matter. We conclude, therefore, that death from old age, by which we mean the gradual weakening and final cessation of vital activities, in *Infusoria* does occur in some forms but is not yet demonstrated for others.

*D. Variations in Paramecium*

Jennings in 1908, from the study of statistical data came to the conclusion that variations in size in Paramecium are brought about through amphimixis and largely from this study, he has developed the argument that conjugation is for the purpose of initiating variations. As this is a conclusion of fundamental biological importance, the ground on which it is based must be absolutely firm and beyond question of doubt.

Jennings material (culture M) came from a small pond. From this a number of pairs were isolated and allowed to multiply after conjugation, seven pure line cultures being obtained from single equal pairs or single ex-conjugants of the caudatum type. About one month after isolation, random samples of about 100 individuals from each line were measured. About three weeks later random samples of 100 individuals (56 in one case) from the same strains were again measured. The data are contained in his table 25. "Examination of this table shows that lines derived from different conjugating pairs or different ex-conjugants do differ from each other at the same periods in the life cycle even though living under identical conditions. The differences are fully as marked as those found among diverse lines derived from individuals not conjugating and taken without reference to the life cycle in which they happen to be" (Jennings '08, p. 494). From the seven pure lines examined, Jennings found that four types or races could be obtained according to the mean length of the random samples. The mean lengths for the two periods are shown below:

	MEANS FIRST MEASUREMENT	MEANS SECOND MEASUREMENT
D.....	176.90	187.03
F 2.....	182.20	199.96
A 2.....	184.64	187.87
F 1.....	193.00	209.64
A 1.....	193.56	203.64
G 1.....	201.40	210.96
L 2.....	206.36	220.56

So far as measurements go Jennings believes that F1 and A1 may be the same, and F2 and A2 the same. In the second series of measurements D and A 2 fall together, otherwise the groups fall in the same order as in the first series and four lines or races result, differing from one another by at least 4 microns in both series of measurements.

Now if similar positive differences are present in the progeny of any one ex-conjugant of *Paramecium caudatum* cultivated under identical conditions as nearly as possible, then the variations found by Jennings in progeny from different ex-conjugants cannot be used as evidence of variations due to amphimixis and the corollary that conjugation is for the purpose of bringing about variations, cannot be deduced.

Table 3 (page 484) shows that our lines measured March 10 fall into three groups, line 20 at one extreme (183.15 microns) and line 21 (223.85 microns) at the other, each separated from the next line by at least 10 microns. The other 14 lines form a group separated from one another by not more than 3 microns.

At the time of the second series of measurements all of the lines were at least 50 generations older. All lines had increased in size but in different degrees so that they do not fall in the same order as in the first series. Line 20 however retains its position as the smallest line while line 21 is removed by one from the largest. With this exception we again have three groups and the same groups as in the first measurement test.

According to Jennings' method therefore, we have at least three distinct races or lines differing in their means by not 4, but from 6 to 10 microns and all derived from one ex-conjugant (Jennings' lines vary from 176.9 to 206.36 and 187.03 to 220.56 differences of 29.47 and 33.53. Ours vary from 183.50 to 223.85 and 195.11 to 231.825 differences of 40.35 and 36.71 respectively). We might choose five lines from these 16 and obtain five races with differences between them (excepting one case where it is  $3.75\mu$ ) of four microns, at each measurement.

Two of Jennings' lines (F1 and F2) might be compared with the sum total of all lines of the J series, that is, the total progeny of an ex-conjugant. If so the population in his cultures would



be characterized by the particular line that chanced to be in the ascendancy at the time of killing and measuring. According to our results such an ascendant line might be like our J20, or 17, or 10, or 8 or 15, each of which would have given decidedly different results from the others and yet all of them would have been possibilities, had we maintained all the progeny of the J ex-conjugant in one culture. The same argument applies, with greater force, to the other five lines in Jennings' table, derived from two ex-conjugants which had paired. In other words the variations adopted by Jennings as evidence of different hereditary groups of *Paramecium caudatum* lie within the variations shown by different lines derived from the same ex-conjugant, and the argument falls completely.

Now as to the interpretation of tables 1, 2, and 3. It is quite evident that the variations in size have little or no value in heredity since all came from the same ancestral cell. The variations in the same pure line are often extreme as is the case with line 31 in which there is a difference in mean length of 25.82 microns, between the two measurements, and in mean breadth of 9.625 microns, a difference which developed within a period of six weeks, or within about 50 generations by division. Some of the lines on the other hand are more stable, remaining large or small. Thus line 21 remains practically the same in size, while seven other lines differ within less than 10 microns. These may be said to be less variable than the other eight lines. Of the eight lines showing less variability, three are strong conjugating lines (1, 7 and 21) which have given conjugation epidemics at every test for six months. In the other set, J 8 is the only strong conjugating line, although five of the other lines gave a few pairs each during one of the recent tests (April).

On the whole, therefore, it seems probable that *Paramecium caudatum* may vary in length from about 140 to about 270 microns, the extremes in our measurements. Had we measured all 32 lines originally derived from the ancestral ex-conjugant, there is reason to believe that the gaps in our table of means (table 3) would have been filled in, giving a unimodal curve with its mode somewhere in the vicinity of 209 microns.

The conclusion is evident from these data that variations in size of *Paramecium caudatum* do not furnish sufficient evidence to warrant the conclusion that variations are the result of amphimixis or, a fortiori, that variability is the primary purpose of conjugation. In order to obtain a basis for such an argument, many series similar to our series J, should be studied in the same way, and if then the modes should fall in different regions of the curve (as they do when aurelia and caudatum are compared), then there might be justifiable grounds for conclusions as to heredity of size.

*E. Rejuvenation or variability?*

The fundamental purpose of conjugation offers an interesting problem for speculation almost as endless apparently, as the vitality of *Paramecium*. The matter has been so frequently discussed in papers by Jennings, Woodruff, Calkins and a host of others that we shall limit the present discussion to the consideration of points raised by the observations made in recent experiments.

Jennings, whose careful and thorough methods of experimentation cannot be criticized, has recently come to the conclusion that "conjugation does not rejuvenate in any simple, direct way. What it does is to produce variation; to produce a greater number of different combinations, having different properties" ('12, p. 573). This conclusion was based largely on the experiment of dividing a conjugating race into two portions. In one of these portions the individuals were allowed to conjugate, and did conjugate four times in the course of a number of months. The individuals of the other portion were cultivated during the same period in slides and were not permitted to conjugate. The latter series weakened; the individuals "multiplied slowly and irregularly and many died." Some of them were then allowed to conjugate with the result that some strains appeared that were more vigorous than the parents; others were weak and died out but even the stronger ones "were notably less vigorous than the strains which had been kept throughout under more natural conditions and had conjugated frequently." We believe that these

results justify an entirely different interpretation from that drawn by Jennings. In the first place the isolated Paramecia weakened, went into the state of depression and all those that had not conjugated finally died. He interprets the weakness and final death to continued cultivation on slides, but he forgets for the moment that Woodruff has cultivated *P. aurelia* similarly on slides for five and one-half years with as yet no signs of depression. His results were fair confirmation of those obtained by Calkins and should be interpreted similarly, not as due solely to the culture method but to the suppression of a normal function of conjugating lines. In the second place the variations appearing in the strains subsequent to conjugation in the weakened race may be interpreted as specific results due to the conditions of the organisms on entering into conjugation. Maupas and Calkins, as stated above, have shown that the center of conjugation activity, the micronucleus, degenerates in an exhausted race and the differences in vigor observed by Jennings in the ex-conjugants of the weakened race may be interpreted, quite as well, as due to differences in the extent to which such degeneration has progressed, than as evidences of variability due to amphimixis. The variations observed by Jennings seem to us, therefore, to be phenomena due possibly to the physical condition of the organisms on entering into conjugation and to have little bearing on the general problem of the purpose of conjugation. The same argument applies in the matter of rejuvenescence—the failure of these weakened Paramecia to rejuvenate after conjugation is no proof that conjugation of normal organisms does not rejuvenate. The portion, in Jennings' experiment, in which the organisms were allowed to conjugate, retained their vigor and this fact, in our opinion, justified Bütschli's conclusion ('76) that conjugation is for the purpose of offsetting an on-coming physiological weakness and death which is certainly what happened with some of the ex-conjugants of the depressed portion.

Jennings ('13) does not regard this result as a case of rejuvenescence. "There was no general rejuvenescence due to conjugation. Three of the six conjugant lines died out within a week,

and a fourth a little later; so that two-thirds of the conjugant lines were dead. But two continued to multiply. But in the meantime all of the ten non-conjugant lines died out" (p. 375). Here then, was a race in depression; they were allowed to conjugate and did conjugate after repeated efforts to make them do so. Two of the six lines that had conjugated continued to live, the other four lines died. Jennings says: "We can hardly speak of rejuvenescence where two-thirds of the ex-conjugants die out. The survival of some of the conjugants may have been due to the greater vigor that was a pre-requisite to their conjugation, the lack of which caused the others not to conjugate. Aside from this we can only say that the results of conjugation were here the same as usual; it induced variation in the reproductive power" (id., p. 275).

Now it seems to us that this is a very clear case of rejuvenescence. Mortality of the ex-conjugants, amounting to 66 per cent was not as great as the mortality found by us in the history of 360 ex-conjugants where it was 82 per cent. Until we know what causes this high mortality, or the mortality of 100 per cent in *Blepharisma* and in *Stylonychia* (Baitsell '12) after conjugation we cannot use mortality as a test of rejuvenescence. The question of vigor which Jennings raises in those able to conjugate, is purely hypothetical; it might have been tested by the method of split conjugations, but this was not tried. Had this been done we believe the lines derived from the split pairs would not have multiplied any more vigorously than the ten control lines which did die out. This experiment therefore appears to be positive evidence of rejuvenescence through conjugation.

Jennings makes the statement: "So far as I have been able to discover, there is no experimental evidence from any other source (viz., that given by Maupas) that conjugation rejuvenates" ('13, p. 374). We would call attention here to one very clearly defined experiment showing successful rejuvenescence after conjugation which was described in Calkins' paper of 1904. Since this seems to have been overlooked by Jennings, and because of its unique character, the description may be repeated here with somewhat fuller details.

The conjugating pair dealt with was derived from the line known as A 1 in Calkins' experiments of 1901-02. This line had been continuously cultivated on slides for 369 generations (from February 1 to December 8, 1901). On December 7 conjugations were found in the stock left over after isolation of the culture individuals and a pair was isolated. On December 8 the two conjugants had separated. One individual lived for fourteen days, dividing 11 times before it died. The other lived for nine months, undergoing 376 divisions during this period. With this line therefore, we have the conditions necessary to test relative vigor of an ex-conjugant and a non-conjugant control from which the conjugant was derived. The ex-conjugant was treated for twenty-four hours with beef extract on December 9, the A series with beef extract on December 14, January 8 and 15. They thus had similar initial treatment, the control line A 1 receiving three treatments with beef extract, the ex-conjugant only one.

The numbers of divisions in ten-day periods of both control and ex-conjugant lines are given in table 8.

The ex-conjugant and control (non-conjugant) lines were carried along under identical conditions of environment, both sets in the same moist chamber, both were cultivated in the same media and changed at the same times. The ex-conjugant showed a remarkably heightened vigor as contrasted with that of the pure line from which it came. This contrast is well shown in the accompanying graph (diagram 8) in which the divisions in successive periods of ten days are plotted for both.

Reference to table 8 will show furthermore, that the non-conjugating line (A 1) is weaker than the ex-conjugant line in both halves of the total period. Thus in the first one hundred and thirty days the ex-conjugant divided 173 times, the non-conjugant control only 150 times. In the last one hundred and forty days the ex-conjugant divided 196 times, the control only 127, giving a total of 396 divisions for the ex-conjugant and only 277 divisions for the non-conjugant control. The evidence thus is very strong for the conclusion that increased vitality or rejuvenescence was brought about through conjugation.

Calkins' paper of 1902 also deals with split conjugations, the method employed being exactly the same as that adopted by Jennings. The details of the experiments however, were not given and are here published from the records of 1902 for the first time.

The conjugating pairs used in the splitting experiments were obtained from mixtures of series A and series B. Series A at this time were in the 376th generation and Series B in the 334th, and the mixtures were made by putting together in Syracuse dishes the stock left over from all lines of A and B after isolating the culture individuals. There is thus, no record of the exact origin of the split conjugating pairs. Of the twelve individuals whose history was followed after the operation of splitting two died within two days, three more died in four days, one in eight days, one in eleven days, one in twenty-five days, one in thirty

TABLE 8

*Ex-conjugant from line A 1 and line A 1 December 10-August 31. Numbers of divisions in periods of 10 days*

	EX-CONJUGANT	CONTROL A 1		EX-CONJUGANT	CONTROL A 1
1st period	10	11	14th period	23	16
2nd period	15	12	15th period	15	15
3rd period	12	12	16th period	22	10
4th period	19	11	17th period	21	15
5th period	14	13	18th period	20	18
6th period	3	9	19th period	16	4
7th period	11	8	20th period	6	8
8th period	12	7	21st period	8	6
9th period	7	12	22nd period	17	8
10th period	15	14	23rd period	11	6
11th period	24	15	24th period	13	6
12th period	15	14	25th period	9	10
13th period	16	12	26th period	9	3
			27th period	6	2
Total 130 days..	173	150			
Total 140 days..				196	127
Total 270 days..	Ex-conjugant 369 divisions		Control A 1 277 divisions		

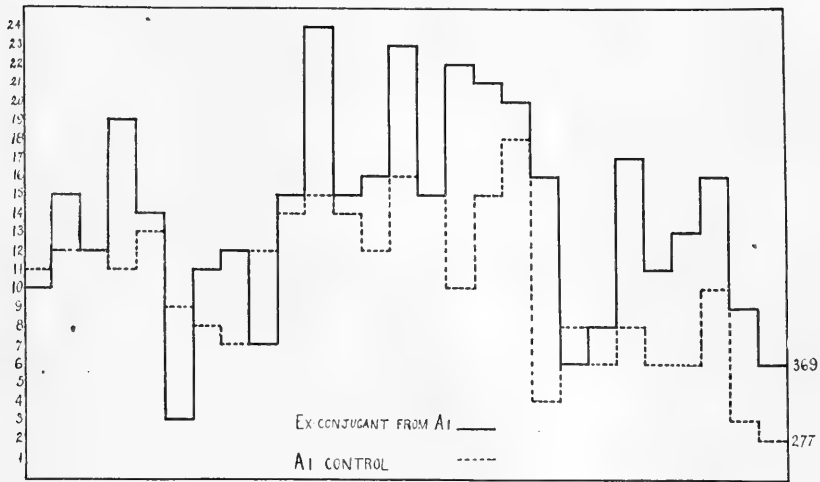


Diagram 8

days, while the remaining three lived for more than two months in culture. One whose history we give below living in isolation cultures for a little more than five months.

From the nature of the mixture it is impossible to compare with certainty the division rate of the split individual with that of the actual line from which it was derived. But we can get fairly close to it indirectly by comparing its division rate with the rates of all of the eight lines which were running at that time. Its ancestral line, and therefore, control line, must have been one of these eight. Table 9 gives the exact number of divisions in consecutive ten day periods for all eight possible ancestral lines (comprising the same dates of course for all lines).

In one hundred and fifty days the split individual divided 180 times. A1 of the regular series divided 181 times in the same period, while none of the other lines came very close, A2 with 169 divisions being the nearest. Jennings has shown that split individuals do not vary from the mean of the stock from which they were selected, hence it is probable that the split ex-conjugant whose history is given above was derived from the A1 line, or the same line from which the ex-conjugant in table 8 was de-

rived approximately at the same time (the ex-conjugant was conjugating December 8, the split pair was conjugating December 12 four days later). From table 8 it can be seen that the ex-conjugant divided 211 times during the one hundred and fifty days that A1 was dividing 181 times and the split conjugant 180 times. If the split conjugant had been derived from any one of the other lines of A or B, the discrepancy would have been still greater.

This experiment demonstrates therefore that conjugation being the only known factor of difference in the individuals compared, was the reason for the greater vigor of the ex-conjugant while the individual ready for conjugation but prevented by splitting, retained the level of vitality of the line from which it was derived.

TABLE 9

*"Split" conjugant, December 14, 1901-May 11, 1902. Number of divisions in 10-day periods of split conjugant and all possible ancestral lines*

	"SPLIT" CONJUGANT	A 1	A 2	A 3	A 4	B 1	B 2	B 3	B 4
1st 10 days	10	10	9	10	10	9	5	4	10
2nd 10 days	19	10	8	4	9	4	9	2	11
3rd 10 days	14	13	9	15	5	4	12	14	16
4th 10 days	16	11	11	14	7	10	11	11	5
5th 10 days	13	12	13	16	16	7	13	11	13
6th 10 days	10	9	8	13	10	11	10	9	10
7th 10 days	10	7	7	10	5	7	4	4	8
8th 10 days	12	8	5	4	5	11	8	3	2
9th 10 days	15	18	20	16	11	11	13	17	17
10th 10 days	16	13	12	14	15	14	15	4	7
11th 10 days	7	15	16	14	4	8	12	13	15
12th 10 days	13	14	13	8	11	14	14	13	13
13th 10 days	10	12	13	3	6	11	10	6	11
14th 10 days	9	18	17	10	8	19	17	13	16
15th 10 days	6	11	8	5	3	15	12	9	5
Total.....	180	181	169	156	125	155	165	133	159



## 9. SUMMARY

1. Pure lines from eleven different series of *Paramecium caudatum* have been studied in cultures for periods of from five to ten months. The pure lines were each derived from a single individual representing either the 4-cell stage or the 32-cell stage of an ex-conjugant. Series J, E', and B are from the 32-cell stages of different ex-conjugants, the J series being the oldest and the most instructive. The pure lines were kept in small vials (permanent cultures) under conditions of temperature and feeding as nearly identical as possible.

2. Relative vitality of the pure lines derived from the same ex-conjugant is shown by *a* mortality in 'cleavage' up to the 32-cell stage, *b* mortality in the permanent cultures, and *c* the daily division rates of representative individuals.

3. *a.* Mortality in the cleavage stages is high, only one series (series J) giving the full set of 32 cells. In the E' series, four died (i.e., one died in the 8-cell stage which should have given four); in the B series four died (see p. 475).

*b.* Mortality in the permanent cultures is also fairly high. Nine died in the J series in ten months. Fifteen died in the B series and ten in the E' series, in five months.

*c.* The daily division rate for the 24 remaining lines of the J series was kept for two different periods, one of thirty days, the other of forty-two days, four months apart. Wide differences were noted in the division rates of the pure lines during the first period, minor differences during the second period. Possibly with increasing age the entire population is approaching a common type.

4. Size variations are well-marked in the different lines. These variations are as definite and as extensive as the variations in progenies from different ex-conjugants. Variations, especially in length, are also wide in many of the same lines at different periods. These periodic variations are more marked in non-conjugating lines than in conjugating lines, the conjugating lines of quadrant A in the J series giving to the entire quadrant a greater stability than in any other quadrant (diagram 2).

5. Conjugation tests are made once a month. Variations in conjugating power are even more marked than are size variations. There is also a difference in conjugating power in progenies from different ex-conjugants, series H' and L for example, are weak series, G, M, Q and T' are strong. In these series no attempt was made to compare more than four pure lines from each ex-conjugant, but of these four there are clear differences in the conjugating power. In series H' only two of the four quadrants comprise conjugating forms. In the T' series one quadrant comprises no conjugating forms, the other three give epidemics at each test; in the M series two quadrants give few pairs, the other two quadrants give epidemics of conjugation at each test. Series E' is also a weak conjugating series.

The most marked differences in conjugating power are shown by the J series. Beginning with the November test and running through until April, every line of quadrant A gave large numbers of pairs while not one pair appeared in any pure line from the three other quadrants.

In the April test some lines of the other quadrants began to develop the power to conjugate, but the number of pairs obtained was small, in marked contrast to the epidemics in all lines of quadrant A (diagram 3).

The conjugation tests are made in all cases with individuals from permanent cultures in which conjugations had not taken place. The tests therefore are made each month with individuals from the same pure lines but of different ages with respect to the ancestral ex-conjugants. The power to conjugate develops in different lines at different ages, this again showing variability in the progeny of an ex-conjugant. Thus in series G conjugations began in one line (quadrant D) three weeks after isolation of the ancestral ex-conjugant; in the other quadrants it did not begin until two months after isolation (diagram 6). In the J series conjugations in J 1, 7, 8, and 21 (quadrant A) began in November, in lines J 15, 16, 17, 20, 25, 29, and 30 conjugations did not appear until April, five months later (diagram 3).

The power to conjugate of the conjugating lines of the J series (quadrant A) is correlated with (a) a lower vitality as measured

by the division rate (diagram 1), and (b) a greater stability in regard to size as shown by the measurements at different periods (diagram 2).

6. Records of the conjugation tests show that the maximum conjugating power of fertile lines is attained from the fifth to the seventh day after seeding the rich cultures and after the second day after transferring from the rich cultures to Syracuse dishes.

7. These differences in physiological activities of pure lines of *Paramecium* point to the explanation of the diverse results and conclusions obtained by different observers with *Paramecium* in culture. Many pure lines die out from a 'natural death;' others, notably the conjugating lines, are relatively weak; still others have a high potential of vitality. Woodruff's *Paramecium aurelia* is evidently a *Paramecium Methuselah* belonging to a non-conjugating line the life history of which is not known in any case.

8. The statement that every protozoon is a potential germ cell is not true. The power to conjugate is not possessed by every *Paramecium* and up to the present it has not been proved that the progeny from every *Paramecium* will develop the power to conjugate. The evidence from Woodruff's culture, and from our J series, indicates that some lines apparently lack even the potential of conjugating power, but this is a matter which continued observation alone will decide.

9. The results of this study show that physiological and morphological variations in the progeny of a single ex-conjugant of *Paramecium caudatum* are fully as extensive as the variations between progenies from different ex-conjugants. The arguments based upon the latter variations to the effect that conjugation is for the purpose of originating variations cannot therefore be sustained.

Experiments here re-described show that the vitality of a given race is increased by conjugation. An ex-conjugant from a pure line that had lived for 369 generations in culture, divided 376 times after conjugation in nine months, while the pure line control that had not conjugated, and from which the ex-conjugant was obtained, divided only 277 times in the same period. Fur-

thermore, a "split" conjugant separated from a pair just beginning conjugation, from the same pure line, divided 180 times, the control non-conjugant divided 181 times and the ex-conjugant divided 211 times in one hundred and fifty days.

10. We do not wish to take the stand that variations do not follow from amphimixis. This fact is too well established in the higher animals and plants to be denied in the case of *Paramecium*, but it has not been proved for *Paramecium*. The only way it can be proved is by a systematic study of variations in many different pure lines from an ex-conjugant and comparing the results of such study with similar results derived from a like study of different ex-conjugants.

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# THE PHYSIOLOGY OF THE CHROMATOPHORES OF FISHES

R. A. SPAETH

THREE TEXT FIGURES AND FOUR PLATES

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## 1. PRELIMINARY

### 1. *Introduction*

In the great majority of researches upon the physiology of the color-changes in vertebrates, the experiments have been made upon living animals. In such cases the experimental conditions have necessarily been complicated by a central nervous inhibition, abnormal circulation, oxygen supply, etc. A direct response of the chromatophores has been claimed by many inves-

tigators but in a number of instances the results have been open to an alternative interpretation. The problem of this research has been to determine the nature of the direct responses of the chromatophores of fishes (especially of *Fundulus heteroclitus* L.), to a series of physiological stimuli.

## 2. *Material*

There are two sharply differentiated types of chromatophores in the adult *F. heteroclitus*, (1) the melanophores, a dark, prominently ramifying variety containing minute granules of a deep brown or black melanin pigment and (2) the xanthophores, a light or yellow, (lipochrome) type in which both ramifications and granular contents are less distinct. The principal variations of shade (light and dark) are dependent upon a proximal and distal migration (Parker '06) of the pigment within the melanophores. (For convenience the term 'contraction' and 'expansion' will be used throughout this paper to designate the light and dark phases respectively.) The distribution of these two kinds of chromatophores is quite constant. Neither occurs in the epidermis. Both types are present in the superficial layers of the derma, that is, that portion lying between the scales and the epidermis. In the deeper layers of the derma, between the body-muscle and the scales, both kinds are again represented. These two types of chromatophores are by no means characteristic of this species. It is highly probable that they occur universally among the color-changing teleosts. If a female, 4 to 6 cms. in length, be examined under a low power of the microscope the deeper melanophores are frequently seen to be fully expanded while the superficial ones are completely contracted. This suggests a physiological differentiation in addition to the morphological one.

No attempt has been made to ascertain the degree of the adaptation of this species when brought upon differently colored bottoms. In an unglazed white vessel or over a bottom of light sand the fishes assume a pale, greenish-yellow hue. This is brought about by a complete contraction of the melanophores



and a diffusion (expansion) of the xanthophores. Over a dull black bottom the fishes become correspondingly black, the melanophores being fully expanded while the xanthophores tend to contract. Thus at the extremes of the color phases the two types of chromatophores may behave in a reciprocal manner—the one expanding when the other contracts.

The relation to the sympathetic nervous system, which has been so frequently described in a variety of fishes, also occurs in this species. Thus, for example, it has been possible (apparently) to sever one of the two branches of the sympathetic system in the haemal arch posterior to the body cavity, causing a loss of control of the chromatophores posterior to the point of the incision and on that side only (cf. v. Frisch '11 a). Complete loss of adaptive changes follows the excision of both eyes.<sup>1</sup>

If a fish which is adapted to a light bottom be decapitated the body becomes intensely dark in the course of a few minutes—the time varying with different individuals. The melanophores remain in this expanded condition for some hours depending, in part, upon the temperature of the water, but eventually they contract and the fish becomes light. Similarly the final condition of fishes which have died in the aquaria is the light one.

The rapidity of the change from the extreme light to the extreme dark phase in a freshly decapitated fish suggested the possibility of observing the microscopic changes within the individual chromatophores. Such fish were brought upon the stage

<sup>1</sup> It seemed of interest to determine at what age the fish begins to show the adaptive color-changes. Many observations of twelve-day embryos failed to show any such change in the melanophores so long as the egg-membrane remained intact, though they were watched both over white and black bottoms. Light was thrown upon the question by the following experiment.

July 18, 1912. From a batch of eggs of *F. heteroclitus* (stripped and fertilized 2 P.M. July 6, 1912) a number of embryos became free between 2 and 4 P.M. All these young were dark dorsally when hatched, especially in the region of the brain. No distinct limits of individual melanophores were visible when first they slipped from the membranes. Two groups of four each were placed in white and black-bottomed Syracuse glasses respectively at 4.30 P.M. At 7.30 P.M. all four fish over the dark bottom were dark as when hatched while all those over the light bottom were light and showed the melanophores, particularly those of the head, to be contracted. Thus within six hours after hatching *F. heteroclitus* is capable of adaptation to the ground-color of its environment.

of the microscope immediately after decapitation and the melanophores could then be seen to be expanding. The dorsal and caudal fins proved to be the most favorable regions for observing this change although the method was far from satisfactory.

### 3. Methods

Following a suggestion of Biedermann and others who had obtained excellent fixed preparations from the scales of fishes, the attempt was made to observe the behavior of the living chromatophores in this way. Owing to particularly favorable topographical conditions the method proved quite satisfactory. The details of the manipulation of the scales were as follows.

A section through the skin of *F. heteroclitus* (parallel with the chief axis of the body) shows the following relations:

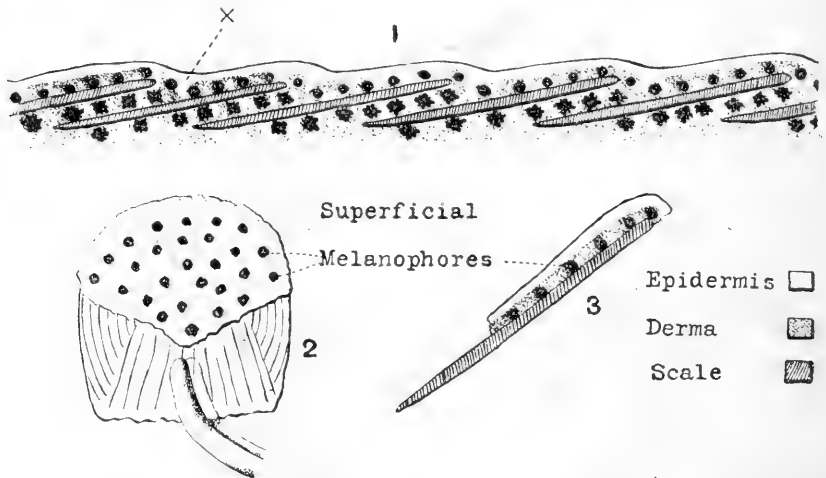


Fig. A Diagrams of the scales in *Fundulus*. 1, position of the scales in a cross-section of the skin; 2, single scale held by forceps; 3, side view of isolated scale.

The scales are so arranged that it is possible by inserting a broad scalpel at the point *x*, to raise the scale, 1, with its superficial layer of chromatophores and epidermis, without injuring or disturbing them in any way. The deeper melanophores (shown as stellate bodies in the figure) remain behind in the proc-

ess, 3. That portion of the scale, 2, which normally lies within the dermal pocket makes a convenient "handle" whereby the scale may be transferred from one solution to another. With very little practice it was found that a number of scales could thus be raised simultaneously, the epidermis serving to hold them together. By removing these scales to the various salt solutions, etc., any changes in the chromatophores could very readily be observed under a low power of the microscope. In removing the scales from the living fish and transferring them to the solutions care was taken to handle them only as shown in 2, figure A. In this way any mechanical stimulus or injury was entirely avoided. Furthermore the scales were always placed face up in the solutions, that is, in the same relative position which they occupy on the surface of the fish. This was done to insure a uniform oxygen supply.

Although there are marked differences in the reactions of scales from different fishes, by using adjacent scales from one individual a satisfactory basis for comparative results was obtained. But even in the same individual, scales from the median, dorsal region vary in the reaction time of their chromatophores from those of the more lateral portions of the body.

In many cases the experiments were started by immersing all the scales in a solution of 0.1 M NaCl which causes a complete and lasting expansion of the melanophores. This was done, for example, in all of the trials with potassium and other salts in which the first specific effect is a contraction of the melanophores. Whenever this was the case and a comparison was to be made between the effects of the different members of a series of salts (for example, the neutral salts of potassium) a preliminary selection of scales was made. Only such scales were chosen for comparison as showed approximately the same size, shape and darkness (that is, number and expansion of melanophores). This selection was easily made without the use of a lens, the scales resting over a light surface.

The fish average from 6 to 10 cms. in length. They were kept in salt-water aquaria throughout the period of experimen-

tation. They were brought, three or four at a time, from the aquaria into the laboratory where they were kept temporarily in a shallow white enamelled dish (6 by 26 cms.). In this, after a brief interval, they assumed the light color phase which served as a uniform starting point. To avoid the carrying over of seawater into the salt solutions the fishes were always rinsed in distilled water before removing the scales.

The salts used throughout these experiments were of the highest quality (Merk, Kahlbaum, and Baker and Adams). Volume molecular solutions (molecular weight in grams up to 1 litre) were made up which were afterwards diluted to the required concentrations with glass distilled water. In a few cases, potassium iodide for example, in which the solutions are unstable at molecular concentration, the solutions were made up as needed from the dry salts.

The majority of the experiments were carried on in Syracuse watch-glasses which were kept covered to prevent evaporation except while under actual observation.

When the actions of several salt solutions were to be compared it became necessary to immerse all the scales simultaneously. This was accomplished by a simple device consisting of glass rods inserted into a large cork. The free ends had been previously heated and flattened. The scales were placed on these flattened ends—there being a rod for each solution. By dipping the ends of the rods simultaneously into the solutions and gently shaking, the scales easily floated off. They were then examined and in case they had been turned face down in this process they were reversed. In this way as many as twenty-five scales (five rods, each carrying five scales), could be manipulated at the same time.

Details of the apparatus used in the oxygen and temperature trials are given in connection with those experiments.

The work has been done chiefly in the Zoölogical Laboratory of Harvard University under the supervision of Prof. G. H. Parker. This opportunity is gladly taken to acknowledge my indebtedness to him for much kindly criticism and suggestion.

During the summer of 1911 the privilege and use of a table at the United States Fisheries Laboratory, Woods Hole, Massachusetts, were granted by Hon. G. M. Bowers, which greatly facilitated the work.

## 2. REACTIONS TO SALT SOLUTIONS AND DISTILLED WATER

### 1. *Effects of sea-water and distilled water*

Among the first experiments performed with the scales taken from the living fish were trials made to determine the effect of sea-water<sup>2</sup> upon the chromatophores. When scales were removed from a light female and immersed in sea-water a very slight expansion of the dark pigment could be seen upon immediate examination. This expansion occurred in all the melanophores of a single scale simultaneously. In no case was it so great as is the expansion in the melanophores of a normal dark fish. When scales from such dark fish were placed in the same sea-water there was no visible change. They remained dark with completely expanded melanophores for some hours (depending upon the temperature of the sea-water). Eventually they began to degenerate from the expanded state. This degeneration was perfectly characteristic. The pigment in the processes of the melanophores which, in the normal, living, expanded condition, is seen as narrow, continuous, dark lines, became broken up into small, spherical or ovoid masses and the melanophores thus gradually lost their individual contours.

The following experiment illustrates the extent of the initial effect in sea-water.

*March 1, 1911*

- 1.33 P.M. Scales were removed from a 10 cm. light female and placed in sea-water. Only a slight outward migration could be seen and this was not active.
- 2.11 and 2.57 P.M. Other scales from the same fish showed the same sluggish behavior as the first set. In all these cases the shifting of the pigment had ceased three minutes after the immersion in sea-water. T. = 17°C.

<sup>2</sup> This sea-water was taken from the marine aquaria in the Museum of Comparative Zoölogy at Cambridge, Mass.

This peculiarly inert behavior of the melanophores suggested an inhibitory or even a toxic effect of the sea-water upon them. Scales were consequently subjected to the action of distilled water. A very definite reaction followed which has proved to be entirely constant.

When scales, taken from a light female, are immersed in distilled water at room temperature (20°C.), a rapid expansion of the pigment is seen. As in the case of sea-water this occurs simultaneously in all the melanophores. The expansion continues for some time—differing with the individual and depending also upon the temperature of the distilled water and the size of the scale. After a longer or shorter period of expansion a complete contraction of all the melanophores follows. This contraction invariably begins at the periphery of the scale and generally at its posterior end (relative to the original position on the body of the fish). Thence it creeps in towards the centre of the scale much like the closing of an iris diaphragm. The melanophores at the center of the scale often continue to expand for some time after those at the periphery have begun to contract. Indeed, individual melanophores lying in the region separating the expanded from the advancing contracted phase frequently show the pigment to be migrating proximally on the peripheral side of the melanophore while on the inner side it continues to move distally. Thus within the limits of a single melanophore the pigment may, for a short time, be migrating reciprocally (figs. 1 to 5, pl. 1).

The contraction which follows the first expansion in distilled water is not, however, the final condition. After another time interval (depending upon the factors previously mentioned), the melanophores relax from the spheriform phase, the pigment extends a short distance out into the processes of the cells (never a complete expansion) and from this semi-expanded state the melanophores rapidly degenerate. This relaxation of the melanophores thus marks the approaching degeneration.

When the melanophores, contracted in distilled water, are examined with an immersion lens, they show the empty processes of the cell from which the pigment has withdrawn to the center

(fig. 6, pl. 1). Since this is not the case when the contraction is brought about by certain salt solutions (potassium salts for example), it is possible that the appearance of the empty processes is due to a selective absorption of water by the melanophores. Considered as an osmotic phenomenon it is evident that a hypotonic fluid is correlated with a contraction of the melanophores.

The changes in the xanthophores are less distinct. The cytolytic effect of the distilled water occurs from the half-expanded state and is never preceded by a complete contraction as in the melanophores.

In attempting to compare the time required for a complete contraction of the melanophores in different individuals it became evident that scales from smaller fish react more rapidly.

*February 3, 1912*

To compare the variation in the time of the complete contraction in distilled water, six females of different sizes were selected and from each of these the large scale lying on the dorsal, median line between the eyes was removed to distilled water.

SIZE OF FISH	TEMPERATURE OF WATER	TIME OF CONTRACTION
<i>cm.</i>	<i>deg. C.</i>	<i>minutes</i>
6.5	19.7	28.5
8.0	19.0	36.0
8.5	20.0	40.0
9.0	19.0	41.2
10.0	19.7	45.0
10.5	20.0	>67.0

It is the actual size of the *scale* however and not that of the fish which determines the time of contraction. This was easily demonstrated by comparing large and small scales from the same individual. In such cases the differences were quite as great as in the above table.

The previous history of the individual and its physiological condition at the time of the experiment may be additional sources of error which make it impossible to attach any considerable significance to differences in the time of reaction even when size,

sex and temperature are constant. These difficulties are obviously eliminated at a stroke when scales from the same individual are compared.

Decapitation of a normal light fish is always followed by a rapid darkening owing to the removal of the nervous inhibition of the melanophores and their subsequent extreme expansion. Obviously when a scale is removed from the living fish, the nervous connections are severed and, unless there be some secondary inhibiting factor, an expansion of the melanophores should follow exactly as in the decapitated fish. This is actually what occurs in the case of distilled water, a uniform and synchronous expansion of all the melanophores. Hence only the peripheral contraction and the second semi-expanded state from which the melanophores degenerate, can be considered as specific effects of distilled water. This characteristic peripheral contraction is due to the gradual advance of the diffusing fluid which seems to penetrate most readily at the edge of the scale where epidermis and derma have been torn from their original attachments. It therefore seems highly probable that the epidermis is impermeable to distilled water.

Additional proof for this impermeability of the epidermis is seen in the case of pithed and decapitated fish which lie in distilled water. Under these circumstances the melanophores remain expanded for hours. Their ultimate degeneration under these conditions is the result of anemia and autolysis and not of the cytolytic effect of distilled water. In certain of the experiments with salt solutions this impermeability of the epidermis is brought out even more strikingly.

The experiments of Garrey ('05) are of interest in this connection. In a number of fish of this species scales were scraped from a considerable area of the body. These fish were then divided into three lots—the first being returned to sea-water, the second to distilled water and the third to a mixture of equal parts of sea and distilled water. All fish of the first and second lots died. At the end of four weeks 70 per cent of those in the dilute sea-water were alive. Garrey attributes the toxic effects of the sea-water and distilled water to changes in the osmotic



pressure of the blood following the action of the hyper- and hypotonic media. The dilute sea-water he believes to approximate the  $\Delta$  of *F. heteroclitus* to a degree which renders it harmless. From these experiments of Garrey's it is clear that the scales are essential to the life of the fish even in sea-water. From the experiments with individual scales in distilled water it seems that not the scale but the epidermis is essentially what protects the fish from the sea- or fresh-water.<sup>3</sup>

Since the migration of the melanin granules in sea-water is practically nil and since there is always a definite response to distilled water, the question naturally followed as to the nature of the inhibiting factor in sea-water which prevents the contraction that invariably occurs in distilled water. Further experiments were carried out with sea-water from other sources,<sup>4</sup> and, whereas these showed a somewhat greater initial expansion, though not so great as in the melanophores of a normally dark fish, a contraction never followed.

A combination of the two types of experiments was now tried. Scales were immersed in sea-water diluted with distilled water in varying proportions in order to eliminate the concentration factor. The following relationships were brought out.

1. When the dilution was slight, 3 parts sea-water: 1 distilled water, a somewhat greater expansion occurred than in the control scales in sea-water but this was not so great as in the case of normal, dark fish.

<sup>3</sup> This fact may throw some light upon the recent controversy concerning the possibility of keeping *F. heteroclitus* alive in fresh water. Obviously fish which showed missing scales would be discarded in such experiments. However, it would be very difficult to determine casually whether or not portions of the epidermis had been rubbed off. This occurs very easily especially if the fish are caught in a seine. It follows therefore that the discrepancies in these experiments in keeping the fish alive in fresh water may have been due to a more or less perfect condition of the epidermis. Scott ('11) has suggested a different explanation for Garrey's results.

<sup>4</sup> The sea-water in these experiments was taken from the taps at the Wood's Hole laboratories (U. S. Fisheries and Marine Biological) and at flood tide at Cape May Point, N. J. This was all slightly more dilute than that of the first seawater experiments which had become somewhat concentrated by evaporation. No freezing-point determinations were made.

2. Further dilution of the sea-water (from 1 part sea-water: 3 distilled water to 1 part sea-water: 14 distilled water) produced a prolonged expansion.

3. The duration of this expansion decreased with still greater dilution (1 part sea-water: 25 distilled water) and at a concentration of 1 part sea: 50 distilled water the presence of the sea-water could only be detected by its protective action; the melanophores of scales in the mixture remained irritable longer than those of the control scales in distilled water.

*August 23, 1912*

- 10.24 A.M. Scales from an 8.5 cm. female immersed in 8 cc. of 3 parts sea-: 1 of distilled water and 8 cc. sea-water respectively. The fish was not previously rinsed in distilled water in this case.
- 11.20 A.M. No differences in the two sets.
- 12.10 A.M. The set in sea-water is distinctly more yellow to the naked eye and shows (under the microscope) the xanthophores more expanded than those in the mixture.
- 12.15 P.M. The scales in the mixture appear slightly darker, with their melanophores more expanded than those in the sea-water: the xanthophores are contracted. This mixture is thus a solution which, after two hours, acts oppositely upon the two sets of chromatophores—expanding the melanophores and contracting the xanthophores.
- 8.40 P.M. Degeneration. Difference in the xanthophores as above. T. = 25.5°C.

At the following and greater dilutions, control scales were run in distilled water rather than sea-water. In this experiment the melanophores in the mixture remain expanded nearly an hour after those in the distilled water have degenerated.

*August 22, 1912*

- 10.58 A.M. Scales from an 8 cm. female were removed to 8 cc. distilled water and 8 cc. of 1 part sea-: 14 distilled water,
- 11.02 A.M. All melanophores were expanding in the mixture while the scales in distilled water were lighter with many peripheral melanophores contracting.
- 11.08 A.M. All the melanophores in the distilled water set were contracted. All in the mixture were still widely expanded.
- 11.11 A.M. Majority in distilled water showed the secondary expansion (relaxation from spheriform phase).

- 11.42 A.M. All the melanophores in the mixture were still fully expanded and all those in the distilled water were degenerating.
- 12.30 P.M. The mixture showed the melanophores still expanded.
- 1.20 P.M. The melanophores were contracted but not degenerating in the mixture.
- 3.25 P.M. The melanophores in the mixture were half expanded but there was no sign of cytolysis. T. = 26°C.

The following two experiments show that even at a dilution of 1 part sea-water to 50 of distilled water (1) the irritability is maintained longer than in distilled water and (2) that the mixture shows a distinct protective action against cytolysis.

*August 23, 1912*

- 8.54 A.M. Scales from an 8 cm. female were immersed in 8 cc. of distilled water and 8 cc. of 1 part sea-: 50 distilled water, respectively.
- 9.04 A.M. Both sets were contracted.
- 9.20 A.M. The melanophores of every scale in the mixture responded to a 1 per cent atropine sulphate stimulus<sup>5</sup> and none were relaxed. All but one scale in distilled water had relaxed and degenerating melanophores and failed to respond to the atropine sulphate stimulus. High power showed empty processes in the melanophores of the mixture exactly as in distilled water (fig. 6, pl. 1). The contraction here was probably due to the dilution—the characteristic hypotonic effect. T. = 25.5°C.

*August 22, 1912*

- 4.55 P.M. Scales from an 8 cm. female were placed in 8 cc. of distilled water and 8 cc. of 1 part sea-: 50 distilled water respectively. No distinct difference was observable at this dilution between the contraction time of the mixture and that in distilled water.
- 5.18 P.M. The relaxation was very distinct in the melanophores of the distilled water set but not in those in the mixture. All scales in the distilled water were more yellow to the naked eye than those of the mixture (expanded xanthophores). T. = 26°C.

The most striking fact about these trials with dilute sea-water is brought out in the experiment of August 22 in which the dilu-

<sup>5</sup> A 1 per cent solution of atropine sulphate causes a rapid expansion of the melanophores which is irreversible by the contracting salts (*vide infra*). Failure to respond to this stimulus was considered a criterion for the loss of irritability.

tion was 1 part of sea-water to 14 parts of distilled water. This solution is hypotonic to the plasma of *Fundulus* though no freezing point determination of the blood of this species has been made. It seems reasonable however that it should fall within the range of the anadromous fishes which show a depression intermediate between purely marine teleosts ( $-0.75^{\circ}\text{C}.$ ) and those living in fresh water ( $-0.53^{\circ}\text{C}.$ ). Assuming the  $\Delta$  of the sea-water used in this experiment to be as great as  $-2^{\circ}\text{C}.$  and that this would vary directly with the dilution, a concentration of 1 part sea-water in 15 of solution would give a  $\Delta$  of approximately  $-0.13^{\circ}\text{C}.$  This is less than one-quarter of the value of the  $\Delta$  of fresh water teleosts, hence it follows that this dilution of sea-water must give a solution hypotonic to the plasma of *Fundulus*. If the effect of this dilute sea-water upon the melanophores were an osmotic one we should expect it to be an hypotonic one as was the case in pure distilled water, that is, it should cause a contraction of the melanophores. As a matter of fact in such dilute sea-water the melanophores are expanded and remain so for a considerable time ( $>1.5$  hours) Hence it follows that in sea-water there is a factor other than the hypertonic one which inhibits the contraction seen in the melanophores when immersed in distilled water.

Experiments were next carried out upon the effects of the individual salts of sea-water. Sodium, potassium, calcium and magnesium chlorides and magnesium sulphate were all used. The concentrations varied from molecular to 0.01 molecular. In the case of NaCl a few trials were made with as high a concentration as 2.5 M.

These experiments showed conclusively that sodium chloride is the only one of the five common salts of sea-water which causes a lasting expansion of the melanophores. All of the other salts produce a contraction. Hence it is sodium chloride which prevents a contraction of the melanophores even in relatively dilute (hypotonic) sea-water. Thus normal sea-water which is generally considered to be a balanced solution, that is, one in which no specific ionic effect predominates over any other, upon dilu-

tion, becomes unbalanced in that the NaCl exerts its individual influence upon the melanophores.

Sea-water preserves the irritability of the melanophores for some hours. Thus the combination of concentration (a hypertonic solution) and the specific sodium chloride effect is only an inhibiting and not a toxic one.

The gradual loss of irritability is brought out in the following experiment in which scales were transferred at regular intervals from sea-water to a KCl solution to see how long the melanophores would continue to respond to this contracting stimulus.

*September 4, 1912*

- 3.30 P.M. Scales from an 8 cm. female were placed in 10 cc. of sea-water. This fish had previously been over a dark bottom for about six hours. All the scales appeared very dark with widely expanded melanophores. Individual scales were now removed from the sea-water to a 0.1 M KCl solution at half-hour intervals beginning at 4 P.M. The first eight trials (until 7.30 P.M.) showed a complete contraction in KCl with an average contracting time of fifteen minutes.
- 9.30 P.M. A slight contraction lasting only three minutes.
- 10 and 10.30 P.M. A very slight contraction was observed in a few melanophores.
- 11.00 P.M. No reaction followed immersion in the KCl solution. All the melanophores remained widely expanded. T. = ca 23°C.

At a somewhat lower temperature and differing with the individual, the irritability of the melanophores may be preserved even longer than six hours. In one case the melanophores responded to the contracting stimulus of  $\text{KNO}_3$  more than eighteen hours after removal from the fish. Further experiments showed that the reactions of the melanophores in artificial sea-water (van't Hoff's solution) were essentially like those in normal sea-water.

In distilled water, on the other hand, the irritability of the melanophores rapidly disappears. Here again the time of the reaction depends in part upon the temperature of the water.

In order to detect the last traces of irritability after exposure to distilled water scales were brought into a 1 per cent solution

of atropine sulphate from time to time. The atropine sulphate is a more violent and rapid expansion stimulus than NaCl which was used in other experiments of the following type.

*October 19, 1912*

- 8.52 A.M. Scales from a 10 cm. female were placed in distilled water.  
9.02 A.M. All the melanophores were contracted. Scales were now immersed in a 1 per cent atropine sulphate solution at 9.05, 9.22, 9.28, 9.30, 9.35, 9.40, 9.44 and 9.59. The first six trials all showed an expansion clearly except the 9.30 scale.  
9.44 A.M. The expansion was very slight.  
9.59 A.M. No expansion. All scales remaining in distilled water showed the characteristic half expanded condition at this time. T. = 19°C. Thus at room temperature the melanophores lose their irritability completely after an exposure of approximately an hour in distilled water.

The results of the foregoing experiments with sea- and distilled water may be summarized as follows:

1. In sea-water practically no migration of pigment occurs either in expanded or contracted melanophores.

2. In distilled water, scales from a light fish show (a) an expansion; (b) a contraction; (c) a secondary expansion and finally (d) degeneration in the melanophores. The first response (a) is due to the elimination of the inhibiting nerve tonus and is independent of the specific effects of distilled water (b-d). This characteristic cycle of reactions is completed in less than an hour at room-temperature. There is evidence for a selective absorption of water by the melanophores which may be the cause of the contraction (b).

3. A gradual dilution of sea-water by the addition of distilled water causes a corresponding expansion of the melanophores. The greater the dilution the longer does this expansion last—up to an optimum concentration. Beyond this, as the proportion of sea-water in the mixtures diminishes, the cytolytic effect of the distilled water becomes evident in the decrease of the duration of the expansion. Finally, at a dilution of one part sea-water to 50 of distilled water, the initial contraction is practically indistinguishable from that in distilled water but the presence

of the small proportion of sea-water appears in its protective action against the characteristic distilled-water degeneration.

4. The specific effect of NaCl accounts for the lasting expansion of the melanophores and the contraction of the xanthophores in dilute sea-water (p. 540).

5. Although upon dilution the relative proportion of the salts remains the same as in normal sea-water (a 'balanced solution' of Loeb), nevertheless the specific effect of the NaCl appears perfectly definitely in the responses of the chromatophores to such dilute solutions.

6. All movements of the melanin granules cease shortly after immersion in sea-water but this is not due to its toxic action for the melanophores may retain their irritability in sea-water more than eighteen hours, at room temperature.

7. In distilled water the melanophores lose their irritability in about an hour, at room temperature.

## 2. *Effects of single electrolytes*

*a. Potassium salts.* A 0.2 M NaCl solution causes a lasting expansion of the melanophores but an isotonic solution of KCl produces a rapid contraction. It is therefore clear that these characteristic reciprocal responses of the melanophores are not dependent upon osmotic differences in the two solutions.

If scales which have been in a 0.1 M NaCl solution until all the melanophores show complete expansion, are immersed in a molecular solution of KCl, a rapid contraction of all the melanophores follows immediately. This contraction is usually not complete however in all the cells of a single scale.

In less concentrated solutions, 0.5 to 0.01 M KCl the contraction is a complete one. Some time after the melanophores have contracted the xanthophores invariably expand. This is quite as characteristic a reaction as the contraction of the melanophores. At so great a dilution as 0.01 M the specific effect of the salt is nearly obscured. Control scales in distilled water show a contraction time which is only slightly greater than that of the scales in 0.01 M KCl. Beyond this concentration the specific KCl effect can no longer be detected with certainty.

The time of contraction in KCl solution of different strengths varies with the concentration.

*September 5, 1912*

9.26 A.M. Scales from an 8 cm. female were immersed in 0.1 M NaCl.  
 10.45 A.M. Sets of 3 matched scales were removed to 8 cc. of 0.2, 0.1, and 0.05 M KCl. The average time of the contracting for nine trials in each solution was as follows:

Solution strength	Average time	
	minutes	seconds
0.2 M.....	2	9
0.1 M.....	3	9
0.05 M.....	4	58

All concentrations of KCl from 0.85 to 0.02 M clearly show a contracting effect upon the melanophores, while NaCl solutions of the same strength produce an expansion of the pigment in every case. Since this cannot be an osmotic effect and the chlor-anion is common to the two salts, it seemed possible that the contraction might be the specific effect of the  $K^+$  ion and the expansion that of the  $Na^+$  ion. If this were so, other neutral K salts might also be expected to exert a contracting influence upon the melanophores.

Trials were now made with 0.1 M KCl and KBr. Both solutions contracted the melanophores in the same time. Not the slightest constant difference in the contraction-time could be detected. The evidence for the specificity of the  $K^+$  ion was this strengthened. A chance observation gave a clue to the difference in the effects of the two anions. After three hours immersion in 0.1 M KCl and 0.1 M KBr solutions, all the scales in the KBr solution showed the melanophores to be slightly expanded while all of those in the KCl remained completely contracted. This difference was quite striking, sufficiently so to be seen clearly with the naked eye. Further experiments proved it to be constant. In KBr a secondary expansion and subsequent degeneration of the melanophores always appeared sooner than in KCl. This secondary expansion was in no way comparable to the expansion produced by NaCl for in the case of the potassium salts it was irreversible.



Similar trials were now made with KI solutions. The effect was again a contraction of the melanophores. The time of this contraction was the same as in the chloride and bromide solutions. No constant difference was observed. A secondary expansion occurred here also but even sooner than in KBr.

After the probable position of the iodide had been established other experiments were made comparing the action of only the KBr and KI since the chloride-bromide sequence had previously been determined.

*April 26, 1912*

Scales from a 9.5 cm. female were put into a 0.1 M NaCl solution. After eighteen minutes 2 matched pairs were immersed in equal volumes of 0.2 M KBr and KI respectively. Thirty-five minutes later the scales in the KI showed a decided expansion of the melanophores while in the KBr they were only slightly expanded. The time of contraction in both cases was one and one-half minutes with no noticeable difference in the two solutions. T. = 20°C.

Thus as regards the maintainance of irritability the anions of these potassium salts showed this order,



Experiments with  $\text{KNO}_3$  showed clearly that the cytolytic activity of its anion was intermediate in character between the  $\text{Br}^-$  and  $\text{I}^-$ . As in the case of the potassium iodide its action was compared with the three halogens simultaneously to determine its approximate position and then matched scales were run against each other in isonic solutions of both the KBr (on one side) and the KI on the other. Thus

*May 8, 1912*

- 11.09 A.M. Scales from a 9 cm. female were removed to a 0.1 M NaCl solution.
- 11.57 A.M. • 4 sets of 4 scales each were immersed in 6 cc. of 0.186 M KCl, KBr, and KI, and 6 cc. of 0.2 M  $\text{KNO}_3$  respectively. All the melanophores were contracted in two and one-half minutes.
- 1.35 P.M. The KI,  $\text{KNO}_3$  and KBr solutions all showed some expanded melanophores. None were expanded in KCl.
- 3.00 P.M. The four scales showing the most expanded melanophores in the four solutions were fixed in 95 per cent alcohol.

4 P.M., 5 P.M., and 6 P.M. Similar sets were killed. In every case the scale having the most widely expanded melanophores was chosen. These scales were all mounted. The series shows very definitely the anion order



Finally the position of KSCN was determined. It fell quite obviously beyond the iodide. A very few experiments in which the action of iodide and sulphocyanate were compared, served to determine this conclusively. Thus the anion series,



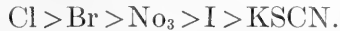
was established for the preservation of irritability of the melanophores in these five neutral salts of potassium.

*October 3, 1912*

- 12.30 P.M. After rinsing in tap- and distilled water scales were removed from a 10 cm. female to a 0.1 M NaCl solution.
- 12.58 P.M. After selecting, five sets of five scales each were immersed in 8 cc. of 0.1 M KCl, KBr, KI<sup>6</sup> and KSCN and 8 cc. of 0.105 M KNO<sub>3</sub>. All the melanophores were contracted after four and one-half minutes. No distinct differences were observable in these contraction times as in the previous experiments. (The selection in this case required less than three minutes and all the scales were drained on filter paper before being placed on the glass dipper. After all the scales were in place the surplus NaCl solution was again drained off.)
- 1.40 P.M. All the scales in KSCN showed the beginning of the expansion. A set of five scales (one from each salt solution) was now killed in 95 per cent alcohol at 2, 3, 4, and 5 P.M. respectively. In making the selection at the end of each hour the darkest scales were chosen in every case and furthermore they were killed in their anion order, beginning with the KSCN scale. This was done in order to throw any possible advantage of the extra time (1-2 minutes) in favor of the less expanded chloride and bromide scales. To obviate any possibility of confusing the individual scales they were placed in separate vials and subsequently were separately mounted under individual cover-glasses, the set from each hour on a different slide. These twenty scales were then photographed individually (plates 2 and 3).

<sup>6</sup> In this case the 0.1 M KI solution was made up fresh for this experiment from a 'Highest Purity' sample of Merck's salt.

In these five neutral salts of potassium the initial effect is a contraction. The time of this contraction is the same for the five salts within the limits of the variations of individual scales. Since there is this common time of contraction and the common cation  $K^+$  in all five salts, it seems highly probable that the initial effect (a contraction) is specific for the  $K^+$  ion. The time of the secondary degeneration effect (expansion) varies with the nature of the anion in the well-known lyotropic order



A little study of the plates (2 and 3) will show that what really occurs is a gradual centripetal diffusion beneath the epidermis. The melanophores play the part of indicators marking the advance of the degenerating area. This is strikingly illustrated in such scales as the KI after one hour and the  $KNO_3$  after two hours in which there is a sharp line separating contracted and expanded melanophores. Whereas in the case of the initial contraction the centripetal diffusion is approximately equal on all sides, the cytolytic effect always appears at the posterior torn edge of the epidermis. As the degeneration proceeds, the epidermis becomes freed from the underlying derma and when all the melanophores are expanded (degenerated) the epidermis is completely detached. It therefore seems quite certain that the diffusion occurs between the epidermis and derma as was first suggested by the experiments with sea- and distilled-water (p. 533).

The experimental evidence thus indicates an independent specific action of cation and anion, the cation effect always appearing first. These effects are furthermore exactly reciprocal.

The question now arose whether the degeneration (cytolytic-expansion) was due to the specific nature of the anions or whether it might not also be dependent upon the cation or some combined interactivity of the two. In order to test this point, further experiments were carried out with the corresponding series of neutral salts of sodium.

*b. Sodium salts.* A 0.1 M solution of NaCl brings about a lasting expansion of the melanophores. A similar expansion occurs in all concentrations of NaCl between 0.02 and 0.83 M.

At 0.9 M concentration a few melanophores show the beginning of an expansion but the centrifugal migration soon ceases. No constant reaction occurs in a molecular solution of NaCl. The epidermis very quickly becomes entirely opaque and the migration of the pigment is completely inhibited.

When scales have been immersed in a 0.1 M NaCl solution for about an hour the xanthophores are completely contracted while the melanophores are widely expanded. Here again is a case in which the melanophores and xanthophores react reciprocally to the same stimulus. This contraction of the xanthophores is quite as characteristic a NaCl reaction as is the expansion of the melanophores.

The expansion produced by a solution of NaCl is dependent upon both the temperature and the oxygen supply. Above 30°C. the specific effect of heat overcomes the expanding tendency of a 0.1 M NaCl solution (p. 563). Similarly in an atmosphere of pure hydrogen the melanophores immersed in a 0.1 M NaCl contract (p. 563). If the temperature and oxygen supply remain constant the chromatophores retain their irritability much longer in a 0.1 M NaCl solution than in KCl of the same concentration.

Believing the expansion in the NaCl solution to be due to a specific effect of the Na<sup>+</sup> ion, experiments were made with other neutral salts of sodium.

*June 22, 1912*

- 11.43 A.M. Scales from a light, 8 cm. female were removed to sea-water.  
 11.48 A.M. 4 sets of three scales each were transferred to 8 cc. of 0.1 M NaCl, NaBr, and NaSCN and 8 cc. of 0.105 M NaNO<sub>3</sub>.  
 12.25 P.M. All these scales showed expanding melanophores and the order of darkness was

Cl > Br > NO<sub>3</sub> > SCN

- Very little expansion was seen in the NaSCN solution.  
 1.30 P.M. Both the NaNO<sub>3</sub> and the NaSCN solutions had the melanophores contracted. In NaCl they were still widely expanded.  
 2.40 P.M. The melanophores in all four solutions were contracted.

In further experiments of this type the same order of darkness appeared repeatedly. Scales were started from both KCl and

$\text{NH}_4\text{Cl}$ . In such cases the melanophores were completely contracted at the beginning of the experiment. These gave essentially the same results as when the scales were first immersed in sea-water. Whereas the scales in  $\text{NaCl}$  and  $\text{NaBr}$  showed approximately the same degree of darkness at first, the melanophores invariably remained expanded longer in the chloride. Hence, as regards the maintenance of the expansion, the sodium salts clearly show the order



In  $\text{NaSCN}$  the melanophores never showed a very great expansion and the secondary contraction always occurred first in solutions of this salt. Thus in striking contrast to the conditions found in the corresponding series of potassium salts in which the initial effect (a contraction) was identical throughout the series, these salts of sodium vary in their initial effect, the melanophores expanding most widely in  $\text{NaCl}$  and least in  $\text{NaSCN}$ . Degeneration always occurs first in  $\text{NaSCN}$ . As in the case of  $\text{KSCN}$  the epithelium becomes freed at the posterior edge of the scale and eventually the xanthophores entirely disappear. The melanophores lose their irritability completely after about twelve hours in  $\text{NaSCN}$  whereas in  $\text{NaCl}$ ,  $\text{NaBr}$ , and  $\text{NaNO}_3$  they may react to atropine sulphate more than five and a half days (136 hrs.) after removal from the fish. After one hundred and sixty hours in these salt solutions the melanophores and xanthophores were still intact, but no longer irritable. In these experiments no precautions were taken to prevent bacterial infection.

When these results are compared with those obtained with the corresponding series of neutral potassium salts the following differences appear:

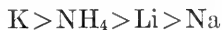
1. Potassium causes a contraction of the melanophores. The time of this contraction is independent of the nature of the anion.
2. Sodium causes an expansion of the melanophores. The duration (and in part the degree) of this expansion varies with the nature of the anion.

3. Degeneration always occurs in the phase opposite to that of the initial one.

*c. Comparative effects of the alkaline chlorides.* After the lyotropic series had appeared so clearly in the case of the anions of the neutral potassium and sodium salts, further experiments were carried out to determine the nature and sequence of the physiological effects of the alkaline metals upon the chromatophores. For this purpose a comparative study was made of the chlorides of these metals. In the earlier experiments sodium, lithium, (ammonium), and potassium were used. Subsequently, through the courtesy of Prof. C. P. Baxter, the chlorides of caesium and rubidium were also secured and compared with the other four salts. Experiments with the first four salts showed the following relationships.

*August 27, 1912*

4:33 P.M. Scales from a 7.5 cm. female were placed in 8 cc. of 0.1 M NaCl, LiCl, NH<sub>4</sub>Cl, and KCl, respectively. These contracted in the order



(T. = 25.5°C.) There were six scales in each set.

4:54 P.M. The xanthophores in the LiCl were contracted but expanded in the other three solutions.

5:40 P.M. The xanthophores were contracted in all but the KCl solution.

5:55 P.M. A relaxation appeared in all the melanophores in NH<sub>4</sub>Cl.

8:30 P.M. The melanophores in KCl showed the beginning of degeneration. In NH<sub>4</sub>Cl the melanophores were partially expanded but without signs of degeneration. The NaCl and LiCl solution both showed contracted melanophores and no sign of degeneration.

12:15 A.M. (August 28, 1912) Marked degeneration appeared in the NH<sub>4</sub>Cl solution.

7:00 A.M. The scales in LiCl showed the melanophores half expanded.

5:15 P.M. The melanophores in NaCl responded to atropine sulphate by expansion (more than twenty-four hours after beginning the experiment).

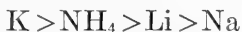
This type of experiment was repeated with entirely uniform results. Although the KCl and NH<sub>4</sub>Cl are clearly distinguish-

able as regards their initial contraction the difference in time required for the secondary degeneration was less obvious. Experiments of the following type were carried out with a view to clearing up this question.

*August 30, 1912*

- 12.25 P.M. Scales from a 7 cm. female were placed in 8 cc. of 0.1 M KCl and  $\text{NH}_4\text{Cl}$ . The melanophores contracted in the KCl sooner than in the  $\text{NH}_4\text{Cl}$  as in previous trials.
- 1.50 P.M. Both sets showed expanded melanophores.
- 3.00 P.M. Degeneration had begun in the KCl. Great expansion but no degeneration appeared in the melanophores of the  $\text{NH}_4\text{Cl}$  set at this time.

From these experiments it eventually appeared that the alkaline cations fall into a regular series as regards (1) their power to contract the melanophores and (2) their susceptibility to degeneration. This series is



KCl contracts the melanophores in the shortest time and in this solution they likewise degenerate most rapidly. NaCl expands the melanophores and they may remain irritable in such a solution for more than twenty-four hours.

Recent experiments with the additional chlorides of caesium and rubidium have shown clearly that the  $\text{Rb}^+$  cation is practically identical with  $\text{K}^+$  in its initial effect upon the melanophores although its time of contraction is shorter than  $\text{NH}_4^+$ . Similarly  $\text{NH}_4^+$  and  $\text{Cs}^+$  are almost indistinguishable although clearly separated from  $\text{Li}^+$ . In the hope of separating the  $\text{RbCl}$  from the KCl effect, experiments were carried out at reduced temperatures. Similar trials were made with  $\text{NH}_4\text{Cl}$  and  $\text{CsCl}$  solutions. None of these attempts succeeded in establishing beyond a doubt the positions of the cations  $\text{Rb}^+$  and  $\text{Cs}^+$  in the alkaline series.

Thus, as regards their contraction effect upon the melanophores the alkaline cations follow the order.



This series is strikingly similar to the familiar sequence



(Höber '11, p. 408).

*d. Comparative effects of magnesium chloride and sulphate.* When scales which have been in a 0.1 M NaCl solution until the melanophores are fully expanded are immersed in a 0.1 M solution of  $MgCl_2$ , a contraction follows. This contraction as in the case of the potassium salts, varies with the temperature of the solution. The question now arose whether salts of magnesium with a different anion would show the same time of contraction as the  $MgCl_2$ . Upon the basis of the proposed theoretical explanation of an independent cation and (secondary) anion action which seems to exist in the case of the five neutral potassium salts, this might have been expected. Experiments were carried out comparing the effects of 0.1 M solutions of  $MgCl_2$  and  $MgSO_4$ . The relatively slight dissociation of  $MgSO_4$  is well known. At this concentration the  $MgCl_2$  is nearly twice (1.7) as highly dissociated as the  $MgSO_4$ . If the contraction effect were a cationic one and dependent upon the number of  $Mg^{++}$  ions in solution the  $MgCl_2$  might be expected to contract the melanophores approximately 1.7 times as rapidly as the  $MgSO_4$  solution.

A brief summary of the experiments follows.

*September 1-3, 1912*

Matched pairs of scales were immersed simultaneously by means of the glass dipper in 0.1 M solutions of  $MgCl_2$  and  $MgSO_4$ . All such scales had previously been treated with 0.1 M NaCl until the melanophores were widely expanded. In no case were the scales in NaCl longer than an hour. Seven different lots of scales were used from females 7.5 to 8.5 cms. long. In fifty successive trials the melanophores of the scales in  $MgCl_2$  did not once contract before those in  $MgSO_4$ . In two cases the contraction times were identical. In the other forty-eight cases the melanophores in  $MgSO_4$  contracted before those in  $MgCl_2$ . The time of contracting in  $MgCl_2$  was divided by the contracting time in  $MgSO_4$  for every pair of scales. This quotient represented the relatively longer time required for the  $MgCl_2$  contraction. For example, two scales were immersed in the solutions at 1.00 P.M.  $MgSO_4$  contracted at 1.21;  $MgCl_2$  contracted at 1.30. Relatively greater con-



tracting time of  $\text{MgCl}_2$ , that is,  $\frac{30}{21} = 1.4$ . The average quotient for these fifty trials was 1.8.

From these figures it is clear that in this case the contraction is not so simple a matter as it seems to be in the potassium salts. Furthermore (1) the cation does not cause the first contraction by its independent action as may be the case with the potassium salts; (2) the nature of the anion has a distinct effect upon the cation; (3) the speed of the reaction is almost (1.8 : 1.7) the reciprocal of the theoretical expectation if the assumption is made that the effect is a function of the number of ions (cations in this case) in solution.

The solutions 0.1 M  $\text{MgCl}_2$  and 0.2 M  $\text{MgSO}_4$  are isionic as regards the number of  $\text{Mg}^{++}$  ions. In fifteen successive trials with matched pairs of scales immersed simultaneously in these solutions the melanophores in  $\text{MgSO}_4$  invariably contracted before those in  $\text{MgCl}_2$ . Here again if the cation action were independent of the nature of the anion, and if it were the cause of this initial contraction (cf. potassium salts) the melanophores should have shown approximately the same time of contraction in the two solutions. This was clearly not the case.

### 3. *Effects of mixed electrolytes*

Since sodium and potassium salts have opposite effects upon the melanophores, experiments were made with mixtures of these salts to determine, if possible, their relative specific strengths.

It was easily shown that equal quantities of potassium and sodium salts do not balance each other in solution. The potassium effect is prepotent.

The proportions and concentrations of both salts were varied in a series of experiments in an attempt to determine the strength at which potassium and sodium would approximately balance each other. It was found that the effect varied according to the previous treatment of the melanophores. Thus, in a solution of 7 cc. of 0.125 M  $\text{NaCl}$  + 1 cc. of 0.125 M  $\text{KCl}$ , contracted melanophores expanded. The  $\text{NaCl}$  seems to predominate. In a solution of 7 cc. 0.125 M  $\text{NaCl}$  + 2 cc. of 0.125 M  $\text{KCl}$  no such

expansion occurred. If scales with completely expanded melanophores were immersed in the former solution a very slow contraction followed. Depending upon the initial condition of the melanophores this solution may thus cause either an expansion or a contraction. If scales were taken directly from a light fish and immersed in this solution, a wide expansion of the melanophores occurred (due, in part, to the removal of the nervous tonus). This was followed at once by a contraction. In the following experiment contracted melanophores could not remain so but their expansion was not complete. Similarly *expanded* melanophores contracted partially.

*February 14, 1913*

- 4.14 P.M. Corresponding scales from the sides of a 9 cm. male were removed to solutions of 0.1 M KCl and 0.1 M NaCl respectively.
- 4.16 P.M. Melanophores contracted in the KCl and expanding in the NaCl solution.
- 4.31 P.M. Three scales from each set were changed to identical solutions (7 cc. 0.1 NaCl + 1 cc. 0.1 KCl).
- 4.51 P.M. A few peripheral melanophores of the set started in KCl showed a slight expansion. All three scales of the other set showed a partial but not a complete contraction.
- 5.06 P.M. All three scales in the KCl set showed all the melanophores partially expanded. All in the NaCl set were partially contracted at this time and slightly darker than the KCl set, though distinctly lighter than the control scales in NaCl.
- 5.36 P.M. The KCl set slightly darker than the NaCl scales. T. = 21.2°C.
- 10.00 A.M. (February 15, 1913) Both sets showed a partial contraction. No difference between them.

Experiments with mixed solutions of other salts of potassium and sodium chloride showed a similar slowing down of the normal potassium contraction by the addition of sodium chloride in a given proportion.

*June 13, 1912*

- 8.40 P.M. Scales from an 8 cm. female were removed to a 0.1 M NaCl solution.
- 9.31 P.M. Six scales were transferred to a solution of 9 cc. NaCl 0.11 M + 2 cc. 0.1 M KSCN.
- 9.41 P.M. All these scales showed the melanophores still expanded.

- 9.56 P.M. Some melanophores were contracting in all six scales.  
 10.34 P.M. The control scales in 0.1 M NaCl showed widely expanded melanophores.  
 8.45 A.M. (June 14, 1912) All the melanophores in the mixture were expanded but incompletely so. This was not the normal degeneration expansion of KSCN for the melanophores retained their contour and a scale brought into 0.1 M KCl showed a slight contraction in a few melanophores.

In NaCl solutions the melanophores normally degenerate from the contracted phase (p. 550). In the following experiment the potassium is propotent at the beginning of the experiment but the end effect is that of a sodium salt.

*June 13, 1912*

- 3.28 P.M. Scales from an 8 cm. female were placed in 0.1 M NaCl.  
 4.31 P.M. Scales were transferred to a solution of 8 cc. 0.5 M NaCl + 2 cc. M KSCN. All the melanophores were contracted in three minutes.  
 10.34 P.M. These were unchanged.  
 9.00 A.M. (June 14, 1912) No change. At noon they showed no response to a ten minute immersion in 1 per cent atropine sulphate solution.

From this and the preceding experiment it is evident that the addition of a certain amount of NaCl to a solution of a potassium salt inhibits the normal degeneration (cytolysis) which has been assumed to be the effect of the anion (p. 547). This action of the NaCl is brought out even more strikingly in the following experiment.

*May 1, 1912*

Scales from an 8 cm. female were immersed in a 0.1 M NaCl solution. 30 minutes after beginning the experiment. Three sets of four scales each were transferred to the following solutions.

A. 2 cc. KBr 0.2 M + 4 cc. water.

B. 2 cc. KBr 0.2 M + 2 cc. 0.1 M NaCl + 2 cc. water.

C. 2 cc. KBr 0.2 M + 4 cc. 0.1 M NaCl.

(The concentration of the KBr is the same, 0.066 M, in all three solutions).

- 45 minutes. All three solutions showed the xanthophores widely expanded and the melanophores contracted with a few stumpy processes. All the scales were in the same condition at this time.

- 2 hours 45 minutes. Conditions were as follows:  
 A. Both melanophores and xanthophores expanded—  
 melanophores slightly.  
 B. No change detected.  
 C. All chromatophores contracted.
- 17 hours 30 minutes. A. Chromatophores completely disintegrated.  
 B. Xanthophores partially contracted(?). Melanophores  
 contracted as before.  
 C. Xanthophores contracted, melanophores about half ex-  
 panded but no sign of degeneration.  
 Single scales from B and C when transferred to a 0.1 M  
 NaCl solution showed complete expansion of the mel-  
 anophores after nine minutes.
- 22 hours. Conditions were as above except that in B, the xanthophores  
 were distinctly more contracted than in C.
- 24 hours. Single scales from B and C were again transferred to a 0.1  
 M NaCl solution. After twelve minutes the scale from  
 B showed widely expanded melanophores while those of  
 the scale from C were only partially expanded. This  
 difference was obvious after seven minutes immersion in  
 the NaCl.
- 26 hours 45 minutes. The two remaining scales in B showed the mel-  
 anophores to be about half expanded. In one of the two  
 scales in C they were very much expanded—the other was  
 unchanged.
- 41 hours. All the chromatophores in both B and C were degenerating.

This same protective action of the NaCl is clearly seen with  
 other salts of potassium also. Thus:

*May 1, 1912*

- Scales from an 8 cm. female were immersed in a 0.1 M NaCl solution.  
 2 hours 30 minutes after beginning the experiment. 2 sets were trans-  
 ferred to A, 4 cc, 0.1 M NaCl + 2 cc. 0.2 M KI, and  
 B, 6 cc. M. 0.066 KI respectively. The melanophores  
 of both sets were contracted in approximately seven and  
 one-half minutes. The xanthophores of A expanded  
 when first immersed.
- 3 hours. The xanthophores of A were obviously contracting.
- 3 hours 20 minutes. All the chromatophores were expanding (degener-  
 ating) in B. In A all were contracted at this time.
- 4 hours. The scales in B showed the chromatophores to be badly dis-  
 integrated. In A the xanthophores were contracted and  
 the melanophores half expanded but there was no sign of  
 degeneration.

This last experiment illustrates a peculiarity of certain mixtures of Na and K solutions which appeared repeatedly. Whereas the melanophores are contracted in such solutions the xanthophores contract also. The former is a typical potassium reaction and the latter a typical sodium reaction. This suggests that these two salts may act selectively upon the two types of chromatophores, the melanophores responding more readily to the potassium stimulus and the xanthophores to the sodium.

Experiments of the following type are of interest in their bearing upon the question of a deferred antagonism between relatively large quantities of potassium salts and small amounts of sodium salts.

*June 12, 1912*

- 8.33 A.M. Scales from a 7.5 cm. female were immersed in a 0.1 M NaCl solution.
- 8.48 A.M. 3 scales were changed to A, 10 cc. 0.2 M KSCN + 1 cc. M NaCl and B 10 cc. 0.2 M KSCN + 1 cc. water respectively. The melanophores of both sets were contracted in one minute and fifty seconds.
- 9.14 A.M. All the melanophores were contracted in A but expanding in B.
- 10.25 A.M. Conditions in A unchanged; in B degeneration had begun.
- 10.55 A.M. Degeneration was very far advanced in KSCN B but in A the melanophores were contracted.

This and numerous other similar experiments with different neutral salts of potassium have shown clearly that the addition of NaCl to a solution of a potassium salt in such quantity as not perceptibly to effect the initial contracting stimulus of the potassium salt, may nevertheless protect the melanophores against what has been clearly shown to be the deleterious (cytolytic) action of its anion (a deferred antagonism). This protective action appears even though the sodium and potassium salt have the same anion. The experiments with the neutral salts of potassium have demonstrated quite clearly that the rapidity of the degeneration varies with the anion. The conclusion therefore follows that in this case the melanophores are protected against the cytolytic action of the anions of potassium salts by the addition of small quantities of a sodium salt (chloride or

bromide) and since the anions of the two salts may be identical it seems highly probable that the  $\text{Na}^+$  cation antagonizes the anion of the potassium salt.

These experiments with mixtures of potassium and sodium salts have thus shown that:

1. The specific effect of potassium is approximately 7 times as great as that of sodium (KCl vs. NaCl at 0.1 M concentration.)

2. The nearest approach to a balanced solution is one that will both expand contracted and contract expanded, melanophores, depending upon their previous treatment. This expansion and contraction is never so great as in the corresponding pure solutions of NaCl and KCl.

3. The melanophores retain their irritability longer in such mixtures than in pure solutions of either constituent.

4. The chromatophores may react to two salts of a mixture simultaneously—the melanophores being contracted by the potassium and the xanthophores by the sodium salt.

5. The addition of an amount of sodium salt which does not appreciably affect the initial contraction of a potassium salt solution may effectively protect the melanophores against the secondary degeneration which is normally brought about by the cytolytic action of the potassium anion. This result suggests an antagonism between cation and anion of different salts.

### 3. PRESSURE EFFECTS

Upon purely theoretical grounds v. Frisch ('11 a) claimed that, since the stimulated phase of the vertebrate melanophores is certainly the contracted one, it would be very surprising to find that the pressure stimulus caused an expansion as several of the older investigators have thought to be the case. He drew a dissecting needle lightly across the surface of both living and dead minnows (*Phoxinus laevis*, L.). The effect was exactly comparable to marking with a pencil upon a sheet of white paper. Examination under the microscope showed the melanophores to have been destroyed along the path of the needle and the black effect to be due to melanin granules which had diffused from the

ruptured melanophores. Thus, what was formerly considered a pressure stimulus he proved to be merely a mechanical diffusion of the pigment and not a true expansion of the melanophores.

v. Frisch does not conclude from these results that the melanophores can not react to a mechanical stimulus. He merely states that if they should react it would be by a contraction, not an expansion.

It was a simple matter to prove, in the case of *F. heteroclitus* the correctness of the conclusion of v. Frisch ('11 a) regarding the supposed 'pressure-expansion' effects of the older investigators (v. Siebold '63, etc.) A needle drawn lightly over the surface of this fish showed an effect similar to the one described by v. Frisch ('11 a) for *Phoxinus*. Subsequent examination with the microscope disclosed many ruptured melanophores and structureless masses of diffused pigment granules which constituted the dark line upon the surface of the fish.

Further experiments with a blunt glass needle and varying degrees of pressure showed conclusively that the pressure stimulus is a contracting one for the melanophores. Scales were immersed in a 0.1 M NaCl solution and the melanophores allowed to expand completely while the xanthophores contracted before the stimulus was applied. With careful manipulation and using scales upon which the melanophores were comparatively far apart (from the lateral portion of the fish) the stimulus could even be applied to a single melanophore. The same melanophore responded repeatedly to the pressure stimulus provided it was not too violent. In such cases the xanthophores within the stimulated area always expanded. Similar experiments performed upon scales immersed in olive oil showed a prompt contraction of the melanophores when stimulated but in this case they did not reexpand as in NaCl. Under normal conditions the melanophores remain expanded in olive oil for some hours. This is probably not due to any specific expanding factor in the oil but merely to the absence of a contracting stimulus. When the nervous inhibition is removed the pigment always migrates distally. This is doubtless the cause of the expansion in olive oil. That this is actually the case seems to be further proved by

the fact that here the pressure effect is practically irreversible. A complete recovery never occurs as it does in the NaCl solution.

Finally experiments were made with the scales immersed in sea-water. Here again the pressure stimulus overcame the expanding tendency of the combined hypertonic medium and the specific effect of NaCl (see p. 533 for the effects of sea-water).

In all these experiments the scales were placed in open Syracuse glasses and the temperature remained constant. The stimulus was applied instantaneously in order to avoid any possibility of reducing the normal oxygen supply. Hence the three factors which are ordinarily so intimately associated with the effect of pressure, that is, access of oxygen, temperature changes, and central nervous inhibition, were all eliminated. Under these conditions pressure causes a contraction of the melanophores and an expansion of the xanthophores in a 0.1 M solution of NaCl. A contraction of the melanophores was also observed in sea-water and olive oil following a pressure stimulus.

#### 4. TEMPERATURE EFFECTS

Until quite recently there has been a remarkable uniformity of opinion among investigators concerning the influence of temperature changes upon the chromatophores. Elevation of temperature was followed by a contraction of the chromatophores while a lowering was claimed to bring about their expansion. Since most of the experiments have been performed upon living animals the usual difficulties of possible psychic stimuli, central nervous influences, etc., have detracted somewhat from the reliability of the conclusions.

The question of a direct stimulation of the chromatophores and the nature of such a reaction is by no means decided.

v. Frisch ('11 b) lays particular stress upon the smaller quantity of absorbed oxygen in a fluid at a high than at a low temperature. Upon this basis he has explained his results with the dead *Phoxinus laevis* in which the higher temperature caused a rapid contraction of the melanophores. The possibility of an additional contracting stimulus due to the heat per se has been



neglected by this author. The problem, upon last analysis, consisted of the separation of a specific heat effect from that obtained by heating the fluid surrounding the chromatophores, and thus driving off a certain amount of the absorbed oxygen.

Two types of experiments were carried out with the scales of *F. heteroclitus* each one being repeated a number of times with uniform results.

*April 11, 1912*

Scales from a 7 cm. female were placed in a 0.1 M NaCl solution until fully expanded. A number of these were now removed to a 3-inch specimen bottle (fig. B) filled with 0.1 M solution of NaCl. This bottle was then closed with a one-hole rubber stopper, care being taken to eliminate every trace of air. A thermometer was now easily inserted into the salt solution through the hole in the stopper and when the apparatus was completed the bottle was without an air-bubble. This bottle was now immersed in warm water until the temperature of the salt-solution had risen to 32°C. At this temperature all the melanophores were completely contracted. The bottle was then removed to cold water until the thermometer within registered 19°C. Even before this temperature was reached the melanophores had begun to expand and at 19°C. this expansion was practically complete. This procedure was repeated ten times on the same set of scales and ten distinct contractions and ten expansions were observed in this way. Such changes in the melanophores were perfectly obvious to the naked eye. At the end of the experiment the bottle was still entirely free from air-bubbles. Sixteen hours after the last expansion the pigment of the melanophores were still active.

In this experiment it is clear that (1) all central nervous system and hence psychic influences were absent; (2) there was no possibility of a loss of oxygen in the salt solution since the bottle was absolutely air tight. Hence it follows that this contraction of the melanophores at 32°C. must have been the result of a specific heat stimulus.

A second type of experiment was as follows.

*April 9, 1912*

A specimen bottle containing about 10 cc. of a 0.1 M NaCl solution was immersed in a water bath in which the temperature was kept constant (at 26°C.) by circulation from a tank regulated by a gas thermostat. After the temperature of this NaCl solution had become constant, scales from a 7.5 cm. female were placed in the specimen bottle. This

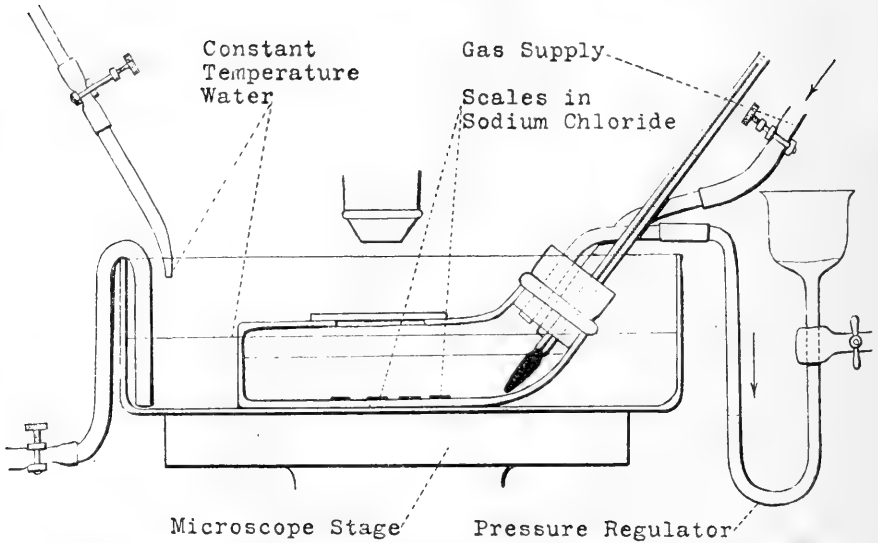


Fig. B Diagram of apparatus for testing the reaction of melanophores to oxygen, to differences of temperature, etc.

was now closed by a three-hole rubber stopper and a stream of pure oxygen was run into the bottle. The oxygen pressure over the salt-solution was kept slightly above one atmosphere by means of a short thistle-tube manometer in which a few cubic centimeters of a 0.1 M NaCl solution served as a valve. The temperature did not vary more than  $\pm 0.25^{\circ}\text{C.}$  during the first forty-five minutes. The melanophores, with a few doubtful exceptions, remained widely expanded throughout this period in all the scales. At the end of forty-five minutes the temperature was gradually raised. The oxygen stream continued to flow. Fifteen minutes later (one hour after the beginning of the experiment) the temperature had risen to  $29^{\circ}\text{C.}$  and all the scales were light with contracted melanophores. The specimen bottle was now removed to cold water. Two and one-half minutes later the temperature had fallen to  $23^{\circ}\text{C.}$  and expansion was well under way in all the melanophores. One hour and five minutes after beginning the experiment all the scales were again dark.

The amount of oxygen absorbed by 10 cc. of water in an atmosphere of oxygen at  $30^{\circ}\text{C.}$  (0.26 cc.) is more than four times as great as the amount (0.06 cc.) absorbed at  $20^{\circ}\text{C.}$  by 10 cc. of water exposed to air (Winkler, '89). Although the actual quantity of oxygen absorbed by a solution of 0.1 M NaCl is less per volume than in water, the same relative conditions obtain (Geffcken '04). In other words at  $30^{\circ}\text{C.}$  in an atmosphere of oxygen a saturated 0.1 M NaCl solution contains more than four times as much oxygen per unit volume as a similar solution exposed to the

air at 20°C. In passing from 18 to 30°C. the change in the percentage of dissociation is less than 0.4 per cent (Noyes and Coolidge '03, p. 374). This error is therefore negligible.

In the above experiment it follows that the contraction of the melanophores could not have been due to an elimination of oxygen from the solution since it occurred at 29°C at which temperature a 0.1 M NaCl solution (under an oxygen pressure of 760 mm.) contains more than four times as much oxygen as a 0.1 M NaCl solution exposed to the air at 20°C. in which the melanophores remain expanded for hours. The change of the dissociation value is negligible. Consequently in this case also, as in the first type of experiment the contraction of the melanophores must be interpreted as a response to the direct, specific stimulation of heat.

These experiments have thus proved (1) that at about 30°C., heat acts as a specific contracting stimulus upon the melanophores; (2) that this contraction is independent of the oxygen content of the fluid in which the melanophores are immersed; (3) that at about 30°C. this specific heat effect antagonizes the opposite effect of 0.1 NaCl.

##### 5. REACTIONS TO OXYGEN

In the experiments with the effects of temperature upon the melanophores of *F. heteroclitus* it was clearly shown that heat causes a contraction. It remained to be proved (1) whether the absence of oxygen would also bring about a contraction and reciprocally, (2) whether the presence of oxygen could actually bring about an expansion of the melanophores from the contracted condition.

*April 16, 1912*

Scales from a 9.5 cm. female were immersed in a 0.1 M NaCl solution. Thirty minutes later scales were removed to two identical specimen bottles each containing about 10 cc. of a 0.1 M NaCl solution. A current of washed hydrogen was now run into one of these bottles. The other bottle served as a control. All the melanophores under the hydrogen atmosphere were completely contracted at the end of thirty minutes. In the control they were fully expanded. The stopper was now removed and the contents were poured (solution and scales with

contracted melanophores) into an open Syracuse glass in the air (any mechanical stimulus due to this manipulation would have caused a contraction). All the melanophores began expanding at once. After six minutes, the expansion was well advanced. Forty minutes later the scales in the air showed completely expanded melanophores and the control set which had been attached to the hydrogen generator when the first set was removed, showed a complete contraction.

The hydrogen used in these experiments as in all others, was obtained from the interaction of metallic (electrolytic) sheet aluminum and a 20 per cent solution of chemically pure KOH made up with distilled water. Before coming in contact with the salt solution the gas was passed through two towers of moist glass beads to prevent any possibility of a transfer of KOH. The water in these towers was tested with phenolphthalin and the salt-solution was similarly tested at the close of the experiment. Neither the NaCl solution nor the water of the second tower gave a hydroxyl test.

The above experiment was repeated a number of times and, with the exception of variations in the time of the response to the absence of oxygen (for example, April 26, 1912; "melanophores contracted in the hydrogen atmosphere in eighteen minutes") the results were the same. In every case the complete elimination of oxygen caused a contraction of the melanophores.

Since the absence of oxygen can overcome the specific expanding effect of a 0.1 M NaCl solution it seemed possible that melanophores which had been contracted in a 0.1 M KCl solution might be reciprocally expanded in an atmosphere of oxygen. The results of such experiments were entirely negative. Other experiments with mixtures of potassium and sodium salts in which there was just enough potassium to keep the melanophores contracted showed no expansion of the melanophores after long exposures to an oxygen atmosphere.

These last experiments suggest that possibly oxygen may act merely in a passive manner and not as a specific expanding stimulus in the same sense that NaCl, atropine sulphate, and methylene blue cause an expansion of the melanophores.

The results of these experiments show (1) that the melanophores are unable to remain expanded in the absence of oxygen;

(2) that this tendency towards a contraction is greater than the specific expanding effect of a 0.1 M NaCl solution and (3) that oxygen differs from NaCl in that it probably acts not as a specific expanding stimulus but passively, since it was found that an expansion of contracted melanophores could not be induced even after a long exposure to an atmosphere of pure oxygen.

#### 6. LIGHT EFFECTS

v. Frisch calls attention to the present unsatisfactory condition of the evidence for the nature of the reaction of melanophores to light. In some cases (trout, v. Frisch '11 a) they absolutely fail to respond; in others (eel, Steinach '91; triton, Hertel '07) they are contracted, and again (chameleon, Brücke '52; *Phrynosoma*, Parker '06; *Crenilabrus*, v. Frisch '12) they may expand upon illumination.

While attempting to photograph, by the light of an arc-lamp, the widely-expanded, living melanophores of *F. heteroclitus* it was found that before the exposure could be made the melanophores had almost completely contracted. The scales were immersed in a NaCl solution at the time of the exposure and when they were removed to the stage of a microscope illuminated by an ordinary gas lamp, the melanophores showed a perfectly distinct reversal of the pigment migration. After a few minutes (depending upon the length of exposure to the arc-light and the strength of the salt solution) all the melanophores were again completely expanded. If these same scales were now exposed a second time to the action of the arc-light, the same reactions followed in the same sequence.

Assuming this contraction in the NaCl solution to have been due to a specific light stimulus, experiments were carried out with NaCl solutions of varying strengths and varying exposures to different intensities of light in the blue-violet region (439–490  $\mu\mu$ ) of a spectrum in an apparatus similar to that described by Day ('11). In all of these experiments the light was reflected from the plane mirror of a compound microscope exactly as in normal field-illumination. Control scales from the same region of the body of the same fish were observed from time to time.

These were illuminated during the actual period of observation by means of a 16 c.p. electric light which had been found to have no visible effect upon the melanophores. This was so arranged as to preclude any possibility of its being a source of error in the spectral illumination.

In no case was a contraction of the melanophores observed which could be unequivocally attributed to the action of the monochromatic light.

It seemed possible that the contraction observed in the earlier experiments might have been due to ultra-violet light from the arc-lamp. Through the courtesy of Mr. W. T. Bovie the question of this possibility was definitely decided.

The source<sup>7</sup> of light for these experiments was a crystal-quartz mercury-vapor lamp. This was placed below the stage of the microscope and a copper jacket was slipped over the lamp-tube with an opening so arranged as to fall immediately below the center of the stage. At the side of this opening an ordinary 'wing-top' or 'fish-tail' burner tip was fastened. Through this a blast of air was blown throughout the experiments. This prevented any possible heat effects. As an additional precaution a thermometer lay within the illuminated field in which the temperature never rose above 23°C.

After the melanophores had been completely expanded in a 0.1 M. NaCl solution in the usual way the scales were exposed to the ultra-violet rays in a cell, the bottom of which consisted of a piece of crystal quartz 1 mm. in thickness, and 4 cm. from the lamp. Repeated exposures of scales placed face up in the solution, that is, with the bony plate of the scale interposed between the source of light and the melanophores, gave no reaction. When, however, the scales were inverted, that is, turned with the melanophores towards the light, in this case the quartz bottom of the cell and the thin epidermis being the only possible sources of interference, a very definite reaction followed.

<sup>7</sup> This lamp was made by Mr. Bovie of Heraeus crystal quartz tubing 8 mm. in diameter with walls 1 mm. thick. The arc was 10 cm. long and the amperage 1.25. The wave-lengths were in the region 185-290  $\mu\mu$ .

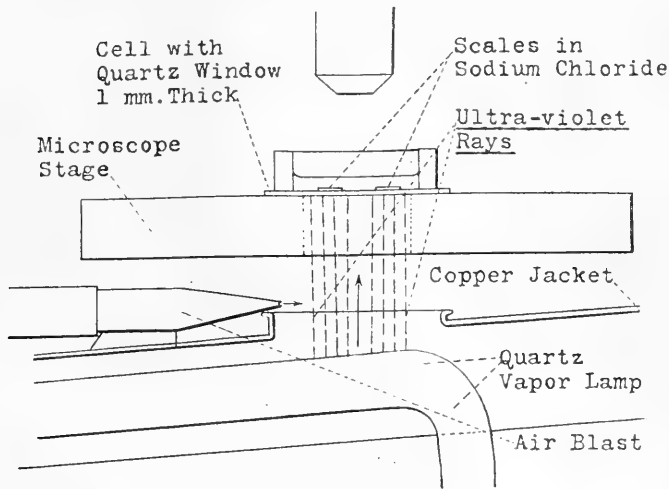


Fig. C Diagram of apparatus for subjecting melanophores to ultra-violet light.

*May 7, 1912*

- 2.15 P.M. Scales from an 8 cm. female were immersed in 0.1 M NaCl.  
 2.35 P.M. 4 scales were exposed to ultra-violet light; 2 inverted and 2 face up. (4 control scales were similarly arranged without exposure to the ultra-violet light). All those scales were immersed in a 0.1 M NaCl solution during the exposure. After 7 minutes both inverted scales in ultra-violet light showed a decided contraction of the melanophores. Neither face-up scale in ultra-violet light showed any contraction. After twenty-one minutes a very decided contraction was visible in both inverted scales in ultra-violet light. The face-up scales showed a very slight contraction at 2 points on the periphery at this time. All four control scales had completely expanded melanophores throughout the experiment. These 8 scales were now fixed in 95 per cent alcohol (Plate 4).

The failure of the response in the scales which were turned face-up suggested that the ultra-violet rays might be absorbed by the bony plate of the scale exactly as they are absorbed by glass. In order to test this point an experiment similar to the last was performed but in this case a thin cover-glass was placed at the bottom of the cell between the scales and the quartz-bottom.

Eight scales were used, four were exposed and four were controls. In each case two of the four were placed face up and two were inverted. The exposure lasted twenty-one minutes and at the end of this time not a single illuminated scale showed any sign of a contraction in the melanophores.

Finally experiments were made to test the reversibility of this ultra-violet light response.

*May 7, 1912 (continued)*

Scales from the 2.15 P.M. set were exposed in a quartz-bottomed cell with an air-blast as before. The exposure began at 3.40 P. M. After ten minutes a very noticeable contraction could be seen in the melanophores of the inverted scales. The cell was now removed and, without touching the scales, it stood exposed to normal daylight. The melanophores of both inverted scales began expanding at once and this expansion was in exactly the reverse order of the contraction by light, that is, it began at the periphery and worked towards the post-median region where the light had first contracted the melanophores. The melanophores which had shown the first response to light were thus the last to recover. Ten minutes after removal from the light about one-half of the melanophores were partially expanded (fig. 2, pl. 4). One scale was now fixed. In the other inverted scale the melanophores showed a complete expansion in twenty minutes (fig. 4, pl. 4).

Possible sources of error might be sought in these experiments (1) in an evaporation of the NaCl solution and (2) in an increase in the dissociation of the NaCl due to the ultra-violet light. Since both of these conditions would actually favor an expansion of the melanophores their contraction in ultra-violet light could not be explained on this basis.

From the above experiments it seems probable that the melanophores, when immersed in NaCl solutions of different concentrations, are not sensitive to exposures to varying intensities of visible light in the region of wave-length 430–490  $\mu\mu$ . When exposed to ultra-violet light (185–290  $\mu\mu$ ) in a 0.1 M NaCl solution, a rapid and reversible contraction of the melanophores follows. Thus, under these conditions, light does not act as a direct stimulus upon the melanophores except in the ultra-violet region of the spectrum.



## 7. ELECTRICAL EFFECTS

The following authors have recorded a contraction of the chromatophores following an electrical stimulation. Brücke ('52) in excised pieces of the skin of the chameleon; Buchholz ('63) in the tails of Cyprinodon fishes (quoted by v. Rynberk '06 b, p. 530); Pouchet ('71) in *Cottus* embryos (quoted by v. Rynberk '06 b, p. 533); Bert ('75) in pieces of the skin of chameleon; Krukenberg ('81) in flat-fishes (Pleuronectidae); Lode ('90, p. 133), in pieces of the fins of medium-sized trout; Biedermann ('92) in the frog (*Rana*, p. 493); v. Rynberk ('06 a) in *Solea* and *Rhomboidichthys* species (quoted by v. Rynberk '06 b, pp. 549-550); Mayerhofer ('09) in *Esox lucius* Lin.; Winkler ('10) in the toad (*Hyla*) by an induction current;<sup>8</sup> v. Frisch ('11 a) in *Phoxinus laevis* L., upon stimulation of the medulla and v. Frisch ('12) in *Trigla corax* and *Crenilabrus pavo* by local stimulation.

The melanophores of *F. heteroclitus* readily respond to an induction stimulus. If this be applied at the medulla just posterior to the cerebellum, a rapid lightening of the fish follows immediately. Other experiments were carried out with scales immersed in NaCl solutions in the usual way until the melanophores were all completely expanded. The stimulus was here applied to the bony portion of the scale. The time of the contraction varied with the strength of the salt solution. When the scales were immersed in sea-water no response followed.

*August 8, 1912*

A Harvard inductorium with closed secondary (one Mesco 'A' dry cell) produced a complete contraction of the melanophores of scales in 0.25 M NaCl solution in thirty seconds and this was reversible. A thirty second stimulus of scales in 0.5 M NaCl solution showed only a very slight and doubtful contraction. In sea-water trials with one, two and three dry cells (in series) and closed secondary gave no reaction. The melanophores remained fully expanded.

<sup>8</sup> Winkler records an expansion of the pigment when stimulated by a galvanic current.

August 8, 1912

- 3.00 P.M. Scales from a dark, 8 cm. female were removed to seawater.  
3.06 P.M. Scales were stimulated with a "Harvard" inductorium, with closed secondary and one dry cell. A stimulation of one and one-half minutes had no appreciable effect.  
4.00 P.M. Another scale stimulated for one minute showed no response.

From these observations it is clear that the conditions in *F. heteroclitus* are essentially the same as in other teleost fishes as regards the response to the induction current. NaCl tends to antagonize the specific contracting effect of the current. This is shown both by experiments with strong (0.5 M) NaCl solutions and by the failure of the melanophores to respond to the electrical stimulus when immersed in sea water.

#### 8. CONCLUSION AND SUMMARY

From these experiments with the chromatophores of *F. heteroclitus* it seems certain that, in addition to the normal pigment migrations which occur in the living fish under the influence of the nervous system, the chromatophores are sensitive to a variety of physiological stimuli which act directly upon the cells. Even the possibility of an intercalated sympathetic reflex-arc seems to be eliminated when the experiments with the various salt solutions and ultra-violet light are considered. In the former case the melanophores invariably showed a reaction which began at the periphery of the scale and proceeded centripedally. Obviously the torn ends of the sympathetic nerve-fibers were exposed to the action of the solutions. Had such broken nerve-endings been stimulated, a synchronous reaction should have followed in the chromatophores. This never occurred. Again in the case of the exposures to ultra-violet light the reaction always began centrally, where the epithelium is thinnest, and proceeded peripherally. The light rays stimulated the melanophores most readily at the centre of the scale. This response, as in the former case, must consequently be considered a direct one (compare figs. 1-5, pl. 1 with figs. 1 and 4, pl. 4).

The preservation of the irritability of the chromatophores in sea-water is in keeping with Loeb's view of a physiologically balanced solution (Loeb '03, p. 409). Upon dilution of the sea-water the specific NaCl effect appears in an expansion of the melanophores. Loeb has recently found ('12) that the toxicity of ions varies with the concentration of the solution. Thus, for example in small concentrations the protective action of  $\text{Ca}^{++}$  is greater than that of  $\text{Na}^+$  but from a definite concentration on,  $\text{Na}^+$  becomes less harmful than  $\text{Ca}^{++}$ . The antagonism of the constituents of sea-water may vary in a similar way which would account for the appearance of the Na expansion in dilute sea-water.

Loeb ('10) found that a solution of KCl of the same concentration as in sea-water, killed *Fundulus* embryos in two days though they lived indefinitely in a NaCl solution of the sea-water concentration. Toxic KCl solutions were rendered harmless by the addition of NaCl in definite quantity. This antagonism was between the cations  $\text{Na}^+$  and  $\text{K}^+$  and not between  $\text{K}^+$  and  $\text{Cl}^-$ .

More recently ('11) Loeb has determined quantitatively the 'Entgiftungskoeffizient' of  $\frac{\text{KCl}}{\text{NaCl}}$  for several concentrations and has found it to be approximately 1/17. He states (p. 469) that some investigators (W. Koch and A. P. Matthews) still claim that the antagonism is always between ions of opposite charges. In this case Loeb considers the antagonism to be between the cations.

Koch ('09, p. 434) states that Loeb overlooked the importance of the anion action when he first discovered the Na-Ca antagonism. The foregoing experiments upon the chromatophores in salt solutions seem to furnish evidence in favor of both views. The neutral series of K salts showed two constant reactions; (1) a contraction and (2) a cytolytic degeneration. The time of the first contraction was the same for isionic solutions of the five neutral salts (p. 547). The time required for the beginning of the degeneration varied with the anion. Since the number of  $\text{K}^+$  ions in all five solutions was identical this result suggested a specific cation (1) and anion (2) effect. It furthermore sug-

gested that an antagonism exists between the  $K^+$  cation and the five anions, which is greatest in the case of the chloranion and least effective with  $SCN^-$ .

A comparison of the chlorides of the six alkalis showed that  $K^+$  and  $Na^+$  are at the extremes of the cation series,  $K^+$  being the most effective contracting stimulus and least effective in its antagonism of the secondary (cytolytic) degeneration. Potassium was found to be approximately seven times as potent in its specific effect as sodium. This was clearly a case of cation antagonism. Thus (1) mixtures of the salts of K and Na showed a definite antagonism of the cations (in agreement with Loeb, '10, '11) and (2) individual neutral salts of K seemed to show an antagonism of cation and anion which varies with the nature of the latter (a distinct influence of the anion as is claimed by Koch '09, and others).

Other experiments with Na and Mg salts showed perfectly clearly that in these cases the first effect (presumably that of the cation) was influenced by the nature of the anion (NaCl vs. NaSCN, p. 549 and  $MgCl_2$  vs.  $MgSO_4$ , p. 553). Na is only about one seventh as potent, specifically, as K. It therefore seems possible that in the case of the contractions which occur in the five neutral salts of K any specific retarding effect of the anion is obscured because of the relatively powerful specific effect of the  $K^+$  ion. Upon this basis the greater activity of NaCl as compared with NaSCN could be explained since in the latter case Na (the least potent contracting stimulus) is coupled with  $SCN^-$ —the most toxic (cytolytic) anion of the series.

The relatively greater activity of  $MgSO_4$  as compared with  $MgCl_2$  is possibly associated with the light dissociation of the former salt. In some respects  $MgSO_4$  shows the characteristics of a lipoid solvent (slight dissociation and narcotic action) but the xanthophores, which contain a lipochrome pigment that dissolves readily in alcohols, etc., remain intact in a  $MgSO_4$  solution for many hours.

Two types of antagonism have been suggested to explain the results of the foregoing experiments with K and Na salts, individually and in combination. In the first of these the antagonism

is between cation and anion of the same salt (Na and K salts); in the second, between different cations of two salts in which the anion may be common. Other experiments with mixtures of Na and K salts have shown that in a solution of a K salt in which the Na salt is present in such quantities as not appreciably to effect the initial K contraction, the presence of the Na may nevertheless appear in its protective action against the cytolytic effect of the K anion, there is thus a third type of antagonism between the cation of one salt and the anion of another (p. 558).

At the extremes of the alkaline cation series, the initial effects of the chlorides of K and Na upon the two types of chromatophores are reciprocal. LiCl, which lies between K and Na, combines the contracting effect upon the melanophores (a specific K effect) with the contraction of the xanthophores (a specific Na effect). It is thus physiologically intermediate between K and Na, showing the effect of K on one type of chromatophore and that of Na on the other. The time required for the contraction in a LiCl (p. 550) solution recalls the retarding effect seen upon adding certain amounts of NaCl solutions of K salts. In such solutions both types of chromatophores were contracted also (p. 557).

The reciprocal specific action of Na and K salts and their mutual antagonism, has frequently appeared in experiments upon other tissues (cf. Zoethout '02; '08). The recent work of Ishikawa ('12) shows conclusively that the effects of Na and K salts upon the chromatophores are not to be compared with the reactions of amoebae to these stimuli. In the later case isotonic solutions produced identical and not reciprocal effects.

Steinach ('92), Franz ('06), Hoffmann ('07) and others have called attention to striking analogies between the responses of the melanophores of fishes and certain types of smooth muscle (sphincter pupillae and the radial muscles of the chromatophores in cephalopods). A series of experiments upon the reactions of the sphincter pupillae and the stomach muscle of *Fundulus* to salt solutions (K and Na) have shown that this analogy fails when carried too far.

The anion sequence (p. 547 and pls. 2 and 3) is the familiar one of Hofmeister ('91), Pauli ('99), Schwarz ('07), Port ('10) and others (Höber, '11, pp. 353-354, 409, 399).

Overton ('04) found that the chlorides of the alkalis preserved the irritability of fresh frogs' muscle in the cation order



With the doubtful exception of  $\text{Cs}^+$  and  $\text{Rb}^+$  this is precisely the order in which the melanophores of *Fundulus* retain their irritability in the chlorides of these metals. Höber ('11, chapt. 10) emphasizes the correlation which this series has brought out between irritability and the state of colloidal aggregation.

In addition to the foregoing experiments upon *F. heteroclitus* the chromatophores of several other species of teleost fishes were tested in solutions of NaCl and KCl.<sup>9</sup> Although none of these species proved so favorable as *F. heteroclitus*, the results showed that the physiological responses of the melanophores are essentially the same in all the forms examined. It therefore seems highly probable that the melanophores of all color-changing teleosts are sensitive to direct physiological stimuli of the type described in this paper.

The results of these experiments upon the responses of the chromatophores of *F. heteroclitus* may be briefly summarized as follows:

1. The two types of chromatophores (melanophores and xanthophores) are normally under the control of the sympathetic nervous system.

2. When scales, bearing the superficial layer of chromatophores, are removed from the fish to sea-water, there is practically no change in the position of the melanophore pigment (p. 533). Distilled water causes a contraction, dilute sea-water

<sup>9</sup> Such experiments were carried out upon the following species—*F. majalis* Walbaum (May Fish); *Cyprinodon variegatus* Lacepede (Pursy Minnow); *Palinurichthys perciformis* Mitchell (Black Rudderfish); *Morone americana* Gmelin (White Perch); *Centropristes striatus* Lin., (Black fish); *Tautoglabrus adspersus* Walbaum (Cunner); *Tautoga onitis* Lin., (Tautog); *Eupomotis gibbosus* Lin., (Freshwater Sunfish).

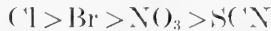
an expansion, of the melanophores. This expansion is due to the specific effect of NaCl (p. 540, pl. 1, figs. 1-6 and p. 547). The irritability is maintained longer (>eighteen hours) in sea-water than in distilled water (one hour) (p. 541 and summary of section 2, 1, p. 543).

3. The neutral salts KCl, KBr, KNO<sub>3</sub>, KI and KSCN all cause a contraction of expanded melanophores and an expansion of the xanthophores which is secondarily followed by a cytolytic degeneration. The time required for the beginning of this degeneration varies in the order



(p. 547 and pls. 2 and 3). The first contraction of the melanophores is probably specific for the K<sup>+</sup> ion (p. 547). There is apparently an antagonism between cation and anion of the same salt in these cases which varies with the nature of the anion.

4. The neutral salts NaCl, NaBr, NaNO<sub>3</sub> and NaSCN produce an expansion of contracted melanophores. The duration of this expansion is greatest in NaCl and least in NaSCN. The other Na salts show an intermediate expansion in the order



Degeneration appears first in the NaSCN. In the other solutions the chromatophores may retain their irritability for more than five days after removal from the fish. The expansion is the specific effect of the Na<sup>+</sup> ion. In contrast to the neutral-salts of potassium the cationic effect is distinctly influenced by the nature of the anion.

5. The alkaline chlorides KCl, (RbCl), NH<sub>4</sub>Cl, (CsCl), LiCl and NaCl vary in their initial effect upon the chromatophores. The first five salts (KCl-LiCl) all bring about contraction of the melanophores and the rapidity of this contraction varies in the order



In NaCl the melanophores expand. The irritability is preserved longest in NaCl solutions. The exact positions in the series of

RbCl and CsCl, is uncertain. They lie very near KCl and  $\text{NH}_4\text{Cl}$ , respectively. LiCl presents physiological properties intermediate between K and NaCl and combines the specific effects of these two salts (p. 550).

6.  $\text{MgCl}$  and  $\text{MgSO}_4$  contract expanded melanophores but the time of this contraction is clearly not a function of the number of  $\text{Mg}^{++}$  ions in solution (p. 552). A comparison of these salts shows that the nature of the anion has a distinct effect upon the time of the first contraction. The difference in the effects of these two salts may be correlated with the greater association of the  $\text{MgSO}_4$ .

7. Mixtures of K and Na salts show two different types of ionic antagonism:

a. Between the cations, in which at 0.1 M concentrations  $\text{K}^+$  appears to be about seven times as potent (specifically) as  $\text{Na}^+$  (p. 558).

b. Between the cation ( $\text{Na}^+$ ) of one salt and the anion of the other salt ( $\text{KSCN}$ ).

8. Pressure causes a contraction of the melanophores and an expansion of the xanthophores when applied to scales immersed in a 0.1 NaCl solution. This reaction is reversible.

9. Increase of temperature produces an acceleration of the contracting effects of distilled water and KCl solutions. At about  $30^\circ\text{C}$ . heat acts as a specific contracting stimulus which is independent (1) of the amount of dissolved oxygen in solution and (2) of the counter-acting stimulus of a 0.1 M NaCl solution.

10. Oxygen is essential for the maintenance of the Na expansion. In its absence the melanophores contract even in a 0.1 M NaCl solution. The action of the oxygen in this case seems to be a passive one since an expansion of contracted melanophores was never induced even after a prolonged exposure to an atmosphere of pure oxygen.

11. Whereas these experiments have thus far shown no evidence of a response of the chromatophores to visible light of varying intensity, an exposure to ultra-violet light (185–290  $\mu\mu$ ) of inverted scales immersed in a 0.1 M NaCl solution produces



a contraction of the melanophores (pl. 4, fig. 1) which is reversible (pl. 4, figs. 4 and 5).

12. An induction current contracts the melanophores. This stimulus is readily overcome by appropriate concentrations of sea-water or of NaCl solutions.

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## PLATE 1

### EFFECTS OF DISTILLED WATER

#### EXPLANATION OF FIGURES

The first five figures are consecutive photographs of a scale from the median dorsal head-region of a 9.3 cm. light female. They show the characteristic peripheral encroachment of distilled water.

1 All melanophores contracted. Photograph taken 1 minute, 49 seconds after the removal of the scale.

2 The characteristic expansion which follows the loss of the nerve tonus. This is the cause of the darkening seen in decapitated fish. Taken 10 minutes, 20 seconds after the removal of the scale.

3 The distilled-water effect is clearly visible at the periphery of the scale where the melanophores have all contracted. Taken 27 minutes, 40 seconds after the removal of the scale.

4 The melanophores at the center of the scale are being contracted by the advancing distilled water. Taken 34 minutes, 15 seconds after the removal of the scale.

5 All the melanophores are contracted. Taken 80 minutes, 44 seconds after the removal of the scale.

6 A single melanophore and a groups of xanthophores. In the former the empty processes may be seen, a characteristic effect of the distilled-water contraction.

## PLATES 2 AND 3

### EFFECTS OF POTASSIUM SALTS

#### EXPLANATION OF FIGURES

These figures show the effects of five neutral salts of potassium. Five sets of four scales each were immersed in isotonic solutions of KCl, KBr, KNO<sub>3</sub>, KI, and KSCN, for one, two, three, and four hours respectively.

After *one* hour the melanophores of the KCl and KBr scales were still contracted; in KNO<sub>3</sub> a few peripheral melanophores showed an expansion which was more advanced in the KI while all the melanophores of the KSCN scale had begun to expand.

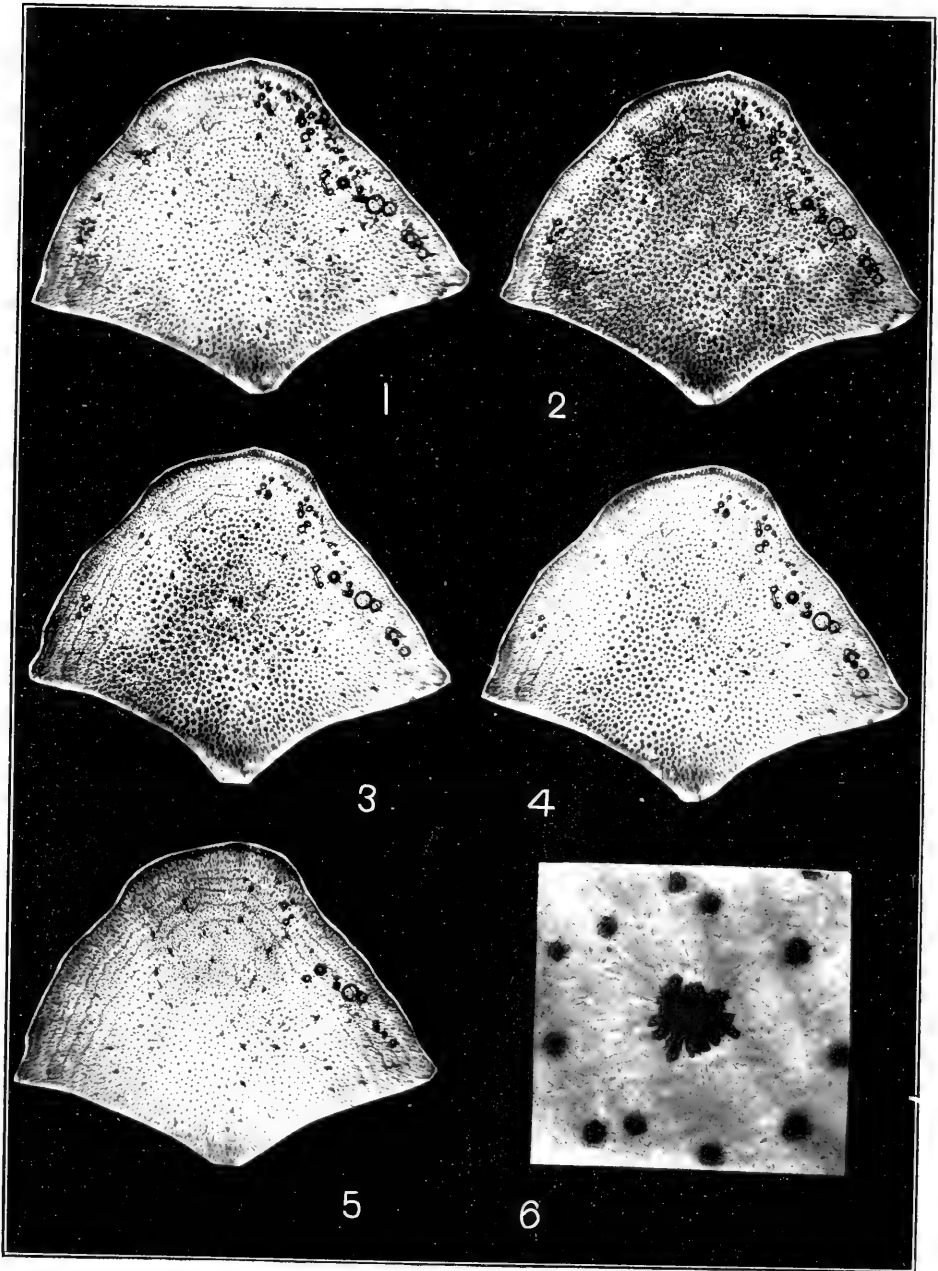
After *two* hours there was still no expansion in KCl, a slight expansion was beginning in the KBr; in KNO<sub>3</sub> all but the most central melanophores showed an expansion and all were expanded in KI and KSCN.

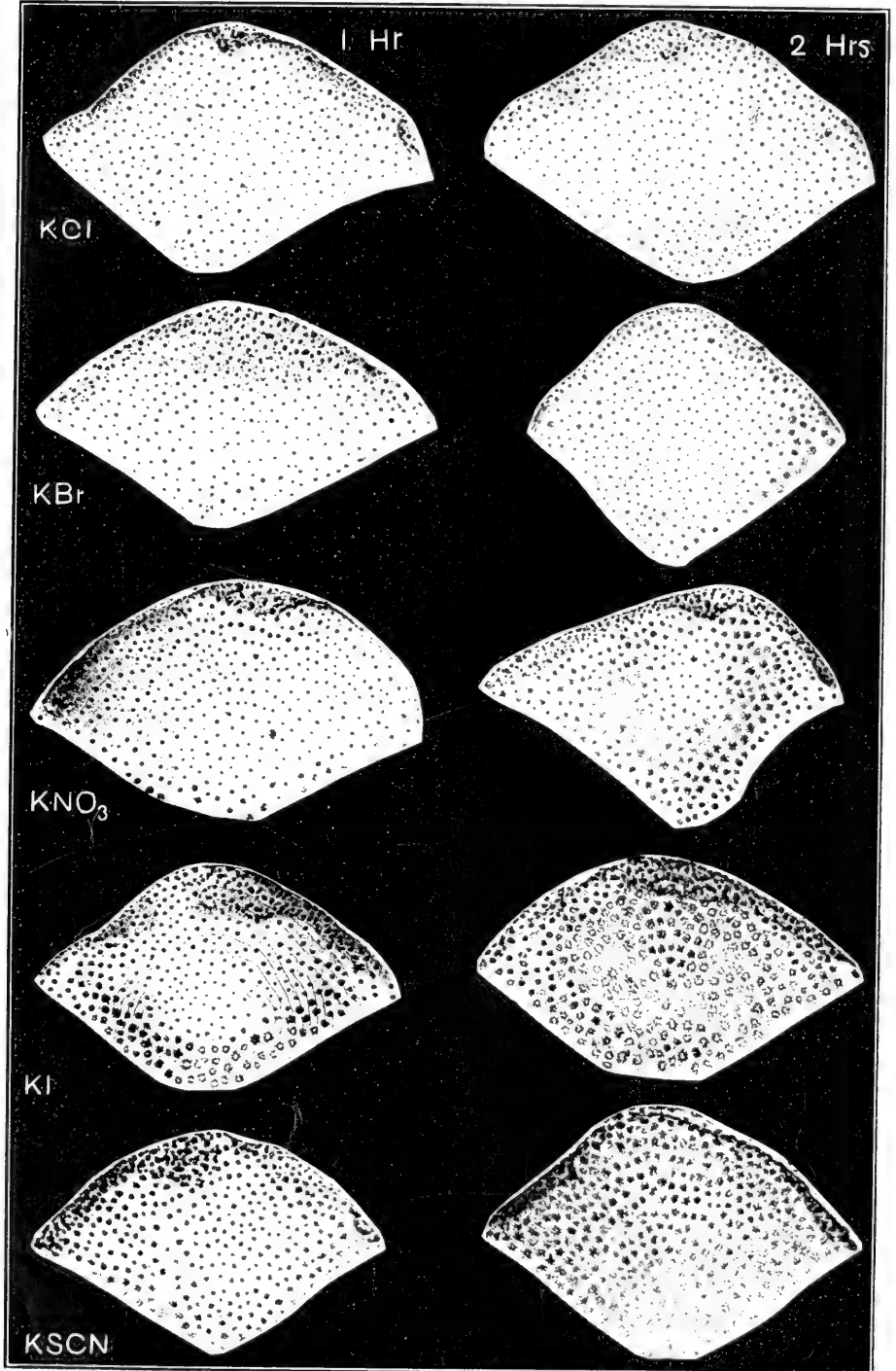
After *three* hours there was still no expansion in KCl; all the others were fully expanded and degeneration was well advanced in KSCN.

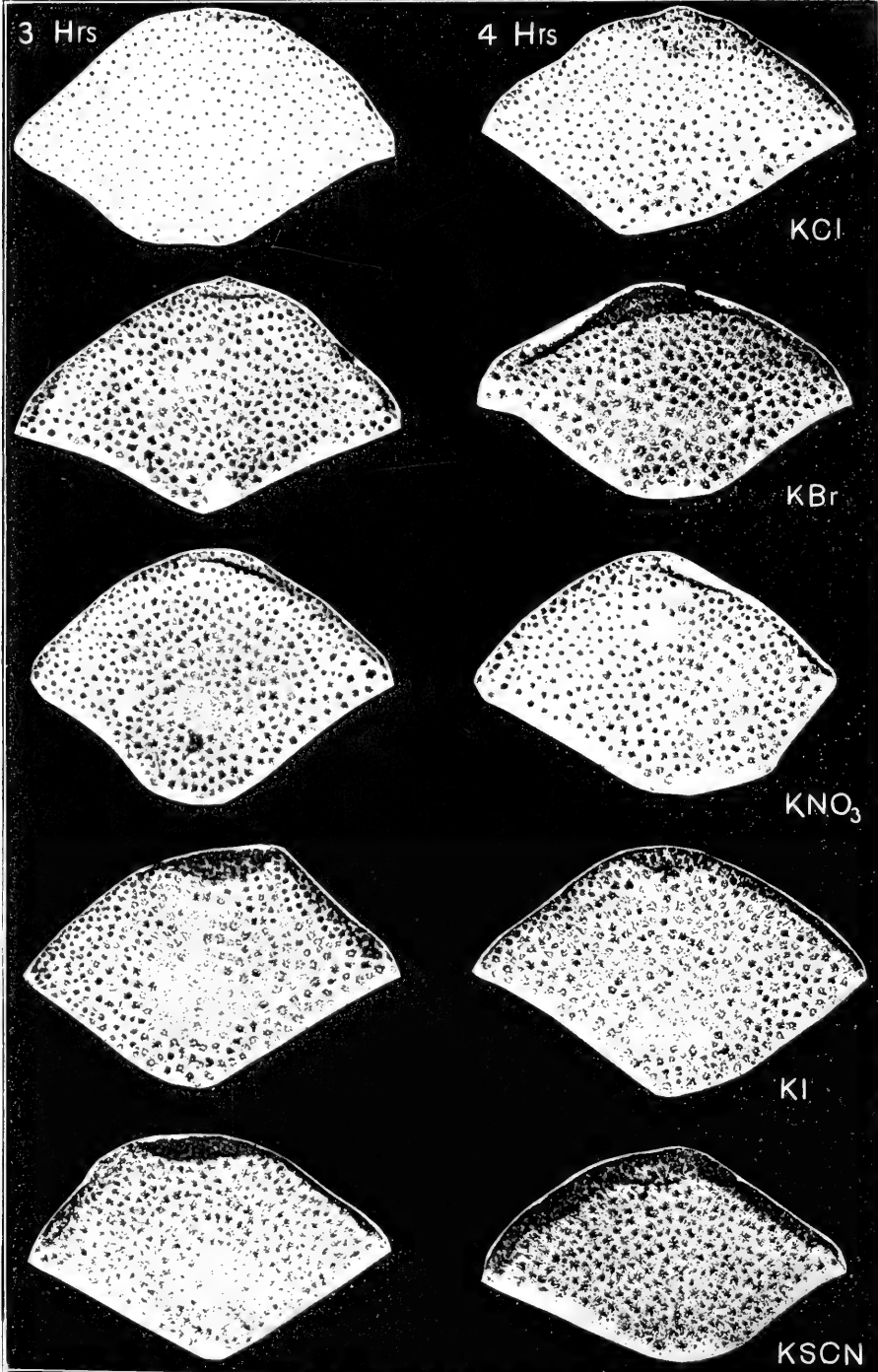
After *four* hours all five scales showed expanded melanophores; in KI and KSCN degeneration was well advanced.

These figures show clearly the protective anion sequence:









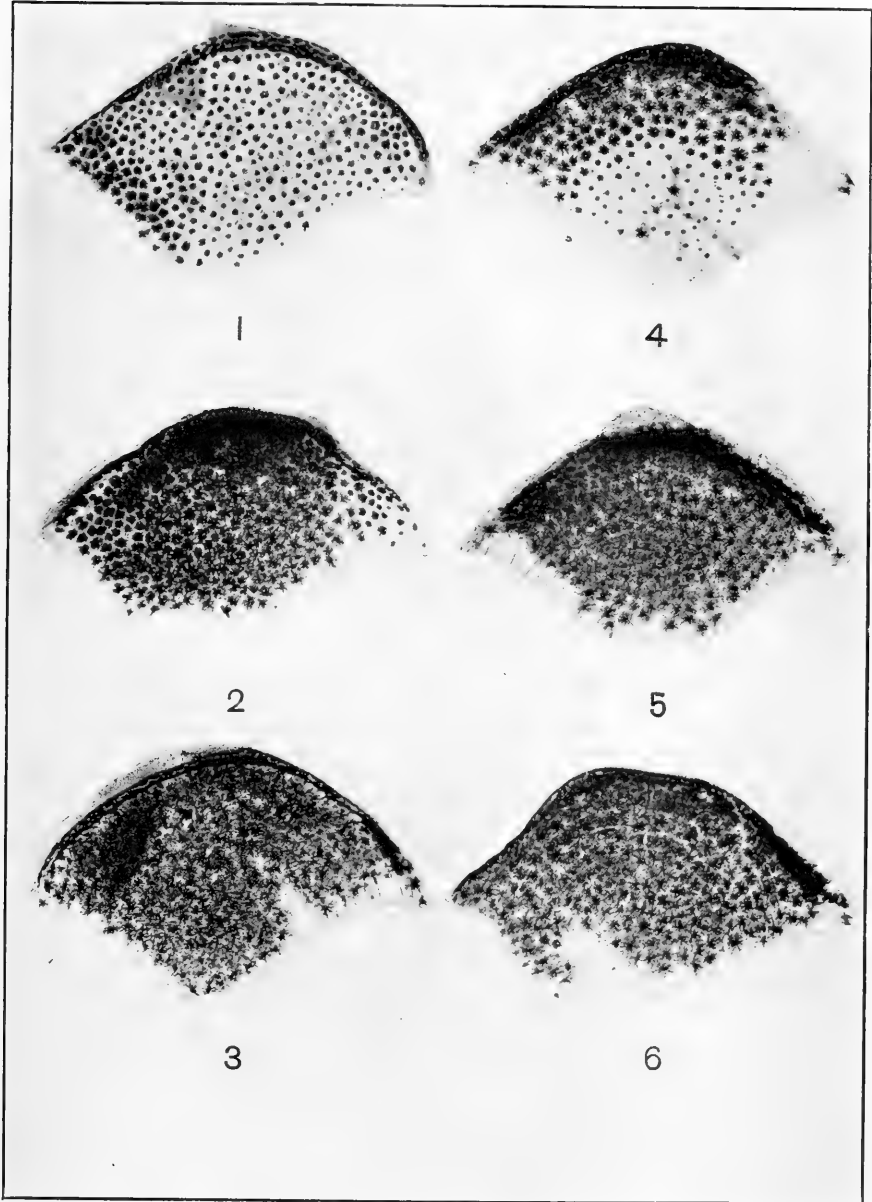
## PLATE 4

### EFFECTS OF ULTRA-VIOLET LIGHT

#### EXPLANATION OF FIGURES

- 1 This scale shows ultra-violet light contraction. It was one of an inverted pair.
- 2 One of the scales exposed to ultra-violet light with that in figure 1 but turned face-up.
- 3 An unexposed control scale in 0.1 M. NaCl.
- 4 One of the inverted scales showing recovery from ultra-violet light contraction 10 minutes after a 10-minute exposure.
- 5 A scale showing complete recovery from ultra-violet light contraction 20 minutes after a 10-minute exposure.
- 6 An unexposed scale in 0.1 M. NaCl.







# NON-DISJUNCTION OF THE SEX CHROMOSOMES OF DROSOPHILA

CALVIN B. BRIDGES

*The Zoölogical Laboratory, Columbia University*

A certain kind of exception that has come up during the course of my work on *Drosophila ampelophila*, with Dr. T. H. Morgan, bears directly upon the problem of sex determination, and especially upon the view that the chromosomes are the carriers of the differentiators of the hereditary characters. The evidence which I am to present deals with the *X*-chromosome<sup>1</sup> of *Drosophila*, concerning which the evidence relating to sex linkage and the linear arrangement of factors is more definite and complete than in any other case. Ordinarily, when a female with white eyes is mated to a wild male with red eyes, the daughters have red, and the sons, white eyes. This is a typical case of the criss-cross inheritance characteristic of sex linkage. The cytological evidence of Miss Stevens shows that *Drosophila* belongs to that group of forms in which the female has two *X*-chromosomes and the male an unpaired *X*-chromosome. In spermatogenesis, half of the spermatozoa receive this *X* and half do not. In oögenesis, each gamete receives one *X*. The fertilization of any egg by a one-*X* sperm, results in a female (*X X*), while any egg fertilized by a no-*X* sperm results in a male (*X—*). Morgan has explained the case of criss-cross inheritance on the ground that the *X*-chromosome is the carrier of all sex linked factors. The sons are matroclinous because they receive their unpaired sex chromosome directly from the mother, and must show all the sex linked characters which she showed. Each daughter, of the case cited, is red because the paternal of her two sex chromosomes bears the dominant red, and the maternal bears the recessive white.

<sup>1</sup>I have used 'X,' 'X-chromosome' and, 'sex-chromosome' interchangeably.

In the exceptional case that I have found, where the mating was like that just described, about 5 per cent of the daughters are like the mother and 5 per cent of the sons are like the father.

This anomolous result may be explained, if, in such mothers, a certain per cent of maturations are of a type characterized by non-disjunction, *ie.*, eggs are formed which contain two sex chromosomes instead of the normal one, and other eggs corresponding to them contain no sex chromosome. If in one of the two maturation divisions of the egg, the two sex chromosomes that usually pass to opposite poles, one going out in the polar body and the other remaining in the egg, should sometimes *not* disjoin from each other, but should lag upon the spindle so that both pass into the polar body or the egg, then there would result eggs with two chromosomes and other eggs with no sex chromosome. The eggs of such a female will then be of three classes, namely, a large normal class with one sex chromosome, and two small equal classes containing respectively, two and no sex chromosomes.

If sex linked characters are determined by factors carried in definite loci in the sex chromosome, then we have a way of tracing the maternal and paternal chromosomes. For example, all the X-chromosomes of a white female, which produces the three kinds of eggs above, are white bearing. Any X-chromosome of the wild male, to which she is mated, is red bearing.

The results of this cross are summarized in the diagram below,<sup>2</sup> and are as follows:

	90 Per cent	5 Per cent	5 Per cent	
Eggs of white female	w	ww	—	
Gametes of red male	W	—		
	<i>Per cent</i>			<i>Per cent</i>
(1) wW      red ♀	90	(2) w —      white ♂		90
(4) wwW    red ♀	5	(3) ww—    white ♀		5
(5) —W     red ♂	5	(6) —      —      ♂		5

(1) The one-X egg fertilized by the one-X sperm will give a female (X X), red, heterozygous for white. (2) The one-X egg

<sup>2</sup> In the diagram the w indicates the sex chromosome which bears white, and the W the red bearing chromosome. The dash represents the no-X gamete.

by the no- $X$  sperm will give a male ( $X-$ ), whose only  $X$  came from his mother, and hence determines him as white. The one- $X$  eggs give, then, the normal criss-cross result characteristic of sex linked inheritance, (white ♀ × red ♂ = red ♀ + white ♂). The two- $X$  egg will give females only, since it is already duplex with respect to the sex differentiator. (3) If this egg is fertilized by a no- $X$  sperm, there should result a female ( $XX$ ), both of whose sex chromosomes are maternal, and would thus produce white. (4) The two- $X$  egg, fertilized by the one- $X$  sperm, will produce an individual with three allelomorphous sex chromosomes, two of which are white, and the other red bearing. This fly will, if viable, be a red female, indistinguishable from the large class above. (5) The no- $X$  egg by the one- $X$  sperm will produce a male ( $X-$ ), differing from the normal male in that his  $X$  is *not* maternal but paternal, and would produce a red fly. (6) The no- $X$  egg by the no- $X$  sperm will produce a zygote with no  $X$ , and such a zygote, I believe, is not viable.

The presence of the white gene in the maternal chromosomes gives us a means of distinguishing between all the classes, except between the three- $X$  and the heterozygous red  $XX$  types.

Non-disjunction of the other chromosomes (autosomes) has not been observed in these crosses.

If 10 per cent of the eggs of such a female matured in a non-disjunctional manner the proportions of the  $F_1$  would be:

Females		Males	
white ♀	red ♀	white ♂	red ♂
5%	95% (or 90%)	90%	5%

The evidence in support of these conclusions is as follows. From time to time in an  $F_1$  where sex-linked characters were concerned, females or males have arisen which I was unable to explain on any current Mendelian hypothesis. Some of these I bred, and the offspring were as hard to explain as the original exceptions. One of the first cases was the following. I crossed a recessive pink female, of a stock which had been pure for some twenty-five generations, to a male which was a triple recessive, namely, white miniature black. The normal  $F_1$  expectation for

this cross is red females and males. Of the factors concerned, pink of the female is in the third chromosome and black of the male is in the second chromosome. Both white and miniature are in the sex chromosome at a distance from each other of approximately 32 units (Sturtevant, '13 a). The normal  $F_1$  female, then, is heterozygous for four factors, white, miniature, black, and pink. The  $F_1$  male is heterozygous for black and pink, but is normal with respect to white and miniature, since his only sex chromosome came from his mother, who was neither white nor miniature. I had three cultures of  $F_1$  and they produced in aggregate the expected red females and males to the number of 251 and 305 respectively. But I found also three white miniature males, one in each culture. Their fathers were white and miniature, but these were *not*  $P_1$  males left over in the culture, because they were very young (young flies are very light in body color and show other characteristic differences from the adult), and occurred after I had emptied the culture bottles several times. Fortunately I bred one of these males to virgin sisters, which were heterozygous for white, miniature, black, and pink. The results showed conclusively that the male had really come from the cross and was not the result of any contamination, in that it gave in the next generation all expected classes from the cross of an  $F_1$  female by a white miniature male heterozygous for black and pink, that is, there appeared blacks and pinks, males and females, whites and miniatures, in all the combinations and permutations expected.

Since my interest was centered primarily in the white and pink characters, and I did not at that time realize the importance of the problem, I counted the offspring of the non-disjunctive male only with respect to white and pink, although the occurrence of blacks and miniatures of both sexes was noted.<sup>3</sup> The count approximated expectation: (4:3:1:4:3:1).

white ♀ (4)	red ♀ (3)	pink ♀ (1)	white ♂ (4)	red ♂ (3)	pink ♀ (1)
106	93	30	111	78	30

<sup>3</sup> For comparison I give the  $F_2$  from normal males by sisters such as those to which I mated the patroclinous male.

red ♀ (6)	pink ♀ (2)	white ♂ (4)	red ♂ (3)	pink ♂ (1)
733	196	354	340	75

There is no question of association here, for the white and pink are in different chromosomes, the sex and the third.

The interpretation of these results is as follows: among the  $P_1$  pink females used there was at least one female which formed some eggs in a non-disjunctional manner with respect to the sex chromosome, but not with respect to the second and third chromosomes. The duplex sex chromosome of the pink female contained only non-mutated genes.<sup>4</sup> Wherefore the female resulting from the fertilization of the two- $X$  egg would be indistinguishable from the other  $F_1$  females, irrespective of the character of the sperm. But the no- $X$  egg, if met by a one- $X$  sperm would produce a male exhibiting all the sex chromosomal characters of the  $P_1$  male which supplied the chromosome. Since the  $P_1$  male was white and miniature, these characters were the ones which showed in the three such males of this experiment.

If this non-disjunction involved the second chromosome, then in  $F_1$  there might appear some black females and males, which was not the case. The white miniature males, moreover, should be black but *simplex*, and in the next generation there should result a great overproduction of blacks, which was not observed. A similar disturbance in the reduction of the third chromosome would have disturbed the  $F_2$  ratio from that observed.

The case just cited illustrates the production and composition of the patroclinous males. The next case gives evidence upon these points for the females. I crossed some white pink females to some eosin vermilion pink males. The expectation in  $F_1$  is white pink males and white-eosin compound pink females heterozygous for vermilion. This cross is reported in this number and an analysis given by Morgan and Bridges. Of the factors concerned, the third chromosomal pink entered the zygote from both sides so that all flies in this experiment are pink. White and vermilion are sex chromosomal and so in expectation all the sons of the white not-vermilion mother are white and likewise not-vermilion. White and eosin are allelomorphous to each other, that is, they occupy the same locus in the sex chromosome. Of the two homologous sex chromosomes of the  $F_1$  female the mater-

<sup>4</sup>Except the gene for non-disjunction itself, as will appear later.

nally derived carried white and the paternally derived carried eosin. The peculiarity of this combination in the female is that in color it looks like an intermediate between white and eosin, hence the name white-eosin compound. Several cultures of the type described above produced in aggregate 2073 white-eosin compound pink females heterozygous for vermilion, and 2037 white pink males. But in addition there were nine white females and two males of a grade of color corresponding to eosin vermilion pink, that is, to the color of the fathers. These two males were sterile so I could not test the correctness of my classification.

With the matroclinous females however, I made the following tests. One of them I mated to her white brothers, and in the next generation got 51 white females and 60 white males. This test only shows that my classification of the female as white was correct, for she gave no eosin sons, as did her ordinary sisters mated to the same males.

Another of these white females I mated to a double recessive male, namely vermilion pink. All the ordinary sisters were heterozygous for vermilion, but if my hypothesis is correct, then the non-disjunctional female should be the exact counterpart of her mother not only in being white, (as the first test showed she was), but also in being pure for not-vermilion, although her father was vermilion. If she were heterozygous for vermilion, it would show in that half of her sons and daughters would be vermilion. As a matter of fact none of her daughters were vermilion and all of her sons were white, with one exception. She *was*, then, the exact counterpart of her mother as far as all sex chromosomal characters were concerned. The one exception, a vermilion pink male, was likewise an exact counterpart of his father. The actual offspring of the second white female by the vermilion pink male of unrelated stock were:

white (pink) ♀	pink ♀	white (pink) ♂	verm. (pink) ♂
2	52	54	1

All the flies are pink because pink entered from both sides. The presence of the one vermilion pink male and the two white



pink females brings out a new point, namely, that a non-disjunctionally produced female manifests her peculiarity directly in  $F_1$  by producing more like herself. The two white females produced here came from the union of the two- $X$  egg with the no- $X$  sperm of the vermillion pink male, and should be entirely maternal and entirely non-paternal in sex chromosomal composition, i.e., they should be exact counterparts once more of their mother. Wherefore, if they should be mated to a male like their father, the same story should be repeated. This test was made:

$P_1$	white pink patroclinous ♀ × vermillion pink (outcross) ♂			
$F_1$	white (pink) ♀	pink ♀	white (pink) ♂	verm. pink ♂
	0	33	25	1

The culture unfortunately ran out before the point was completely established, but the appearance of the one patroclinous vermillion pink male makes it certain that white pink females would ultimately have appeared.

The other of these two white pink females (the first was mated to the vermillion pink ♂) was mated to an eosin male of an unrelated stock. The story was repeated, but with a very important modification, namely, that the non-disjunctional males were eosin. The results were as follows:

$P_1$	white pink ♀ (non-disjunctionally derived) × eosin ♂ (pure stock)			
$F_1$	white ♀			
	(heterozygous for pink)	white-eosin compound ♀	white ♂	eosin ♂
	1	119	101	4

The results show that the non-disjunctionally derived female produced non-disjunctional females once more, and that like her mother she was homozygous for white (all female offspring white-eosin compound and all normal sons white), and that the sex chromosomal characters of the non-disjunctional sons do not depend in any degree upon the composition of their mother but only upon that of their father, in the first case cited, white miniature, here eosin. Moreover, of sister females, one produced vermillion and the other eosin patroclinous sons when mated to

vermilion and eosin males respectively. Thus for four sex chromosomal factors the same conclusions hold.

Since it has been shown that the non-disjunctionally produced female hands her peculiarity on directly to her  $F_1$  non-disjunctional daughters, we can test the non-disjunctional sons in that respect. The offspring of the white miniature exceptional male give us no information on the question, because the classes of offspring which would show the effect can not be separated from the large normal class already present. The vermilion pink male, however, I mated to wild females. The first generation consisted of red females 223 and red males 159 and *no* non-disjunctionals.  $F_2$  was like that from a normal stock vermilion pink male, namely:

red ♀	pink ♀	red ♂	vermilion ♂	pink ♂	vermilion pink ♂
45	22	27	27	6	9

The fact that the non-disjunctional female gives non-disjunctionals in  $F_1$  is the result one would expect *if the cause of this peculiar oögenesis were itself a sex chromosomal gene*. If such a gene were present in each of the sex chromosomes of the original female all her exceptional daughters would of course have the same condition and thus the line would be perpetuated parthenogenetically, so to speak, indefinitely. But with regard to the exceptional male it should be otherwise, for he gets *no* sex chromosome from his mother and consequently can neither have nor transmit the non-disjunction gene.

Before passing to the next instances, I can present some evidence on the composition of the normal pink females and white males produced in the cross of the white pink non-disjunctional female to the vermilion pink male (out-cross). A few of the normal pink females and white males produced:

pink ♀ (4)	white ♀ (4)	white ♂ (4)	verm. pink ♂ (3)	pink ♂ (1)
74	77	66	62	15

The simple ratio is altered somewhat by association of white and vermilion, but, is otherwise a wholly normal result.

Two other white pink females (of the nine) I mated separately to wild males. They produced:

white ♀	red ♀	white ♂	red ♂
7	192	131	8
5	151	147	11
—	—	—	—
12	343	278	19

These two instances illustrate once more the fact that the sex chromosomal character of the non-disjunctional female depends solely upon the sex chromosomal character of her mother and in nowise upon that of her father. But with the non-disjunctionally produced male the result is exactly reversed, for each time the character of the non-disjunctional male depends *not* upon the characteristics of the mother (other than non-disjunction itself) but is a duplicate of that of his father, in this case completely normal.

If the third chromosome is not involved in this non-disjunction then we should expect that as far as the distribution of pink is concerned, the results would be exactly that of a normal cross, that is, both the non-disjunctional females and males should be heterozygous for pink, because their mother was homozygous for pink and their father was not-pink. To test this point further I crossed three non-disjunctionally produced females (of the twelve) to non-disjunctionally produced males (of the nineteen). The next generation was:

white ♀	red ♀ (3)	pink ♀ (1)	white ♂ (4)	red ♂	pink ♂
1	52	37	115	0	0
7	101	42	141	0	0
—	—	—	—	—	—
8	153	79	256	0	0

One can see here in the same cross both non-disjunction and normal segregation, but with respect to different chromosomes. The non-disjunction results are, the sons all white to correspond to the composition of their mother, and the occurrence of the white non-disjunctional females. The production of pink females shows that *both* parents were heterozygous for pink, since if

either one were pure for not-pink, (as in the next case below) *no* pinks would have appeared in the offspring. Had the numbers been large enough, exceptional males should have appeared in the proportion of three red to one pink among themselves.

A second non-disjunctional white female (of the twelve) I crossed to a wild male and obtained:

white ♀	red ♂	white ♂	red ♂
15	159	146	13

Here *no* pink appeared although the cross was externally the same as the previous one. The interpretation is that the male in the first case differed from the wild male in that it was heterozygous for pink.

To effectually show that the non-disjunctional males were heterozygous for pink I mated four (of the nineteen) to stock pink females, and as I expected, half of the females and half of the males were pink:

red ♀	pink ♀	red ♂	pink ♂
70	69	63	62

To show, once more, that it was the female only that was responsible for the production of exceptions in  $F_1$  I simulated the cross of non-disjunctional white pink female by non-disjunctional male given above by testing a non-disjunctional brother of the first by a white female of *another* stock. The result was simply red females 105 and white males 74, with no exceptions.

A third male (of the nineteen) I tested to an eosin female with still no exceptions appearing in  $F_1$  among the 143 red females and 146 eosin males.

There still remained to be tested only the normally produced females (343) and males (278). Two pairs from these gave the ordinary Mendelian result:

white ♀ (4)	red ♀ (3)	pink ♀ (1)	white ♂ (4)	red ♂ (3)	pink ♂ (1)
52	43	15	59	44	23
45	37	15	48	29	12
—	—	—	—	—	—
97	80	30	107	73	35

I continued tests of this line of females for three generations more as follows: From the fifteen non-disjunctional females I selected three which I mated to four red brothers. If both the females and males were heterozygous for pink I should get pink females among the offspring and these in fact I found as follows:

white ♀	red ♀	pink ♀	white ♂	red ♂
4	99	17	134	6*
10	158	29	173	11
—	—	—	—	—
14	257	46	307	17

\*In addition to these red males there was a red eyed lateral gynandromorph, which is being figured by Morgan in "Heredity and Sex."

From among these fourteen matroclinous white females I mated two to four of their red brothers. The results were:

white ♀	pink ♀	red ♀	white ♂	red ♂
9	17	134	95	5

It is quite probable that only one of these flies was heterozygous for pink, since theoretically, only half of the fourteen flies should be heterozygous for pink. For they came from a cross of heterozygous non-disjunctional female to a wild male (not-pink).

The five cases like the last sum up to:

white ♀	pink ♀	red ♀	white ♂	red ♂	pink ♂
22	142	544	658	22	0

Expectation calls for fifteen red to seven pink among the exceptional males. I think that probably the non-appearance of the pink males has no significance.

Three more of the fourteen white females I now mated to red males of wild stock. The results were

white ♀	red ♀	white ♂	red ♂
15	132	95	9
4	98	80	0
—	—	—	—
19	230	175	9

From the nineteen exceptional white females here produced, I mated three to wild males and obtained:

white ♀	red ♀	white ♂	red ♂
2	128	86	2
3	131	157	2
—	—	—	—
5	259	243	4

The cross of white pink females by eosin vermilion pink males was originally planned only on a small scale for the purpose of making observations on the eye color of the daughters. When there appeared a few matroclinous daughters, the scale of the experiment was increased. However, the color of the males which would correspond to the exceptional females is very close to white, and it would be easy to miss some of them among the normally produced whites. Accordingly, some of the females from the white pink stock were mated to wild males. I raised four cultures, using as the mothers in each case five white pink females. The results show that non-disjunction was wide-spread in that stock and had maintained itself there during the six months (some fifteen generations) between the two crosses. The results are tabulated below:

P <sub>1</sub>	white pink ♀		wild ♂	
F <sub>1</sub>	white ♀	red ♀	white ♂	red ♂
	7	212	207	6
	1	187	186	2
	1	191	247	3
	2	220	205	8
	—	—	—	—
	11	910	845	19

That it is the same phenomenon here is shown by the results of mating a white daughter (of the seven) to wild males.

P <sub>1</sub>	white ♀		wild ♂	
F <sub>1</sub>	white ♀	red ♀	white ♂	red ♂
	3	99	64	3

Half of these white females should be heterozygous for pink if they really were produced in the manner assumed. One bred to a pink male showed that she was in fact heterozygous for pink.

P <sub>1</sub>	white ♀ (of the three)			pink ♂	
F <sub>1</sub>	white ♀	red ♀	pink ♀	white ♂	red ♂
	2	70	71	126	2

I have been able to detect non-disjunction in certain other stocks, notably eosin. Of the females tested, one gave the following offspring by a white miniature male.

F <sub>1</sub>	eosin ♀	white-eos. comp. ♀	eosin ♂ (long)	white min. ♂
	7	154	134	2

These seven eosin females contained no trace of the paternal white and miniature, for one mated to a wild male gave no white or miniature sons, but only eosin not-miniature sons, and further patroclinous sons, which were of course red.

P <sub>1</sub>	eosin ♀ (of the seven)		wild ♂	
F <sub>1</sub>	eosin ♀	red ♀	eosin ♂	red ♂
	3	118	120	5

The purity of the eosin daughters is shown even more clearly by back-crossing three other females (of the seven) to white miniature males. One would ordinarily expect half the males and half the females to be miniature and white, with the proportions showing the association of white and miniature. But no such effect was produced, as one can see in the progeny:

eosin ♀	white-eos. comp. ♀	eosin ♂	white min. ♂
15	133	135	9

The white-eosin compound is a very efficient aid in studies such as these, since it enables one to tell at a glance the composition of the female whether pure eosin, pure white, or white-eosin; determining in this case that the thirteen daughters were pure eosin and not-white, and that the 133 daughters were true hybrids, i.e., white-eosin compounds.

For the sake of maintaining stock, I have continued this line a little further by breeding one of the three eosin females to one of her red brothers.

P <sub>1</sub>	eosin ♀		red ♂	
F <sub>1</sub>	eosin ♀	red ♀	eosin ♂	red ♂
	5	81	76	6

The next generation in the same manner was:

eosin ♀	red ♀	eosin ♂	red ♂
8	132	139	14

Since it makes no difference what male is used, I paired two of the eight eosin females to white males and obtained:

eosin ♀	white-eos. comp. ♀	eosin ♂	white ♂
10	99	110	10
6*	91	93	14

\* "In addition to these six eosin females there appeared a female with a penis like that figured in "Heredity and sex." The eye color was eosin ( $w^e-w^e$ ), It is possible that this was a three chromosomal form, or else caused by somatic non-disjunction. Neither gynandromorph would breed.

I shall cite here several cases of non-disjunction which have appeared in other experiments, and which show that although the phenomenon is infrequent, it is wide-spread in diverse stocks

P <sub>1</sub>		eosin ♀ ♀		white vermilion pink ♂ ♂
F <sub>1</sub>	eosin ♀	white-eos. comp. ♀	eosin ♂	white verm. ♂
	2	273	260	2
		298*	277	1

P <sub>1</sub>		white ♀ ♀		eosin vermilion pink ♂ ♂
F <sub>1</sub>	white ♀	white-eos. comp. ♀	white ♂	eos. verm. ♂
	0	151	142	1

\* There appeared here one female with one eye white and one eye eosin. Her offspring by a wild male were:

red ♀ (6)	white ♂ (3)	eosin ♂ (2)	eos. verm. ♂ (1)
119	46	39	18

I had found females of this type before, but had not determined whether the condition might be transmitted, so as to appear in F<sub>2</sub>. Three pairs of F<sub>1</sub> red female by eosin vermilion brothers gave:

	wh.-eos.	wh.-eos.						
red ♀	comp. ♀	pink ♀	pink ♀	white ♂	red ♂	verm. ♂	pink ♂	verm. pink ♂
63	50	14	14	69	30	20	10	3
37	51	32	10	33	36	12	7	2
65	55			72	38	19		

In each of the first two bottles appeared a pink eyed female splotted with white. One of these was sterile, and the other gave by a pink brother only unspotted pinks for three generations.

	pink ♀	pink ♂
F <sub>3</sub>	26	22
F <sub>6</sub>	132	105
F <sub>5</sub>	112	118

Mosaic forms can be explained by *somatic* non-disjunctions.



P <sub>1</sub>	vermilion pink ♀ ♀ eosin vermilion pink ♂ ♂		
F <sub>1</sub>	verm. pink ♀ heteroz. for		
	eosin	verm. pink ♂	eos. verm. pink ♂
	307	290	1
P <sub>1</sub>	pink ♀ ♀		white ♂ ♂
F <sub>1</sub>	red ♀	red ♂	white ♂
	168	159	2
	17	12	4*

\* This culture was from a pair.

P <sub>1</sub>	pink ♀ ♀		white verm. pink ♂ ♂
F <sub>1</sub>	pink ♀	pink ♂	white (verm. pink) ♂
	260	231	1

This one white male I mated to wild females and in F<sub>2</sub> obtained

red ♀	pink ♀	white ♂	red ♂	verm. ♂	pink ♂	verm. pink ♂
99	30	64	25	14	13	4

This is an extremely close approximation to the result expected on the assumption that the grandfather was vermilion and pink as well as white. Thus, although to sight the vermilion was masked by the white, its reappearance in F<sub>2</sub> makes another instance where the whole set of sex-linked factors of the father appeared in his exceptional sons in their original combination exactly. The simplest hypothesis is one which assumes a common carrier for all sex-linked factors and the sex differentiator. Just such a carrier is offered by the sex chromosome.

Dexter ('12, p. 190), crossed a yellow female heterozygous for white to a gray white male. There were produced:

gray red ♀	gray white ♀	yellow red ♂	yellow white ♂	gray wh. ♂
116	116	82	100	2

These gray white males which Dexter was unable to account for in any way, are exactly like their father, as is characteristic of non-disjunctional sons.

When the white eyed mutant first appeared, it was mated to normal red females. In F<sub>1</sub> there appeared five white males, which Morgan explained as further mutations. It seems to me more likely that these are non-disjunctional males.

It is possible that some of the anomolous appearances of flies in the cultures of Morgan and Cattell may be explained in this way.

The case referred to by Sturtevant ('13 b) is probably one of non-disjunction coming from miniature white stock.

There are many other cases similar to these, some of which involve other sex-linked factors than those already given.

The sum of all the  $F_1$  counts derived from single females which were themselves matroclinous is as follows:

matroclinous ♀	hybrid ♀	matroclinous ♂	patroclinous ♂
133	2934	2677	138

This gives the percentage of exceptions as 4.83, or roughly 5 per cent.

At first glance it would seem that these exceptions are the result of partial sex linkage, that is, that we are dealing with a linkage ratio in which sex is one of the characters. That this is not the case, can be seen from the fact that the results are independent of what particular sex linked factor is used, while in linkage, each different combination has a characteristic value, for instance, the percentage of crossover of white and yellow is about 1.2 per cent, of white and vermilion about 33 per cent, of white and miniature about 35 per cent, and of white and rudimentary about 44 per cent. If the sex differentiator gives 5 per cent of crossover with white it ought to give  $33 + 5$  or  $33 - 5$  with vermilion,  $35 + 5$  or  $35 - 5$  with miniature and, so on. From the fact that I get no such variation depending on the particular factor used, I conclude that this phenomenon is not one of partial sex linkage. It is obvious, however, that non-disjunction forms an alternative hypothesis for all the cases which have been described as partial sex linkage. In some of these cases it is to be preferred, but no decisive choice can be made in any particular case between the two hypotheses, unless, as in *Drosophila*, there are present *more than one sex linked factor*.

Morgan and Sturtevant have shown that a certain number of sex linked factors can be arranged in a consistent linear series if the percentage of crossovers be used as the measure of the dis-

tance between the factors, for example, if A and B give 20 per cent of crossover and A and C give 10 then B and C will give approximately<sup>5</sup> either  $20 + 10$  or  $20 - 10$  as the percentage of crossover. Suppose the experiment gives 10 per cent. Then the order becomes A, B, C, and not A C B. This order when once discovered is found to hold when tested out with any other sex-linked factor. If we know how far a new factor D is from A and B we can predict the percentage of crossover of C - D, or if we know its distance from A and C we can predict B - D. At present the series of known sex-linked factors numbers twenty-eight. So far as tested, these factors behave as though they occupied loci in a *linear series with fixed order and distances*, and we believe that this behavior is explicable on the view that the sex chromosome is the material basis of the series.

The bearing of non-disjunction upon this question is that *the series of factors which has been shown to be linear, behaves here as though it were a unit*. The *whole* series that the mother bore descends to her matroclinous daughters, and the *whole* series of the father to his patroclinous sons.

It will have been noticed that in all these cases of non-disjunction, *sex* behaves in a fashion strictly parallel to that of any sex-linked character. The non-disjunctive eosin or white *female* transmitted her femaleness to her matroclinous daughters in exactly the same manner that she transmitted her eosin or white eyes. This behavior requires a common vehicle for the transmission of sex and a linear series of sex linked characters, and such a vehicle is furnished by the sex chromosome.

If we accept the conclusion that the matroclinous daughters are the result of the fertilization of two-X eggs by the no-X spermatozoa, and the patroclinous sons are the result of the fertilization of no-X eggs by one-X spermatozoa, then we must inquire what would result from the fertilization of the two-X eggs by the one-X spermatozoa, and from the fertilization of the no-X eggs by the no-X spermatozoa. In the first case an individual with three, and in the second case with no sex chromo-

<sup>5</sup> Inaccurate for long distances because of double crossing over.

somes should result. In my experiments I have seen nothing to correspond to the last case, and do not know what to look for, on the dubious assumption that such a fly could live. The three chromosomal form, I believe, would be a female on the assumption that two sex chromosomes bearing the sex differentiator produce a female, and that the extra chromosome would not change this result. If we suppose that the result is a female, what characters will she show? A two-*X* egg from an eosin female fertilized by a red male would have two eosin factors and the dominant red allelomorph. It seems likely that this female would be red, perhaps diluted somewhat. I have never been able to discover any eye color differences in the large class of red females. Several taken at random, were tested individually with wild males. If one of these females were three chromosomal I expected that there would be two eosin to one red male, and that there would be a preponderance of females, in the ratio of about two females to one male instead of the normal 1 : 1 ratio. The thirty cases tested gave, however, only the regular proportion, 2 red ♀ : 1 red ♂ : 1 eosin ♂. Using another and better test, I am about to renew the search for these three-*X* females.

Although, in *Drosophila*, there is as yet no direct cytological evidence concerning non-disjunction of chromosomes, in other forms, there is cytological evidence in harmony with what the genetic evidence demands in the present case. The work of Prof. E. B. Wilson ('09) on *Metapodius* furnishes two such instances. Here the number and size of the chromosomes in each individual is constant, but this number ranges from 21 to 28, the variation being due to loss of a *Y*-chromosome from the typical 22-chromosome form to give the 21 form, and the addition of one to six supernumerary *Y*-chromosomes to give the higher groups. In the 22-chromosome form, Wilson observed a few second spermatocyte divisions where both *X* and *Y* passed to the same pole. The result would be spermatozoa containing respectively twelve and ten chromosomes. Wilson has shown that if these spermatozoa are functional, the whole series of variations follows. The production of spermatozoa with two or with no idiochromosomes

is directly comparable to the production of two- $X$  and no- $X$  eggs.

Wilson ('10) found an individual of *Metapodius* in which there were three homologous  $m$ -chromosomes. He explained this individual in the same manner as the result of the union of an exceptional two- $m$  gamete and the regular one- $m$  gamete.

In the case of *Diabrotica*, Miss Stevens ('08) found a variation in the number of supernumeraries from zero to four, with inequalities of the distribution in gametogenesis such as Wilson found in *Metapodius*.

The closest parallel, is perhaps in the case of *Ascaris megacephala* as reported by Frolowa ('12). Here the  $X$  is attached to the end of an autosome, as it perhaps is in *Drosophila*. However, Frolowa found in exceptional eggs that both sex chromosomes became attached to one member of the pair of autosomes instead of one  $X$  to each member. During maturation, there will arise two- $X$  and no- $X$  eggs determined by which autosome of the two, passes out into the polar body. Her diagram for *Ascaris* fulfils all the requirements for the case of non-disjunction in *Drosophila*, and, from cytological grounds, she reaches nearly the same conclusion with respect to the fate of the two- $X$  and no- $X$  eggs, as I do from the side of genetics.

The phenomenon of non-disjunction is most readily discovered in any form when the non-disjunction occurs in the sex chromosome, but it might occur with respect to any autosome, where it would only be detected in certain cases. I have a case which may be non-disjunction of the third chromosome of *Drosophila*, and probably cases of so called "monolepsis" or "faux hybrides" will be found to depend upon this phenomenon. Some forms of parthenogenesis probably differ from non-disjunction only in that the whole number of chromosomes is concerned and not one, or a limited number.

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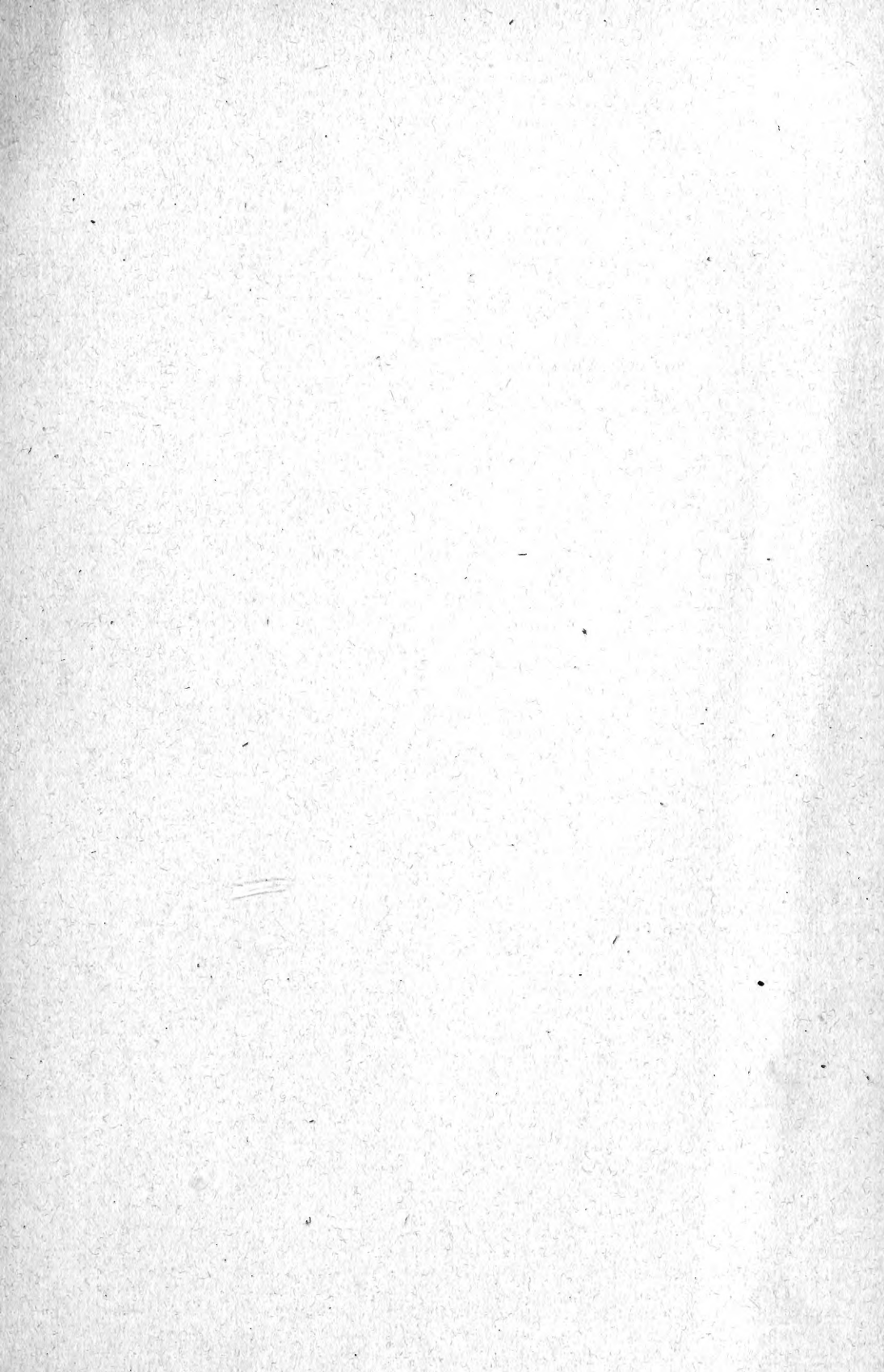
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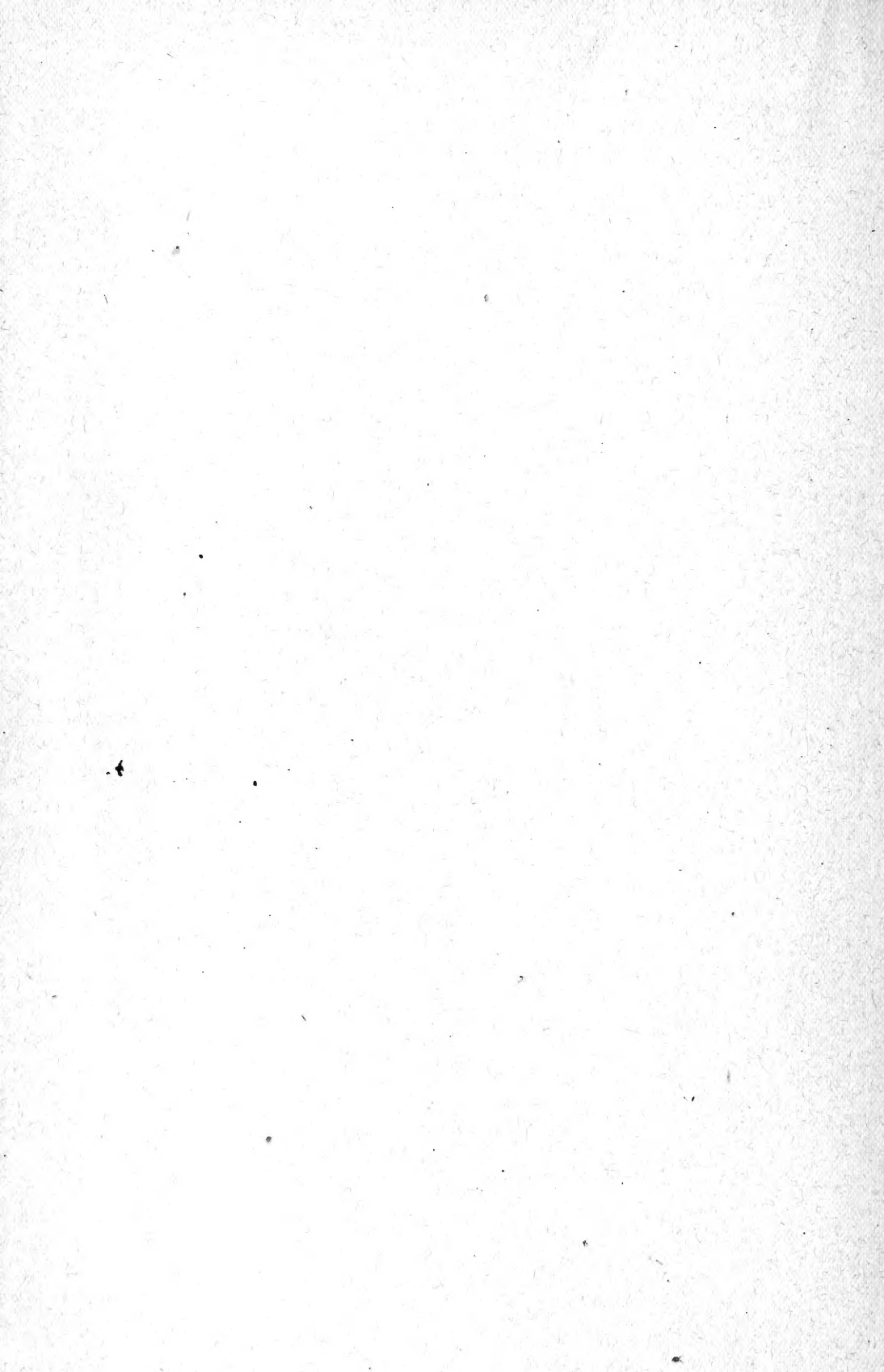
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