VII. *Transmission of Environmental Effects from Parent to Offspring in* Simocephalus vetulus.

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(Received October 15, 1912,—Read January 23, 1913.)

Contents.

1. Introduction.

Two distinct sets of phenomena can be distinguished, each of which gives the appearance of the inheritance of acquired characters. *Somatic induction* (Detto; = "Somatische Reizleitung" of Plate) corresponds to Lamarckian inheritance in its strictest sense, where the soma acquires a certain character in response to a certain stimulus, and then influences the germ plasm in such a way as to cause it to produce offspring exhibiting the same character even in the absence of the special stimulus that was needed to produce it in the ancestor. *Parallel induction* (Detto; = "Simultanreize" of Plate) is applied to cases in which the same external stimulus affects both germ plasm and somatoplasm in the same way, simultaneously but independently. Whereas probably most zoologists think that the existence of somatic induction has not been demonstrated, many well-established cases of parallel induction

Published separately, February 10, 1913.

have been described. Probably all cases of persistence of the effects of a previous environment on offspring bred in a different environment also come under this heading.

In the following pages I have described some experiments on parallel induction carried on on a large scale and with measurable characters, lending themselves therefore to biometrical treatment. Special attention has been paid to the all-important subject of controls, as will be described in the proper place. The organism I have employed is *Simocephalus vetulus*, a common Daphnid which adapts itself easily to breeding in captivity.

These experiments have so far occupied a period of three years, and were begun in 1909. The main results of the experiment on the first character dealt with were attained during the first few months of the experiment, and the delay in publication has been due to the difficulty experienced for a long time in repeating the production of the abnormality dealt with, in order to get a greater mass of material. In the meantime the work has grown, and has been extended to two other characters, influenced in different ways. During the time that has elapsed since the experiments began, two or three papers by WOLTERECK, dealing with closely related matters, have appeared. The relation of my experiments to those of WOLTERECK is discussed in the general part of the paper.

Three separate experiments have been carried out. Experiment A concerns a curious abnormality, consisting of a reflexion of the valves of the carapace, and Experiments B and C concern the size of the animal at birth. The characters were influenced strongly by known means, and then the persistence of the effect tested (by comparison with contemporary controls) in the generations produced after return to control* conditions. The results in the three experiments are very similar and, especially in the case of Experiment B, are on a large enough scale to be trustworthy. The most precise experiments on the production and physiology of the variation have been made in the case of the valvular reflexion (for production see especially Table III), but the general inheritance is shown most satisfactorily in Experiment B (Table VI), owing to the large numbers used and the uniformity of the control conditions.

2. BIOLOGY OF *Simocephalus vetulus*, SO FAR AS IT BEARS SPECIALLY ON THE EXPERIMENTS DESCRIBED.

a. *Sexual Conditions.*

All the individuals recorded in these experiments were produced parthenogenetically. Much work has been published on the subject of sexual cycles in Cladocera, and I hope to return to this question in a later paper. So far, my results on this score have been in accord with the very extensive experiments of WOLTERECK. At present I need only say that the line from which came all the specimens recorded

* By control conditions I mean always, unless otherwise stated, the comparatively "normal" conditions described on pp. 325—326.

in Experiments A and B is now in its 26th generation in captivity, and probably a much larger number of generations has elapsed from the original ephippial female, as the parthenogenetic female which was used to start the line was taken from a large tank in which sexual forms have been very rare. These 26 generations have occupied just over a year. The line does not show the least tendency to increased production of sexual forms nor to degeneration, though some authors, from incomplete experiments, have supposed that these phenomena would have shown themselves at a much earlier stage. Many thousands of specimens have been bred from the line in question, but as it is very difficult to tell the sex of *Simocephalus* in its first instar, and as the great majority of these specimens were measured in that instar and then rejected, we must confine ourselves to those individuals which became adult for definite information about sex. Altogether one or more specimens from 404 broods have been allowed to become adult, and of these only four gave males. So far from these appearing in later generations, or in later broods of a given generation, as some authors, *e.g.* PAPANICOLAU, suppose to be normally the case, the broods in which males appeared were as follows: One was a first brood of the 5th generation, one a first of the 6th, one a second of 6th, and one a first of the 8th. The remaining 400 broods all gave parthenogenetic females only. Not a single ephippial female has yet appeared in this line. This lack of sexual forms I ascribe to the favourable conditions under which the line has been bred.

b. *Growth and Egg Production.*

The eggs of *Simocephalus* are laid in batches. As soon as one batch is laid the ovaries are left devoid of yolked ova. Yolk deposition then begins in a new batch (consisting of from one to about thirty), and in four days at ordinary room temperature and with abundant food this is ready to be laid. As is well known, the eggs are laid, not directly into the water, but into a brood pouch. In two to three days the eggs hatch, and the embryos remain another couple of days in the brood pouch and then are born, *i.e.* pass out of the brood pouch to the exterior. In the following pages the words laying, hatching, and birth are often used, and in the senses just indicated. The process of giving birth to a batch of young is followed by an ecdysis of the parent and the laying of a new batch of eggs, the three processes generally being completed within a half hour.

When the young are born they are still in an embryonic state, but within a few minutes of birth they cast an excessively thin, transparent, and delicate cuticle. Immediately this cuticle is cast, they assume the form of the young *Simocephalus*. The animal reaches the adult condition by successive ecdyses. It is necessary for the description of these experiments to have an expression for the different stages of growth, and I have used the word *instar* of entomologists for this purpose. The following is a summary of the most important periods in the life of a *Simocephalus* at ordinary room temperature and with abundance of food :—

1. At moment of birth it is in its last embryonic instar.
2. Birth is followed immediately by the ecdysis of the embryonic cuticle, resulting in
3. First free living instar, lasting about three days (spoken of hereafter as the "first instar").
4. Ecdysis.
5. Second instar, lasting about three days.
6. Ecdysis.
7. Third instar, lasting 3–4 days. In this instar the first batch of eggs undergo their ovarian growth (yolk deposition), and I speak of it therefore as the "adolescent instar."
8. Ecdysis, followed immediately by
9. Laying of first batch of eggs and assumption of fourth instar = first adult instar. Four days after the beginning of this instar
10. Birth of the first batch of young takes place, followed immediately by an ecdysis and
11. Laying of second batch of eggs and assumption of second adult instar, and so on.

The number of adult instars may be very large, probably not infrequently as many as twenty, though I have not myself made precise observations above ten. The number of eggs in a batch varies greatly with the amount of food supplied. Other things being equal, it is less in the first batch than in the later ones. In the first it rarely exceeds a dozen, but later broods may contain 30–40 if the food supply is very abundant.

All growth takes place in the few minutes after ecdysis. Once the new cuticle has hardened, the dimensions of the animal are fixed for that instar. I have proved this by repeated measurements. The animal continues to grow at each ecdysis throughout life.

c. *The Supposed Nourishment of the Embryo by the Parent during Development in the Brood Pouch.*

Weismann considered that all Cladocera nourish their developing embryos in the brood pouch by a secretion passed into the pouch by the mother. In the case of such forms as *Moina*, *Polyphemus*, and *Bythotrephes* he has probably proved his point, but I feel fairly satisfied that in *Simocephalus* (and also *Daphnia*) the embryo receives no nourishment from its parent.

Weismann based his conclusion that nourishment is passed from parent to offspring even in these two genera from a number of separate pieces of evidence, of which the following are the chief:—(1) If the eggs are extracted from the brood pouch, they fail to give rise to living young, death taking place either before or after hatching, but in any case before the first free living instar is reached; (2) the embryo is considerably larger than the egg from which it develops; (3) the winter eggs (which develop

outside the brood pouch) are much larger than the summer eggs, and yet the young emerging from the former are scarcely appreciably larger (60 : 55); (4) the fluid in the brood pouch sometimes appears yellowish.

As regards the first argument, my own experience as to the development of eggs which have been extracted from the brood pouch constitutes the strongest possible evidence against the theory of the maternal nourishment of the embryo. It is true that eggs extracted from the brood pouch within a few hours of oviposition very often die—almost always if they are placed in a watch-glass or other shallow vessel. Death, however, takes place as often before hatching (*i.e.* before the eggs could have received nourishment even if left in the maternal brood pouch) as after. Moreover, the small percentage of cases in which the development of such extracted eggs pursued a normal course appeared to constitute evidence against the cause of death of the others being lack of maternal nourishment. A number of different methods were subsequently tried in the hope of obtaining evidence of a more precise character. Mechanical stimuli of various sorts, running water, heat, cold, proved unsuccessful. At last a very simple method was found by which extracted eggs can be made to develop in a comparatively large percentage of cases. It consists in removing the eggs, as soon as they have been extracted from the brood pouch, into a deep vessel instead of the shallow watch-glasses which had been used almost exclusively before. The vessels used were narrow test-tubes. The following experiments illustrate the power of eggs to develop outside the brood pouch :—

1910.

Jan. 21.—*S. vetulus.* Five eggs laid between 9 and 10 A.M. Eggs extracted from brood pouch at 4 P.M. same day.

,, 24.—All five hatched.

,, 25.—Ant. 2 free, making feeble movements.

,, 27.—All normal *Simocephalus* of first instar.

1911.

June 8.—*S. vetulus.* About nineteen eggs laid, 5–6 P.M. Extracted from brood pouch 6.45 P.M. same day.

,, 14.—Six normal young, remaining eggs not having hatched.

May 1.—*S. exspinosus.* Twelve eggs laid 1 P.M. Extracted 2 P.M.

,, 7.—Twelve normal young of first instar.

,, 1.—*S. exspinosus.* Thirteen eggs laid 10.40 A.M. Extracted 12.10 P.M.

,, 4.—All hatched.

,, 7.—Thirteen normal young of first instar.

April 10.—*D. pulex*, two specimens. One laid nine eggs, 10.20 A.M. Extracted 10.50 A.M. Other laid thirteen eggs, 11.30 A.M.–12.30 P.M.. Extracted 12.30 P.M. Seventeen eggs from these two specimens put in tube together.

,, 12.—Hatched.

,, 14.—Fourteen normal young of first instar.

In the case of all these three species the young obtained from eggs which developed outside the brood pouch do not differ from those normally born. The latter contain a large amount of unabsorbed yolk and fat in their tissues at birth, and young obtained from extracted eggs have about the same amount, though actual measurements of the amount present were not made. Moreover, there is no constant difference in size between the young obtained in the two ways.

Thus one of WEISMANN'S strongest pieces of evidence—the behaviour of eggs extracted from the brood pouch—is seen really to furnish very strong evidence that no nourishment passes from the mother to the embryos in the pouch.*

It is of course evident that the death-rate amongst eggs and embryos removed from the brood pouch is much higher than in the case of those developing normally, and the examples given are the most successful experiments which were made. The cause of death is rather obscure, but it is most probably due to injury of the vitelline membrane of the egg or soft cuticle of the newly hatched embryo by contact with the glass of the vessel in which they are lying. The evidence for this conclusion is that both eggs and young embryos tend even while alive to adhere to the glass, it often being very difficult to dislodge them, while in the brood pouch they are freely movable over the lining of the pouch.

In this connection it may be mentioned that HÄCKER states that eggs of *Cyclops* will not develop if the egg sacs are removed from the parent. Here, at any rate, there is no question of receiving nutriment from the mother.

WEISMANN'S remaining arguments need only be touched upon very shortly. He himself admits that the difference in size between embryo and eggs may be due to absorption of water, and, as a similar difference is found to exist between these stages when development has taken place outside the brood pouch, we shall not be running much risk in supposing that this is the case.

The argument drawn from the nearly equal size of the young developed from winter and summer eggs was founded upon a single measurement of the winter egg young. And even if substantiated, as it quite probably could be, it could not by itself be taken as important evidence that the brood pouch embryo receives nourishment from the parent. I have found in my own experiments that a variation of as much as 20 per cent. of the length of parthenogenetic young is not very rare.

As to the argument that the fluid in the brood pouch is sometimes yellow, I can only say that I have only observed this extremely rarely, and then only in injured specimens in which it appeared to be due to extravasated blood.

* It is worth recording that the posterior spine of the embryo *Daphnia* is bent down at right angles to its future position, so as to lie close alongside the posterior border of the carapace instead of projecting from it. Now this position of the spine might easily be supposed to be caused directly by the pressure on the still soft spine of the walls of the brood pouch and of the neighbouring young. Yet this spine is similarly bent in the embryos developed from eggs extracted from the brood pouch before hatching.

Moreover, there is no definite apparatus for passing nourishment into the brood pouch, though such is present in *Moina*, *Polyphemus*, and *Bythotrephes*. Finally, the pouch in *Simocephalus* is most incompletely closed, so that enormous waste of nutritive material would take place if it were secreted into it. This was shown by putting individuals into water in which carmine was thickly suspended. On putting them back into pure water after a short stay in the carmine, the brood pouch may be seen to contain a larger or smaller amount of carmine granules. These are gradually got rid of (some, however, adhering to the inner surface of the carapace) mostly by spasmodic flexions of the abdomen, which the animal carries out periodically, and which open the brood pouch widely to the exterior. In case it may be said that these flexions were stimulated by the presence of the grains in the pouch, and do not occur naturally except when giving birth to young, it should be stated that I have carefully watched *Simocephalus* hanging on to the vertical glass wall of an aquarium, and have seen that under normal conditions it does perform these flexions periodically, for the purpose apparently of getting rid of the useless solid matter which has been drawn into the carapace chamber with the respiratory stream.

I have given the evidence rather fully for the conclusion that in *Simocephalus* (and *Daphnia*) the eggs and embryos are not dependent on nourishment received from the mother during their development in the brood pouch, partly because such nourishment might be thought to account for part of the "inheritance" to be described later (though, of course, only for the F_1, not in any case for F_2 or F_3; see also p. 337, footnote); and partly because I wish to correct what I believe to be an error which has found its way into text-books, and which is accepted without question by workers on these animals, *e.g.* WOLTERECK (10), KUTTNER.

3. METHOD OF BREEDING EMPLOYED.

Except where the contrary is stated, the specimens used in these experiments were bred in cylindrical glass tubes, 10 × 3 cm., when corked containing about 50 c.c. of water and 15 c.c. of air. One specimen only was kept in each tube, and when a brood was produced it was removed within 24 hours. Such tubes make excellent and most convenient breeding cages for *Simocephalus* and *Daphnia* of various species, and during the last seven or eight years I have bred tens of thousands of individuals in these tubes. For food an inexhaustible supply was at hand from a heated tank in which are living a number of *Lepidosiren paradoxa*, fed daily with *Anodonta*. The water in this tank, which is changed weekly, is turbid with mud and organic matter, and has proved an excellent medium for cultivating Cladocera of various kinds, as shown by the very low death-rates and regularity of reproduction obtained. A jar of water was taken from this tank on alternate days, strained through linen to remove Rotifers, Lynceids, etc., which swarmed in it, and used to renew the water every alternate day in the breeding tubes. The *Simocephalus* was picked out of the

tube with a pipette, the water poured off, and then the fresh water and the animal returned. The jar containing the water was well shaken up before filling each tube, so that every *Simocephalus* was provided on the same day with approximately the same amount of a practically identical food medium. The tubes were kept in a rack in the laboratory, and when it was a case of comparing controls with specimens whose ancestors had been treated in different ways, the tubes containing the various classes of individuals were well shuffled on the rack in order to distribute any minimal differences of temperature, light intensity, etc., which might occur in the area occupied by the rack. In this way the conditions under which the animals under comparison were living were as similar as they could well be made. These are the conditions under which the majority of specimens were kept, and wherever the animals were kept under different conditions—*e.g.* those in which the various abnormalities described were produced—these conditions will be described.

4. Experiment A.—Reflexion of the Valves of the Carapace.

a. *Nature and Cause of the Variation.*

This variation, or abnormality as it may certainly be called, is a very curious one. It consists in a change in the curvature of the valves of the carapace, so that a transverse section of the creature is bell-shaped instead of oval (figs. 3 and 4). If a normal *Simocephalus* is kept alive in a watch-glass containing enough water to allow of its free movement, when it comes to rest it takes up a position lying balanced on its back. One looks through the microscope, therefore, on to the ventral surface of the animal, and obtains the view shown in fig. 1. From this point of view the thoracic appendages are nearly but not quite concealed by the carapace, for the valves of the latter are not in contact in the mid-ventral line, but leave a tolerably wide slit between them, through which can be seen these appendages making their rhythmical respiratory movements.

The slit left between the edges of the valves is protected by stiff setæ which project inwards from a setigerous ridge near the edge of each valve and by interlacing with those of the other side form a sort of sieve (fig. 1). It is probable that these have the function of preventing large objects passing into the carapace cavity, for if a *Simocephalus* is watched lying in its usual position on its back, sometimes a small creature, such as a small Cypris or Chydorus may be seen to crawl over it, and I have seen such an organism, quite small enough to drop through the slit between the valves into the carapace cavity, creep over the sieve apparatus which protects it.

In the abnormal individuals under consideration, the reflexion of the valves causes the thorax and abdomen to be fully exposed as the animal lies on its back (fig. 2), and the protective setæ no longer project inwards to form the sieve apparatus, but

are turned outward and must be functionless. Sometimes only one valve is reflexed, the other being normal.

In spite of the obvious abnormality of this condition affected animals appear quite healthy, and reproduce freely. They take rather longer than normal specimens to

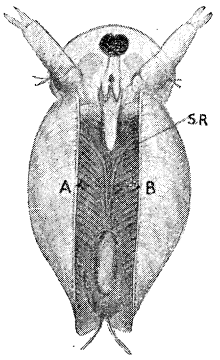

FIG. 1.

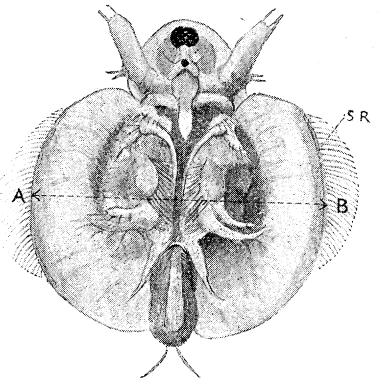

FIG. 2.

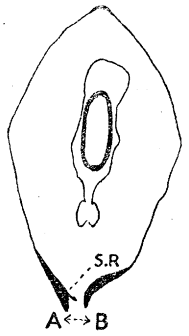

FIG. 3.

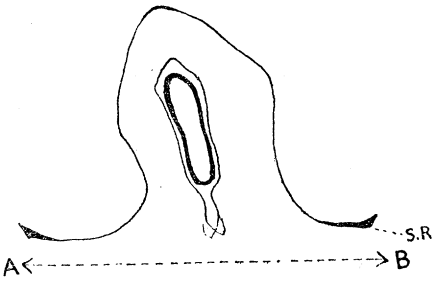

FIG. 4.

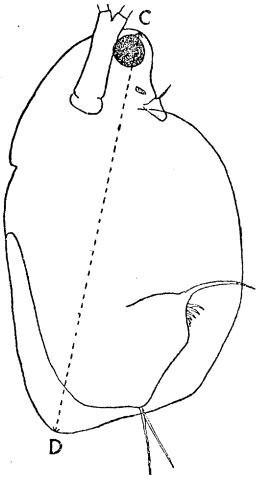

FIG. 5.

FIG. 1.—Ventral view of a living, normal, *Simocephalus vetulus*. Drawn under anæsthesia.

FIG. 2.—Similar view of a specimen with reflexed valves. The L/W ratio of this specimen was 1·12.

FIG. 3.—Transverse section of a normal specimen; the section passes through the bend of the abdomen. Sublim.-acetic.

FIG. 4.—Similar section through a specimen with reflexed valves. L/W = 1·37. Sublim.-acetic.

FIG. 5.—Side view of a normal specimen in its first instar. This figure is on a larger scale than the others.

(A–B, Line along which intervalvular width, W, is measured. C–D, line along which length, L, is measured. SR, setigerous ridge.)

(All the figures are drawn with the Abbé Camera.)

attain to maturity, however, as is well shown when one member of a brood is more abnormal than its sisters.

The cause of the abnormality in question seems to be the nature of the food. It was first observed in specimens living in the breeding tubes already described, not

in *Lepidosiren* tank water, however, but in a culture of Protophyta grown in a mixture of cowdung, soot, and water. Later, this culture was replaced by one grown in the chemical solution recommended by Klebs for the cultivation of *Chlamydomonas*. No attempt was made to obtain pure cultures of any one organism, and several species were probably always present, the food of the *Simocephalus* consisting for the most part of *Protococcoidea* and *Confervoidea*.

Besides *S. vetulus*, the abnormality was also observed in *S. exspinosus* living in the same medium, but no experiments were made on this species. Several species of *Daphnia* were kept from time to time in the same medium, but the abnormality was never observed in these.

The valvular reflexion is almost certainly caused by the nature of the food ingested, and not by the chemical composition of the medium. For if the organisms growing in the Klebs' solution are centrifuged and washed two or three times with tap water (being centrifuged again after each washing), and are then added to tap water (or sterilised water from an aquarium), this proves just as potent to produce the abnormality as if the entire culture—organisms and culture medium together—had been used.

Although the effect is thus produced by the nature of the food it is impossible to give precise directions for obtaining it. Many protophyte cultures prove very potent to bring it about, while others—apparently similar—have no such power. I find that a given culture generally retains the power for a few weeks or months, and then loses it. Doubtless the precise factor (species, stage in life-history, of food organisms ?) needed could be isolated, but I have not done so. Once, and once only, out of thousands of individuals, a brood with strongly reflexed valves was born in a medium in which no protophyta were known to be present, and in which they were certainly not abundant. All the individuals in this brood were, however, pathological, and all died in a few days.

b. *Method and Reliability of Measurements.*

There are two ways in which the reflexion of the valves may be recorded. First, as each individual is passed under the microscope it can be noticed whether the valves are reflexed or not. This, however, takes no account of the degree of reflexion. Secondly, it is possible to measure the "intervalvular width," *i.e.* the distance between the edges of the valves (the distance AB in figs. 1–4). A difficulty comes in here, namely, that the width between the two edges of the valves is affected by two separate factors: (1) the curvature of the valves and (2) the state of contraction or relaxation of the adductor muscles.

Simocephalus is able by a strong muscular effort to bring the edges of the valves practically into contact, closing the carapace cavity ventrally. Although it is generally possible to determine whether the muscles are completely relaxed or not, it is not always so. Consequently a source of inaccuracy is present here.

As the intervalvular distance increases with the general increase in size of the animal, the measurements are all given as the ratio length : intervalvular width, so as to allow of comparison of animals of different sizes. The mean L/W ratio in the first instar for specimens bred in the *Lepidosiren* water tubes is about 4·5. In the most pronounced cases of the abnormality the reflexion of the valves is carried to an extraordinary extreme. I have measured many specimens with a ratio below 1·5, the extreme going down even to 1.

The measurement of length is a simple one. The animal is put in a flat-bottomed watch-glass, and the water drained off with a pipette till the animal falls on its side, incapable of movement. The greatest length of the animal—*i.e.* from front of head to posterodorsal angle of carapace—is then easily measured with an eyepiece micrometer (see fig. 5). A slight complication comes in with the very abnormal specimens (those with L/W ratio below about 1·5), as in these the valves are too widely recurved to allow the animal to fall on its side. When the water is drained off it is stranded on its back, and the length measurement has to be made on the animal lying in this position. Owing to the greatest length being along a line which makes an angle with the dorsal line of the animal, the side length measurements give a slightly higher figure than back measurements. From the determination of the lengths of a large number of individuals by both methods, it was found that to convert back to side length measurements it is necessary to multiply the former by 1·06. In Table IV the columns "All Broods Born in Protophyte Culture" and "F_1 I" contain many measurements corrected in this manner, but none appear in any of the other columns.

The intervalvular width is measured thus :—The animal is placed in a shallow watch-glass, in enough water to keep it well covered when in its natural position of ventral side uppermost. Having got it covered by the eyepiece micrometer scale, it is necessary to watch it carefully for a few seconds, or it may be longer, to ascertain that the adductor muscles are in the position of rest. In most cases this is not a difficult thing to do approximately, as it is seldom that the valves are kept closed for more than a few seconds. Should they be in this condition they will be seen to widen out gradually till they come to a state of rest, when the distance between the two edges is read off on the scale. Individuals which have been much knocked about are apt to keep their valves obstinately half shut. It is seldom that the mistake will be made of obtaining too great a value for the measurement, for though *Simocephalus* is able to open its valves slightly beyond the normal resting position, the movement is a spasmodic one, resembling a yawn and quickly over. This action generally accompanies the act of defæcation or of ejection of solid accumulation from the carapace cavity.

The accuracy of the measurements used in the following Experiment A is therefore not so great as that of those usually employed in biometrical work, and in order that the reader may see for himself what reliability may be placed upon them, Tables I and II are given here. While it will be seen from them that the inaccuracy is

considerable, it must be remembered that it is trifling when account is taken of the enormous difference between fully reflexed* and normal individuals; and also that in testing the "inheritance" of the reflexion in descendants in control conditions, large numbers of specimens have been used, so that it may be taken that the plus and minus errors will be approximately equal in the compared classes of individuals.

Table I deals with a brood of eight individuals which were measured in their first instar. After measurement each was put in a separate marked tube, and later anæsthetised with acetone chloroform and measured again. In four cases the L/W ratio before and after anæsthetising are the same; in the other four the second ratio is lower than the first, showing that in the first measurement the adductor muscles were not fully relaxed.

TABLE I.

	L/W ratio.	
	Before anæsthetising.	Under anæsthesia.
1	3·09	3·09
2	3·25	3·25
3	3·58	3·58
4	3·89	3·68
5	3·89	3·50
6	4·37	3·89
7	4·75	4·75
8	4·93	4·11

Table II shows 15 individuals, taken from various broods, each of which was measured and then put into a marked tube, and after a certain lapse of time measured again, put back into its tube, and after another interval again measured. The remeasurements were always made without referring first to the mark of identification on the tube. The measurements were, of course, all made within the same instar (the first).

* Of course, in the case of these, the *determination* of reflexion does not depend upon measurement, but is visible at once from the shape of the valves and the position of the rows of straining setæ—*i.e.* turned outwards instead of inwards.

TABLE II.

Specimen.	First measurement.	Number of hours elapsed between first and second measurements.	Second measurement.	Number of hours elapsed between second and third measurements.	Third measurement.
1	1·19	3½	1·20	4	1·24
2	1·21	3½	1·21	4	1·21
3	1·63	3½	1·58	4	1·52
4	1·98	3½	1·81	4	1·91
5	2·11	3½	2·00	3½	2·00
6	2·42	3½	2·27	3½	2·30
7	2·71	3	2·88	3½	2·71
8	2·94	3	2·72	3½	2·78
9	3·71	2	3·87	5	3·71
10	4·14	2	4·14	5	4·14
11	4·35	2	4·53	5	3·95
12	4·48	2	4·78	5	4·20
13	5·00	2	4·65	5	4·39
14	5·00	2	5·06	5	4·44
15	5·79	2	5·06	2½	6·23

c. *Production and Transmission of the Variation.*

The general course of acquirement and transmission of the character is illustrated by the simple experiment shown in Table III.

Specimens 1–8 in the first vertical column were eight sisters, belonging to the same control brood. They were born on February 9 and their L/W ratios determined on that day. These are given in the column headed February 9. They were then divided into two sets. Nos. 1–4 were put singly into the usual control tubes, Nos. 5–8 were put singly into tubes of sterilised aquarium water, to which were added centrifuged protophyta from Klebs' culture solution.

The L/W ratios were again determined in the adolescent instar (February 16 for the controls, February 14 for the others). These are given in the third vertical column. A slight complication arises here, owing to the fact that in normal animals the L/W ratio does not remain constant throughout life, but gradually increases. Hence in order to make comparisons between measurements of the same animal in different instars, it is necessary to allow for this natural increase. This is most easily done by reducing the later ratios by a factor which represents the mean increase for normal specimens for the number of instars which have intervened between the two measurements. Thus it was found necessary to multiply the adolescent (third instar) ratios by 0·807 to make them comparable with the first instar measurements. All the eight ratios recorded on February 14–16 are therefore the actual ratios, multiplied by 0·807. It will be noticed that in every case the ratios of Nos. 5–8, feeding on the protophyta in the Klebs' culture solution, have dropped—*i.e.* the valves are becoming reflexed. The mean ratio for the four specimens has fallen from 4·54 to 3·63.

TABLE III.—Showing Somatic Acquirement of Reflexion of the Valves, and its Transmission to Offspring after Parents have been Returned to Normal Conditions. For full explanation see text.

Specimen.	L/W, first instar.	L/W, adolescent instar.	L/W, first adult instar.	Mean L/W of first broods in first instar.	Number of specimens in first broods.
A. Controls, *i.e.* born in normal condition and left there all their lives.					
	Feb. 9.	Feb. 16.	Feb. 18.	Feb. 22.	
1	4·61	3·99	5·01	4·67	6
2	4·10	3·98	5·16	4·12	4
3	4·61	5·38	4·01	4·37	5
4	4·94	4·91	4·08	4·25	6
Mean . . .	**4·56**	**4·56**	**4·56**	**4·35**	
B. Sisters of the above controls, born in normal conditions, but placed in protophyte culture within 24 hours of birth. Left in culture till within 14 hours of laying first batches of eggs, when they were put back into control conditions identical with those for Nos. 1–4.					
	Feb. 9.	Feb. 14.	Feb. 18.	Feb. 20–21.	
5	4·35	2·84	1·42	1·61	5
6	4·61	2·96	2·32	1·49	12
7	4·61	4·28	2·37	2·04	8
8	4·61	4·44	2·01	2·20	7
Mean . . .	**4·54**	**3·63**	**2·03**	**1·83**	

Nos. 5–8 were left in the protophyte culture till their ovaries were well developed and the eggs evidently about to be laid. Then (on February 15) they were removed to control conditions, identical with those in which Nos. 1–4 were living. The eggs were laid in all four cases within 14 hours after removal from the protophyte culture.

In the fourth vertical column, under February 18, are given the L/W ratios of the eight sisters in their first adult instar. To make these comparable with the first measurements, they are all multiplied by 0·474.* It will be observed that the ratios of Nos. 5–8 have dropped enormously, showing the somatic acquirement of the

* Although the increase in the ratio with age has been apparent throughout the other experiments also, I had not satisfactory data from these for determining the actual increase from instar to instar, so that the factors used here were determined from Nos. 1–4 themselves, which accounts for the identity of the mean ratio for the four specimens in the three measurements. Although the use of such a small number of observations is somewhat unsatisfactory, this is immaterial for purposes of comparison between Nos. 1–8, as all have been treated by the same factor.

character we are considering. These third measurements of Nos. 5–8 were of course made on the animals after putting back into control conditions, as this was done at the end of the adolescent instar. The build which a *Simocephalus* assumes after any ecdysis is, however, determined by the conditions under which it passed its previous instars. Now Nos. 5–8 were all removed from the protophyte culture tubes a few hours before laying their eggs, and therefore before the ecdysis which separates the adolescent from the first adult instar, and thus the ratio assumed in the latter instar is due to the previous sojourn in the protophyte medium.

The first broods from the eight sisters—now all living under the same control conditions—were born on February 20–22, and the mean L/W ratios of each brood in its first instar are given under these dates. The broods of Nos. 1–4 are of course quite normal. Those of Nos. 5–8, however, exhibit very pronounced reflexion of the valves, as indicated by the very low ratios. Thus *although the individuals were removed to control conditions before the eggs were laid, nevertheless the young developing from these eggs exhibit the same abnormality as that which their parents had acquired during their ontogeny as a direct result of their environment.* This result has been confirmed over and over again in all the three sets of experiments. (See Tables IV, VI, and VII.)

An experiment similar on the whole to that described in Table III (and, indeed, including that experiment) but on a statistical scale, and arranged so as to test the persistence of the effect for three generations, is shown in Table IV. The 1120 specimens there recorded were all descended by parthenogenesis from the same original female. The table is divided into two portions, according to the way in which the controls, and the individuals which were tested against them, were kept. The individuals referred to in the upper half of the table (November 14–January 11) were not kept in the usual way (p. 325) but in cylinders of glass, 10×3 cm., closed at each end with muslin and suspended in a large aquarium. The controls and individuals compared with them being thus suspended side by side in the same aquarium, with the water in it in free communication through the muslin ends with the insides of the tubes, the conditions under which they were living must have been practically identical. The only drawback to this method was the paucity of food in the aquarium, which caused the number of young per brood to fall off very much towards the end of the experiment, and also much prolonged the time taken to reach maturity. The individuals referred to in the lower part of the table (December 24–February 25), controls and controlled alike, were kept in the usual way, as described on p. 325.

The table is divided into horizontal rows, each corresponding to one week, so as to allow of comparison between nearly synchronous births. As will be seen from the controls, the mean ratio varies considerably from time to time, even under control conditions—probably from slight variations in temperature and food supply. Consequently it is not satisfactory to average the ratios of all the individuals in each class and compare

TABLE IV.—Showing Transmission, etc., of the Reflexion of the Valves. Under line between weeks January 4–11 and December 24–30 divides the table into

Week.	Controls, all broods.			All broods born in protophyte culture.			First broods $F_1 = F_1$ I.		
	Mean L/W.	No. of broods.	No. of individuals.	Mean L/W.	No. of broods.	No. of individuals.	Mean L/W.	No. of broods.	No. of individuals.
Nov. 14–22 (9 days) .	...	...	...	1·49 – 4·73*	3	28			
Nov. 23–29 . . .	6·22	4	39	1·66 – 4·56	1	10	3·29 – 2·93	11	54
Nov. 30–Dec. 6 . .	7·36	5	39	...	...	...	3·80 – 3·56	2	4
Dec. 7–13	7·03	2	3	...	...	...	2·95 – 4·08	3	4
Dec. 14–20	6·36	1	1	...	...	...	...	...	...
Dec. 21–27	7·11	3	5	...	...	...	...	...	...
Dec. 28–Jan. 3 . .	5·62	1	1	...	...	...	...	...	...
Jan. 4–11	5·07	1	1	...	...	...	...	...	...
Dec. 24–30	4·88	1	6	2·02 – 2·86	2	15			
Dec. 31–Jan. 6 . .	4·32	3	16	1·67 – 2·65	1	11	2·10 – 2·22	2	22
Jan. 7–13	4·83	5	28	...	...	...	2·88 – 1·95	5	33
Jan. 14–20	4·59	10	69	...	...	...	2·47 – 2·12	3	14
Jan. 21–27	5·54	5	21	...	...	...	1·88 – 3·66	1	6
Jan. 28–Feb. 3 . .	4·35	4	26	...	...	...	...	...	...
Feb. 4–10	4·82	9	47	...	...	...	...	...	...
Feb. 11–17	4·16	2	15	...	...	...	...	...	...
Feb. 18–25 (8 days) .	4·24	8	55	...	...	...	1·82 – 2·42	4	31
Totals . . .	...	65	372	...	7	64	...	31	168
Mean weekly difference from controls .	...			– 3·87			– 2·76		

* The difference in the first period was calculated from the control mean

each mean is shown its difference from the control means for the same week. The the two portions mentioned on p. 333. For full explanation see the text.

Second broods F_1, = F_1 II.			Third broods F_1, = F_1 III.			F_2, all broods.			F_3, all broods.		
Mean L/W.	No. of broods.	No. of individuals.	Mean L/W.	No. of broods.	No. of individuals.	Mean L/W.	No. of broods.	No. of individuals.	Mean L/W.	No. of broods.	No. of individuals.
6·55 −0·81	8	32	7·13 −0·23	7	44						
...	...	...	...	...	...	5·62 −0·74	1	2			
...	...	...	...	...	...	5·17 −1·94	1	1			
...	...	...	...	...	...	5·34 −0·28	2	3			
...	...	...	...	...	...	5·50 +0·43	1	1			
4·88 +0·56	1	5									
4·93 +0·10	2	12	6·40 +1·57	1	3	4·6 −0·23	1	3			
5·83 +1·24	1	6	4·81 +0·22	2	13	4·57 −0·02	4	32			
4·35 −1·19	1	4	5·00 −0·54	2	8	4·65 −0·89	14	50	4·67 −0·87	1	6
...	...	...	...	...	...	4·43 +0·08	23	143	4·85 +0·50	3	23
...	...	...	...	...	...	4·64 −0·18	13	73	5·28 +0·46	5	18
...	...	...	...	...	...	4·41 +0·25	2	7			
4·58 +0·34	3	27									
...	16	86	...	12	68	...	62	315	...	9	47
−0·29			−0·06			−0·25			+0·33		

for the succeeding week, as there were no contemporary control broods.

the values arrived at in this way. Especially it will be noticed that the ratios of the controls in the aquarium are uniformly higher than those in the Lepidosiren water. (The table was also worked out for three-day periods and gave closely similar results.)

As an example of the way in which the table is to be read, we may take the week January 7–13. In that week five control broods were born, with a total of 28 individuals. The mean L/W ratio of each brood (as always, in the first instar) was found, and then the mean of the five means, which gives the value 4·83. Throughout the table the means are similarly means of brood means and not of individuals, though, of course, the results would have been closely similar if the other method had been used.

This method was chosen because each brood forms a fairly closely correlated group of individuals, and to find the mean of all the individuals born in any period would be to give too great a weight to broods containing a large number of individuals. When the number in a brood was more than 10, only the first 10 taken at random were measured in almost all cases.

In this week no broods were born in the protophyte culture. Five broods are entered under the F_1 I column. These are the first broods born from individuals belonging to broods born in protophyte culture in an earlier week, which were removed from this medium to control conditions with eggs ready to be laid, but never with eggs already laid.* As we saw in Table III, the valvular reflexion still persists, the mean of the five brood means being 2·88, or 1·95 less than that of the corresponding controls. Two F_1 II broods were born this week—that is to say, the second broods after removal of the parents from the protophyte culture. The mean of the two brood means is 0·1 above that of the corresponding controls. One third brood of the same generation was born that week, averaging 1·57 above the control mean. Under the F_2 column there is one brood shown with three individuals in it, averaging 4·6, or 0·23 below the corresponding controls. All the F_2 broods are the offspring of isolated F_1 I specimens, kept, of course, under control conditions. (At birth, as we have seen, these F_1 I individuals have strongly reflexed valves. At each successive ecdysis, however, the degree of reflexion becomes less, and by the time maturity is reached they are about normal.) Some specimens belonging to first broods of F_2 were isolated and provided the F_3 generation—none of which were born in the week in question.

Under the columns headed "Controls," "All broods born in protophyte culture," "F_2" and "F_3," all broods are included without discrimination into first, second, etc., broods. (Analysis of the control broods showed, as was to be expected, that there is no significant difference between the mean ratios of broods of different orders.)

* Some of the F_1 specimens are descended from parents which were not born in the protophyte medium, but were put into it when young, and acquired the variation ontogenetically (like Nos. 5–8 in Table III). Others had a considerable protophyte fed ancestry. It appears to make no difference to the persistence of the reflexion how long the ancestry has been subject to the influence.

In the case of F_1 the broods have to be registered separately, as the eggs from which the first broods came matured in the ovary in the protophyte culture, and those of the subsequent ones in control conditions.

The bottom line of the table gives the mean weekly difference of the brood means of each class from the corresponding control mean. On the average, the broods born in the protophyte culture have a ratio 3·87 lower than that of the controls. The F_1 I broods have on the average a mean ratio 2·76 below the control mean—still a very great difference, but not so great as that between the controls and the protophyte broods. Three possible reasons for the apparent partial disappearance of the abnormality present themselves :—

(1) The F_1 I young developed in brood pouches of parents in control conditions, and hence, as we have seen, exposed by the incomplete closure of the brood pouch to the control medium instead of to the protophyte medium. As, however, the reflexion effect is produced by the food and not by the medium itself, and as the young do not feed till they have assumed the first instar, in which they are measured, it is very unlikely that this is the reason. The possibility is, moreover, negatived by an experiment the converse of the one described, where normal specimens were put from control conditions into protophyte culture with eggs ready for laying. The first broods born under these conditions are completely normal.*

(2) The composition of the eggs in the two cases probably differs slightly, because the parents of the F_1 generation were removed to control conditions a few hours before completion of the ovarian growth, while their parents were in the protophyte culture during the whole of this period.

(3) It may be due to mere error of random sampling, the number of broods in the two classes being small and their variability very large.

From the summary line it appears that the effect still persists, but in a rapidly diminishing degree, in the second and third broods of F_1, but the results in the different weeks are so irregular and the total mean difference so small that the table itself cannot be said to give inequivocal evidence, especially when the fairly high degree of inaccuracy in the original measurements is considered. The whole summary line, however, bears such a striking resemblance to that of Table VI that there can be little doubt that it substantially represents the course of transmission. This being so, we see that the effect still persists in F_2. The reaction in F_3 is very interesting, and again confirmed by Table VI. The significance of this is discussed later.

At the Fourth International Genetic Conference in Paris, 1911, I gave an account of an earlier experiment performed in 1909 similar to that just described. The reflexion of the valves was produced in this case by a protophyte culture in cowdung and soot, and its persistence tested on individuals suspended in cages in an

* This experiment also disposes of the possibility that the persistence of the abnormality might be due to maternal influences on the developing embryos, even if such influence was possible through the supposed maternal secretion.

aquarium, as in the first part of Table IV. The result, except for F_3, which consisted of two individuals only and may therefore be neglected, was precisely the same as that shown in Table IV, but as it was on a much smaller scale, and less satisfactory in that the controls were not known to belong to the same pure line as the specimens which were tested against them, I have not given a detailed account of it here.

5. Experiment B.—Reduction of Length of Animal Caused by High Temperature.

a. *Nature and Causes of the Variation.*

The character dealt with in this, the most extensive of all the experiments, is the simple one of the length of the *Simocephalus* in its first instar. This length depends mainly or entirely on the size of the egg from which the young developed, though it is conceivable that it might be influenced to a much slighter degree by the stage of yolk absorption at which the animal is born and assumes the first instar. Besides the *à priori* probability, there is convincing indirect evidence, from my experiments, of this dependence of size of young on size of egg, but I have not found it practicable to show it directly, owing to the difficulty of getting a satisfactory measurement of the eggs. These begin to increase in volume directly they are laid, and go on doing so till they are hatched—presumably by absorption of water. Hence, in order to compare the sizes of the eggs of two parents, it is necessary to be sure that they are the same number of hours or even minutes old. I have measured newly laid eggs at intervals of less than an hour and found a very sensible increase. Under these circumstances it is not practicable to collect comparable measurements of eggs on a statistical scale.

The means used for inducing the variation in this character was differences of temperature, the two temperatures used being ordinary room heat (about 15·5°–18·5° C.), and one of about 28·5°–31·5° C.

The individuals used in this experiment, and recorded in Table VI, were all kept in the usual tubes and in Lepidosiren water, and treated as described on p. 325, except in the case of those individuals which were subjected to the high temperature. The tubes containing these were floated in a heated tank kept at the above-mentioned temperature.

The effect of the high temperature is to diminish very greatly the size of the young in their first, and indeed in all, instars. It also enormously increases the rate of the life cycle—*i.e.* from birth of parent to birth of young—which is completed in 6 days at 30° and in 14 at 16°. The number of young per brood is diminished. Among broods born in control conditions, there is a high negative correlation between the size of the young composing a brood and their number, large size being correlated with small number. Hence, the diminution in size at the high temperature is physiologically even more noteworthy than it appears, being accompanied as it is by a decrease in the number per brood.

Another factor affecting the size of the young is the order of the brood. On the average, the first broods have the smallest young, and the size increases up to the fourth brood, after which the increase appears to cease, though I have not abundant enough comparable material of broods of a higher order than this to make very definite statements about it. As the number of young is also lowest in the first brood, and increases in later ones, it is obvious that the correlation between size and number of young only applies when broods of the same order are compared.

TABLE V.—Showing Mean Lengths of Young of Broods of Different Orders. Unit of Measurement = 0·018 mm. Wherever there were more than 10 specimens in a brood, only 10 (at random) were measured.

Order of brood.	Number of broods.	Number of individuals.	Standard deviation.	Mean length.
First broods	63	396	1·686	43·184 ± 0·057
Second broods	56	429	1·752	44·012 ± 0·057
Third broods	43	349	2·036	44·366 ± 0·073
Fourth broods	30	223	2·697	44·448 ± 0·123

Table V shows the relation between the mean sizes of the young of control brood of the orders 1–4. The few fifth broods which are available for purposes of comparison are practically identical with the fourth broods. It is plain from this table that one cannot compare broods unless they are of the same order, or unless an allowance is made for the difference. In Table VI the columns "Controls," "All broods born at high temperature," "F_2" and "F_3" include broods of all orders 1–5, but they are all reduced to comparable values by multiplying the mean lengths of all second broods by 0·981 (43·184/44·012, Table V), third broods by 0·973, and fourth and fifth broods by 0·972. The second to fifth broods of F_1 are, of course, also treated in the same way.*

b. *Method and Reliability of Measurements.*

The measurement of length was made in the same way as that of the same dimension in Experiment A, and always in the first instar. No special source of inaccuracy was present like that which troubled us in that experiment. All the 2015 individuals recorded were descended by parthenogenesis from a single original female, the same one as provided the specimens used in Experiment A.

c. *Production and Transmission of the Variation.*

This is shown in Table VI. The variation was produced by taking new-born young from control broods and placing them in tubes floating in the hot bath, as

* PAPANICOLAU (6, p. 746) found a gradual increase in the size of the summer eggs as degenerative phenomena and tendency to sexual reproduction set in. Such an increase (as judged by the size of the newly hatched young) has been as completely absent in my experiment as degeneration and tendency to sexuality.

already described. These specimens are slightly smaller at maturity than the controls—*i.e.* the smallness is partially acquired ontogenetically like the reflexion of the valves, and like that character it is "inherited" by the young. The general course and result of the experiment are the same as those of the preceding one, but the results are smoother, probably owing partly to the larger number of individuals,

TABLE VI.—Showing Transmission, etc., of Small Size caused by High Temperature. Unit of

Period.	Control, all broods.			All broods born at high temperatures.			First broods F_1 = F_1 I.			Second broods F_1 = F_1 II.		
	Mean length.	No. of broods.	No. of individuals.	Mean length.	No. of broods.	No. of individuals.	Mean length.	No. of broods.	No. of individuals.	Mean length.	No. of broods.	No. of individuals.
Feb. 23-26 . .	43·00	6	52									
Feb. 28-Mar. 1	...	...	...	34·23 −8·75*	5	28						
Mar. 2-5 . .	...	...	...	34·77 −8·21*	4	14						
Mar. 6-9 . .	42·97	7	27	35·42 −7·55	7	22	36·25 −6·72	1	4			
Mar. 10-13 . .	44·08	5	28	36·71 −7·37	7	27	36·50 −7·58	3	7			
Mar. 14-17 . .	42·16	2	10	...	...	...	35·62 −6·54	2	5	39·03 −3·13	3	19
Mar. 18-21 . .	41·18	5	30	36·01 −5·17	2	10	...	...	...	39·43 −1·75	2	7
Mar. 22-25 . .	43·24	12	68	35·79 −7·45	2	6	37·33 −5·91	1	3	...	...	...
Mar. 26-29 . .	43·28	6	21	36·95 −6·33	2	8	...	...	...	38·99 −4·29	1	1
Mar. 30-Apr. 2	44·03	6	28	...	...	...	...	...	...	...	...	...
Apr. 3-6 . .	43·85	9	61	...	...	...	...	...	...	...	...	...
Apr. 7-10 . .	42·88	12	101	...	...	...	...	...	...	...	...	...
Apr. 11-14 . .	43·42	15	100	...	...	...	...	...	...	...	...	...
Apr. 15-18 . .	43·79	14	132	35·27 −8·52	21	136	...	...	...	...	...	...
Apr. 19-22 . .	45·03	11	98	...	...	...	36·82 −8·21	4	34	...	...	...
Apr. 23-26 . .	43·94	6	37	...	...	...	...	...	...	...	...	...
May 2-4 . .	42·85	5	50	...	...	...	...	...	...	...	...	...
Totals . .	...	121	843	...	50	251	...	11	53	...	6	27
Mean difference from controls for all periods.	...	...	...	−7·96	...	...	−7·39	...	...	−2·86	...	...
Means for whole experiment .	43·45±0·12	...	...	35·47±0·13	...	...	36·51	...	...	39·16	...	...
Difference from controls . .	...	...	...	−7·98±0·18	...	...	−6·94	...	...	−4·29	...	...

* There being no control broods in the first two periods, the differences are calculated

partly to the greater accuracy of the measurements, and partly to the greater uniformity of the abnormal conditions used to produce the abnormality, the temperature being much more easily controlled than the flora of the Klebs' solution. For the same reasons as before Table VI is divided up into short periods, and the means given are means of brood means, not means of individuals (see p. 336). The greater

Measurement = 0·018 mm. Arrangement of table as in Table IV; for full explanation see text.

Third broods F_1 = F_1 III.			Fourth broods F_1 = F_1 IV.			Fifth broods F_1 = F_1 V.			F_2, all broods.			F_3, all broods.		
Mean length.	No. of broods.	No. of individuals.	Mean length.	No. of broods.	No. of individuals.	Mean length.	No. of broods.	No. of individuals.	Mean length.	No. of broods.	No. of individuals.	Mean length.	No. of broods.	No. of individuals.
43·09 +1·91	2	5												
43·01 −0·23	1	5	40·92 −2·32	1	5	...	...	...	43·03 −0·21	3	10			
...	...	...	40·39 −2·89	2	7	...	...	...	43·70 +0·42	7	17			
41·60 −2·43	1	4	...	...	...	43·16 −0·87	3	11	43·81 −0·22	6	21			
38·75 −5·10	1	3	40·54 −3·31	2	10	...	...	...	43·08 −0·77	16	119			
...	...	...	...	...	...	43·25 +0·37	1	2	41·81 −1·07	13	88	45·01 +2·13	10	87
...	...	...	...	...	...	...	...	...	43·66 +0·24	8	50	45·14 +1·72	7	52
...	...	...	...	...	...	...	...	...	43·14 −0·65	13	81	45·55 +1·76	7	62
...	...	...	...	...	...	...	...	...	44·34 −0·69	7	51	46·38 +1·35	1	9
...	...	...	...	...	...	...	...	...	43·58 −0·36	5	33			
...	...	...	...	...	...	...	...	...	42·65 −0·20	11	109			
...	5	17	...	5	22	...	4	13	...	89	579	...	25	210
−0·79	...	...	−2·94	...	...	−0·56	...	...	−0·46	...	...	+1·88		
41·91	...	...	40·56	...	...	43·18	...	...	43·14±0·13	...	...	45·23±0·16		
−1·54	...	...	−2·89	...	...	−0·27	...	...	−0·31±0·18	...	...	+1·78±0·20		

from the mean of the controls born in the preceding and succeeding periods.

number of broods made it possible to divide the table into four-day periods instead of weeks, and this is an advantage, as four days is the normal interval between two successive broods of the same female at room temperature. Where there were more than ten specimens in any brood, only the first ten, taken at random, were measured.

The table is to be read in the same way as Table IV. Take, for example, the period March 22–25. During these four days, 12 control broods were born, totalling 68 individuals. The mean of the 12 brood means was 43·24, the unit of measurement (one division of the micrometer scale) having the value of 0·018 mm. During the same four-day period two broods consisting of six individuals were born at the high temperature, the mean of the two brood means being only 35·79, being therefore less than their controls by 7·45.

During the same period one first brood of F_1 was born. As in the previous experiment, the broods in this column come from specimens which were kept under the abnormal conditions (here high temperature) till the eggs were ripe for laying but not yet laid. Then they were removed to the same room as the controls, and placed side by side and well shuffled with them in the same rack. The eggs were in all cases laid a few hours after removing to these normal conditions. The mean length of the F_1 I brood born in the period in question was 37·33, being therefore 5·91 less than that of the corresponding controls. There were no second F_1 broods in this period, but there was one third and one fourth, both averaging less than their controls. Three F_2 broods were born within the same period, the means of the three brood means being 0·21 less than that of the controls. All the F_2 broods in this table were the offspring of specimens isolated from first broods of the F_1 generation and of course kept strictly under control conditions. The F_3 broods (none of which were born in the period in question) were similarly descended from specimens of first broods of F_2.

The third line from the bottom of the table shows the mean difference for all the four-day periods between the brood means of each class and the corresponding control means. This line corresponds to the last line of Table IV. The last two lines of Table VI present the total data in rather a different form, but with a closely similar result. Here I have found the mean for the whole of each class without dividing it up into four-day periods.*

A general survey of the whole table shows the following:—

(1) The specimens born at the higher temperature are very much smaller than those born at room temperature.

(2) The specimens developed from eggs laid by parents a few hours after removal from the higher to the lower temperature are almost as small as those born at the higher temperature. The possible reasons for the slight apparent recovery are probably the same as those discussed on p. 337.

(3) The subsequent eggs laid by the same parents still under control conditions

* Readers who desire a condensed tabular summary of the results of this experiment will find it in these two lines.

still remain affected by the smallness-producing conditions, though to a rapidly diminishing extent (F_1 II–F_1 V). The number of broods is small but the difference in size from the controls too marked to be due to mere chance. It may be pointed out that the F_1 III broods born March 18–21 are larger than the controls chiefly because of a single highly aberrant control brood with a mean of only 34·01 born in that period.

(4) The individuals belonging to the F_2 broods are also, on the whole, smaller than the controls. The difference is not very great but it is large enough and moreover regular enough (in eight out of ten periods) to be probably significant. As judged by the difference between the total F_2 mean and total control mean shown in the last line of the table, it cannot indeed be said to be significant, being less than twice the probable error. It must be remembered, however, that the probable errors of the means were calculated from the number of broods. If they had been calculated from the number of individuals, they would have been less than half as great.

(5) There is a very significant and constant reaction in the opposite direction in F_3.*

(6) The results are in perfect accord with those of Experiment A.

These experiments differ from most heredity experiments, in that measurements were made of young individuals instead of adults. The results would have been the same in principle if adult measurements had been used here—at any rate, in this experiment, which is the only one of the three for which I have the necessary data. All the specimens kept for parents were measured again in the first adult instar, with the following result: Those born and bred at the higher temperature are smaller in all instars than the controls. The F_1 from these in control conditions are smaller, not only in the first, but also in the first adult, instar. As we have seen, the F_2 individuals are smaller in their first instar, but, on the other hand, they are distinctly larger in the first adult instar. That is to say, the effect of the high temperature is overcome, and the reaction sets in during the growth of the soma of the F_2 generation, and appears in the F_3 young.

* There is little doubt that the factors given on p. 339 for commuting measurements of later broods so as to make them strictly comparable with those of first broods are fairly accurate. Whether this is so or not is immaterial where the comparison lies between F_1, F_2, and controls, as in these three classes there were about the same proportion of broods of the different orders I–V present. In the case of F_3, however, only two broods were taken from each parent, instead of five as generally in the other classes. Hence, if the above-mentioned factors were seriously wrong (which I think is out of the question, having regard to the large numbers of individuals used and their distribution over a considerable number of generations), the comparison between F_3 and the controls would be seriously affected as there is a much larger proportion of commuted measurements in the latter class than in the former. In order to satisfy myself on this point I therefore made up a table similar to Table VI, but comparing only broods of the same order. The result gave the mean difference of (F_3 *minus* controls) as +1·19 instead of +1·88. This new value is still, of course, quite large enough to be significant, even if it is accepted as the most probable one. There can, however, be little doubt that the figure given in Table VI is the more correct one, as in comparing only broods of the same order the number of control broods for the four periods in which F_3 occurs is reduced from 52 to 19, and this small number, I think, introduces a far greater source of error than that which may be due to slight errors in the factors given on p. 339.

6. Experiment C.—Reduction of Length, caused by Living in Klebs' Solution.*

This experiment deals with only 81 broods and 403 specimens, and, apart from the small number of individuals, is in addition somewhat unsatisfactory, as it is not certain that the controls belonged to the same genotypes as the individuals compared with them. Table VII includes the descendants of six parthenogenetic females, which were of no known relationship with one another, though, as they were all

Table VII.—Showing Transmission, etc., of Small Size caused by Living in Klebs' Solution.

Week.	Controls, all broods.			Born in Klebs solution, all broods.			First broods F_1, = F_1 I.			Second broods F_1, = F_1 II.		
	Mean length.	No. of broods.	No. of individuals.	Mean length.	No. of broods.	No. of individuals.	Mean length.	No. of broods.	No. of individuals.	Mean length.	No. of broods.	No. of individuals.
June 20–26 . . .	47·44	5	22	41·08 −6·36	4	18	43·93 −3·51	3	16	47·45 +0·01	2	13
June 27–July 3 .	45·79	3	28	41·93 −3·86	2	20	...	...	...	45·14 −0·65	2	14
July 4–10 . . .	47·37	5	25	...	...	...	...	...	...	...	...	...
July 11–17 . . .	45·95	9	34	43·35 −2·6	2	14	42·01 −3·94	4	21	...	...	...
July 18–24 . . .	47·04	4	20	...	...	...	...	...	...	...	...	...
July 25–31 . . .	46·22	8	39	37·56 −8·66	1	8	37·54 −8·68	1	9	...	...	...
Totals . . .	...	34	168	...	9	60	...	8	46	...	4	27
Mean weekly difference from controls . . .	...	...	...	−5·22	...	...	−4·37	...	...	−0·32	...	...
Mean for whole experiment . .	46·58±0·19	...	...	41·38	...	...	42·17	...	...	46·30	...	...
Difference from controls . . .	...	...	...	−5·20	...	...	−4·41	...	...	−0·28	...	...

taken from the same aquarium, it is not improbable that they belonged to the same pure line. In view of these drawbacks, this experiment will be disposed of very briefly.

I found accidentally in June, 1911, that certain of my protophyte cultures (not pure) in Klebs' solution had the effect of shortening the new-born young instead of reflexing the valves. The diminution affected the carapace more than the rest of

* This experiment was presented, in a rather different form, to the Fourth International Genetic Conference, Paris, 1911.

the body. In consequence of this, the abdomen, which is normally fully covered by the carapace, projects slightly behind it. In a few extreme cases, the carapace was so much reduced and mis-shapen that the abdomen was half naked. The measurements were made in the same way as the length measurements in the previous experiment, *i.e.* from head to posterior angle of the carapace. While in the former experiment this measure represents the greatest length of the animal, in the present case it does not always do so, as the end of the carapace is not always the most posterior point.

Unit of measurement = 0·018 mm. Arrangement of table as in Tables IV and VI.

Third broods F_1, = F_1 III.			Fourth broods F_1 = F_1 IV.			Fifth broods F_1, = F_1 V.			F_2, all broods.			F_3, first broods.		
Mean length.	No. of broods.	No. of individuals.	Mean length.	No. of broods.	No. of individuals.	Mean length.	No. of broods.	No. of individuals.	Mean length.	No. of broods.	No. of individuals.	Mean length.	No. of broods.	No. of individuals.
44·95	2	16												
−0·84														
51·50	1	2	49·50	2	7	48·62	2	13	47·08	3	9			
+4·13			+2·13			+1·25			−0·29					
...	...	...	50·06	1	4	...	...	...	46·65	6	22			
			+4·11						+0·70					
49·41	2	7	...	...	...	...	...	...	...	...	...	45·67	1	3
+2·37												−1·37		
44·27	1	3	...	...	...	...	...	...	46·52	4	12	48·87	1	4
−1·95									+0·30			+2·65		
...	6	28	...	3	11	...	2	13	...	13	43	...	2	7
+0·87	...	...	+2·79	...	...	+1·25	...	...	+0·35	...	...	+0·64		
47·42	...	...	49·69	...	...	48·62	...	...	46·71	...	...	47·27		
+0·84	...	...	+3·11	...	...	+2·04	...	...	+0·13	...	...	+0·69		

The course of the experiment was exactly similar to that of Experiments A and B, and Table VII is to be read in the same way as Tables IV and VI.

It will be seen that the result is in the main similar to that of the former experiments, but that the reaction appears to set in earlier, being already visible in the later F_1 broods, and also in F_2. It must be remembered, however, that the controls were not known to belong to the same pure lines as the individuals in the other columns (which were themselves possibly a mixture of three lines), so that it is possible that the former belonged to smaller genotypes than the latter. Even if this

were the case, it would not affect the general result that the reduction persists for a short time, and is followed by a reaction, but would make the reaction appear to set in earlier than if all the individuals belonged to the same genotype.

7. Literature.

Here I shall only mention those experiments most similar to my own.

Woltereck has published two papers on parallel induction in Cladocera within the period during which my own experiments have been in progress. He has taken the genera *Daphnia* and *Hyalodaphnia*, and used the character of the shape of the head, which under certain conditions becomes drawn out into a pointed helmet-like crest. The development of this crest is favoured by abundant nutrition, and in *D. longispina* the F_1 broods of parents removed from high to low nutritive conditions are crested. The F_2 broods are always completely uncrested—and by implication no effect is observable in the later generations. Even after 40 generations of crest production by gŏod feeding the effect did not persist beyond F_1. Woltereck also cut off one antenna of *Hyalodaphnia cucullata* living in a highly nutritive medium, thus impairing its power of feeding. The young (F_1) born from these starved parents were uncrested, though developed in the rich culture. The F_2 had partly recovered their crest, " die dritte Generation (F_3) aber erwies sich immer wieder als vollständig normal " (in this case, fully crested). This persistence of the effect in F_2 appears to have been observed in the descendants of one operated female only. His general results are, however, obviously fairly similar to the ones I have recorded, but Woltereck does not present his results in a statistical way—nor, indeed, does he appear to have made a long series of actual measurements, but contents himself with giving figures of typical specimens. The fact that he does not mention any reaction in F_3, and only occasionally a persistence in F_2, does not therefore show that these were not present in the degree described in my own experiments, which could only have been detected by measurements on a large scale. It is not necessary, however, to suppose that the characters and methods employed by Woltereck must have given similar results to my own.

The fact that individuals can acquire the tall crest ontogenetically when placed in the rich culture makes this a case of parallel induction.

Kammerer's remarkable series of experiments on the inheritance of impressed characters is well known. Some of these are clearly referable to parallel induction. His experiment most similar to those described here was carried out on *Lacerta muralis*. A normal, white-bellied female can be made to take on a red belly by being subjected to a high temperature. The acquirement of this character takes about a year. On being put back into normal (cold) temperature the colour very gradually fades away. Red-bellied females removed back to normal conditions were bred from, and gave a high percentage of red-bellied female offspring, especially in their earlier broods. On the whole, the percentage decreased slightly in their later broods.

No F_2 had been obtained when the experiment was published. Controls gave no red-bellied females in their offspring.

His similar experiments on *Lacerta fiumana* are especially interesting, as here the impressed character (white instead of red belly in the male) was transmitted through the male parent. Again only the F_1 had been tested.

The experiments of FISCHER and STANDFUSS on *Lepidoptera* are probably the best known cases of parallel induction.

8. SUMMARY AND DISCUSSION.

In discussing the general nature of parallel induction it must be noted that there is a phenomenon called by WOLTERECK *Pre-induction*, which is of essentially the same nature. In this case, however, though the character which subsequently appears in the offspring is induced by the action of environment on the ovarian eggs, it is not necessarily acquired also by the soma of the parent. This is plainly an unimportant distinction physiologically, merely indicating that the soma has passed the stage at which it can be influenced in the manner in question. In pre-induction of this sort there is, of course, no appearance of inheritance of acquired characters.

Briefly reviewing the results of the three described experiments we note the following points :—

In the case of the valvular reflexion it was shown that the reflexion was due to the food, for the abnormality appears when the protophyte food is washed free from the culture medium and added to the tap- or sterilised aquarium-water which is itself impotent to produce the effect. In the case of the body length (Experiment B) the cause was the temperature, as the individuals which showed reduction in size were provided with the same food and medium as their controls. Whether the cause of the reduction in length in Experiment C was due to food or medium was not tested. Individuals placed in the various abnormal environments in their first instar acquired the definite abnormal features in their own somas in later instars—at any rate in Experiments A and B. Simultaneously, the eggs in their ovaries were influenced in such a way that the young developed from them presented at birth the same abnormality as that which their parents had acquired in their lifetime. It made little difference whether the young developed from eggs laid after removal of the parents to control conditions, or were born in the abnormal environment, so long as the eggs underwent their ovarian growth while the parents were under the influence of the environment. In the subsequent broods of parents removed to control conditions the effect of the abnormal conditions appeared in rapidly diminishing intensity. In the next generation in control conditions (F_2), the abnormal effect still persisted in Experiments A and B but to a very slight degree. In all three experiments a very decided reaction appeared in F_3.

In the case of valvular reflexion an experiment exactly the reverse of the one described was made. Females with ripe eggs were removed from control conditions

into the protophyte culture. The young developed from these eggs were fully normal, showing the persistence of the effects of normal environment. Subsequent broods of these females in the culture became successively more and more reflexed, *i.e.* the normality wore off in the same way as we saw the abnormality do. This experiment was not carried beyond the F_1 generation.

Let us consider the bearing of these data upon the general questions of inheritance and variation. Any result of the functioning of living protoplasm—for example, the formation of a soma—depends upon both the composition of the living units of the protoplasm and their environment. True variations (as distinct from recombinations) in the somatic structure may therefore be due to a change either in the composition of the living units (genes), or in their environment. Mutations giving rise to new genotypes are supposed to be of the former nature. When such a mutation occurs, it must surely imply that a living unit has changed its composition, and now forms a new living unit capable of assimilation, growth, and reproduction, and hence of pure breeding. The other cause of variation—a change in the environment while the living units remain the same—is probably far commoner. Such a change, if effective, will probably result in the formation of unusual metabolic products included in the living protoplasm, and thus the visible external variation produced may have as its immediate cause either the changed environment itself, or the altered protoplasmic inclusions. In the case of parallel induction, it seems that the environment works indirectly, through the mediation of these (non-living) products, which when once formed are not immediately got rid of, but are passed on passively included in the protoplasm of the gamete. Non-transmissible environmental effects may be either direct effects, which therefore cease to be produced immediately the environment is changed, or if indirect ones acting through changed metabolic products, these are eliminated at once and so cease to operate after their formation discontinues.

The variations produced in parallel induction seem to be indirect environmental effects. All the experiments point to their being due, not to a change in the living, assimilating, and self-reproducing unit, but to the inclusion in the soma and in the egg of some non-living substance. When included in the egg it passes passively into the soma which develops from it, and thus produces the same effect on it as it produced on the soma of the parent which acquired the character in question.

The experiments of Sitowski with Sudan red present a probable analogy. He stained the food of caterpillars of *Tineola biselliela* with Sudan red. The fat of the living animals was stained red by the dye, and so were also their eggs. The next generation, developing from these eggs, was also stained red.

It is highly probable that parallel induction is due to the accumulation in the soma and in the gamete of non-living substances in an analogous way. The gradual wearing off of the "abnormal" effects in generations removed to "normal" conditions may be due in a slight degree to the mere dilution of our hypothetical substances, caused by the increase in the bulk of protoplasm without a corresponding

increase in the amount of the substances. The experiments indicate, however, that the protoplasm plays a more active part, and that the substances are not merely diluted or eliminated. The reaction in F_3 is only explicable on the assumption that the presence of these substances stimulates the formation of antibodies.

The experiment of KAMMERER on *Lacerta fiumana*, where a conspicuous somatic variation was transmitted in this way through the male gamete (F_1 only tested), shows that these substances can exert a very powerful effect when present in only minimal quantities, thus naturally suggesting that they are of an enzyme nature.

The conception of the inclusion in the protoplasm of soma or gonad of substances exerting a formative influence on growth is, of course, an old one. CUNNINGHAM'S "hormone" theory of the origin and inheritance of certain adaptive characters approaches in some respects the hypothesis suggested above for the explanation of parallel induction, with the great exception that the former theory demands that the production of these "hormones" for an "indefinite number of generations" can finally result in such a fixed character as the erect position in man or the tendency to horn production in the sexually mature stag. This, of course, is going far beyond known facts, and seems to require the assumption that the "hormone" can act for an indefinitely long time after it has ceased to be actively produced.

To account for his results with *Daphnia*, WOLTERECK has proposed a complicated biochemical scheme of interaction between gene and environment. I can only say that it does not seem to me to account for the persistence of the induced effects in F_2 (observed apparently only in one exceptional case by WOLTERECK) and that it accounts not at all for the reaction observed in my experiments in F_3.

PLATE (7, p. 338) considers that the existence of parallel induction as exhibited in FISCHER'S and STANDFUSS' experiments proves the truth of the determinant theory, for he argues that the structure of the cells of the germ-plasm and wing rudiment are so different that it is inconceivable that the same external conditions should produce the same effects on both, though it is reasonable to suppose that the wing colour determinants could be similarly affected lying in the germ-plasm and in the imaginal discs. He therefore agrees with WEISMANN'S explanation of these phenomena. The mode of action I have suggested is, of course, independent of the determinant theory.

It is plain that in parallel induction we have a factor which causes a correlation between the characters of parent and offspring, and an appearance of inheritance with regression to the mean, which are not really due to inheritance in any legitimate sense of the word. I will not here enter into a discussion of this interesting point, preferring to defer it till the completion of an experiment, already far advanced, dealing with the general question of parthenogenetic inheritance in Cladocera. For the same reason I have not entered into an exhaustive consideration of the phenomena related to parallel induction.

Attention was drawn in the introduction to the now well recognised necessity of

distinguishing between parallel and somatic induction when discussing the problem of the inheritance of acquired characters.

It need hardly be emphasised that there is absolutely no evidence to show that continued action of the abnormal environment—for example, high temperature—however long the animals were subjected to it, would ever make the species permanently smaller, *i.e.* remaining smaller for an indefinite time after return to the lower temperature. A permanent change like that can only be brought about by the mutation of the living unit to a fresh unit capable of itself of assimilation, growth, and reproduction. If the explanation of the obtained effects given above be correct, the constitution of the living protoplasm is in no way directly altered by the changed environment.

We may sum up the question of transmissible environmental effects as follows :—

(1) A changed environment (in its widest sense) may produce a visible variation in the soma indirectly by altering the nature of the metabolic products included in the living protoplasm. These in turn react with the protoplasm, and therefore effect changes in its product, the soma.

(2) Whenever the environment acts simultaneously on soma and gonad a similar alteration in metabolic inclusions of somato- and germ- plasm takes place.

(3) These metabolic substances included in the gamete naturally pass into the developing soma, which, therefore, shows the same variation as its parent did, even though removed from the environment in question.

(4) These substances may produce a powerful effect, though present only in minimal quantities.

(5) They may be of such a nature as to stimulate the formation of antibodies, thus causing a reaction in later generations.

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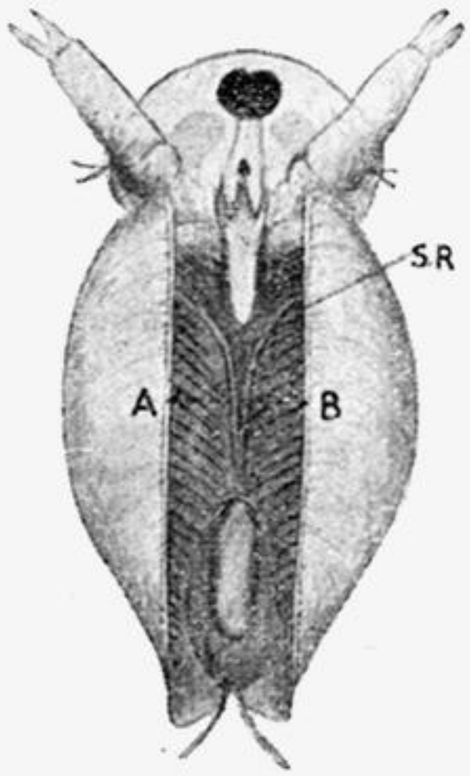

FIG. 1.

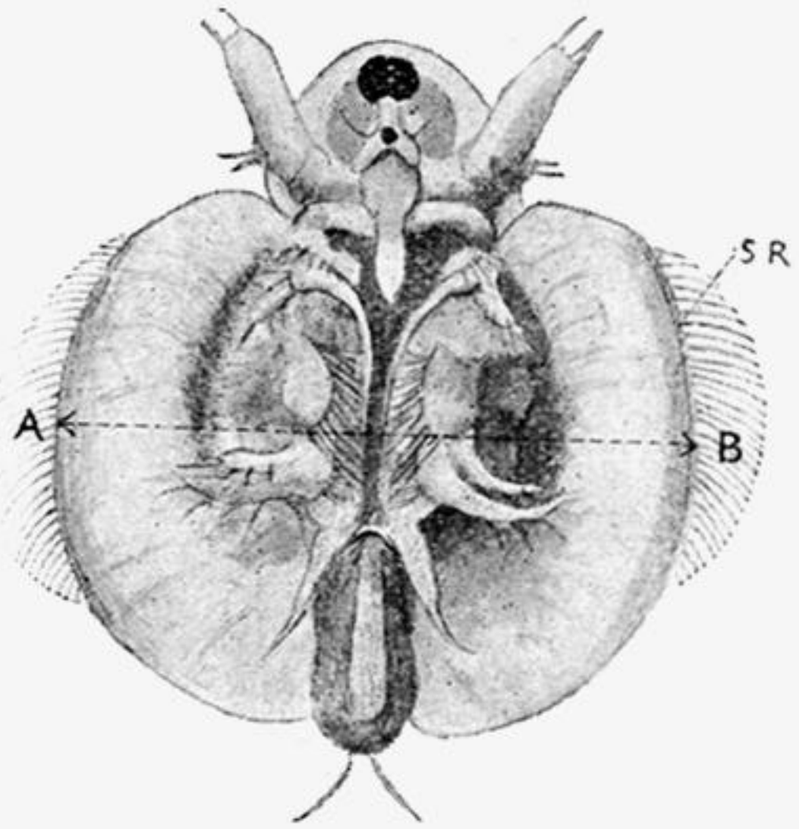

FIG. 2.

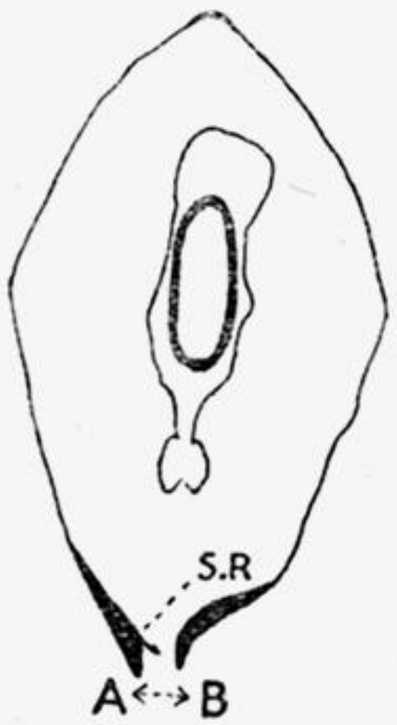

FIG. 3.

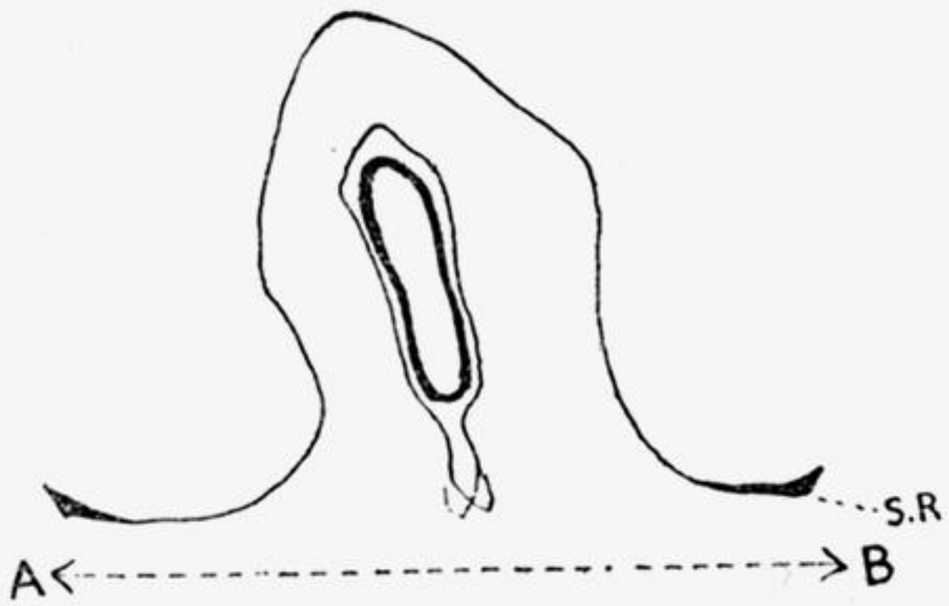

FIG. 4.

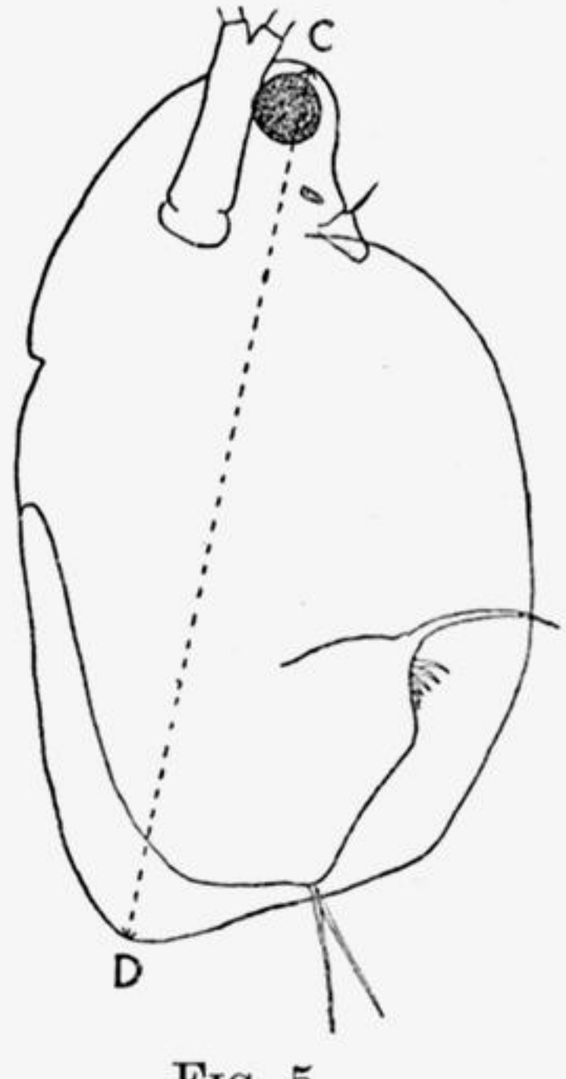

FIG. 5.

FIG. 1.—Ventral view of a living, normal, *Simocephalus vetulus*. Drawn under anæsthesia.

FIG. 2.—Similar view of a specimen with reflexed valves. The L/W ratio of this specimen was 1·12.

FIG. 3.—Transverse section of a normal specimen; the section passes through the bend of the abdomen. Sublim.-acetic.

FIG. 4.—Similar section through a specimen with reflexed valves. L/W = 1·37. Sublim.-acetic.

FIG. 5.—Side view of a normal specimen in its first instar. This figure is on a larger scale than the others.

(A–B, Line along which intervalvular width, W, is measured. C–D, line along which length, L, is measured. SR, setigerous ridge.)

(All the figures are drawn with the Abbé Camera.)