THE QUESTION OF THE EQUALITY OF SOMATIC AND GERM NUCLEI IN RESPECT TO HEREDITY AND SURVIVAL, ON THE BASIS OF STUDIES IN A SOIL PROTOZOON.

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With 15 Figures.

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From MAUPAS (1886) and R. HERTWIG (1889, 1914) we know that Ciliata protozoa have two sorts of nuclei: somatic and germ nuclei. Furthermore, that the role of the somatic, or macronucleus is the preservation of the cells and vegetative propagation by amitotic division. The germ nucleus, or micronucleus, goes over by mitotic equatic division into the vegetatively propagating successors. But, as HERTWIG and others have established, it keeps the potentiality that during sexual reproduction the reduced haploid germ nucleus, which we call the micronucleus, reciprocally unites with the germ nucleus of its conjugating partner and recreates the somatic or macronucleus which has disintegrated. WOODRUFF (1921) and others after him established the same process in the course of endomixis or parthenogenesis. This fact was established in the beginning simply by observation, but later various authors confirmed it experimentally, removing the germ nucleus and observing in the presence of the somatic nucleus alone the vegetative and propagative life-functions of animals. (CALKINS, 1930, DAWSON, 1919, 20, PATTEN, 1921, WOODRUFF, 1921, SCHWARTZ, 1939, TZE-TUAN CHEN, 1940, REYNOLDS, 1938, PIEKARSKI, 1939, KIMBALL, 1941.) From the foregoing it appeared that the somatic, or macronucleus itself alone could assure the entire life function, with the exception of sexual reproduction and parthenogenesis. In this case (i. e., sexual propagation and parthenogenesis) the somatic nucleus is destroyed and, the germ nucleus not being present, there is nothing with which to re-build and so the animal dies. In reality, therefore, these experiments seemed to confirm the theory of nuclear dimorphism founded by R. HERTWIG: the macronucleus as somatic nucleus disappears without leaving a trace,

but the micronucleus is deathless, as according also to WEISMANN (1892).

In my experiments, as follows, I propound that, contrary to existing opinions, the somatic nucleus is equal to the germ nucleus in a soil cilia protozoön in respect to heredity and survival when the micronucleus has been experimentally eliminated from the organism.

CHOICE OF EXPERIMENTAL OBJECT AND EXPERIMENTAL TECHNIC.

For experimental purposes a 100-micron-sized soil protozoön (the hypotrichous *Kahlia simplex* HORVÁTH 1934, 1935—36) is used. These animals have 2 macro and 2 micro nuclei. (In reality more are demonstrable, but only 2 can function.) They are easily cultivated in a water extract prepared from a soil in which the microorganism lives. The simplest method of preparing is by adding 1 litre water to ½ kgm. soil, shaking it several times, 12 hours later filtering it through a filter paper, then to the filtered solution adding 0.3 g. gelatine and dissolving it warm. The animal is in the best condition for cultivation when it divides 4 times daily; i. e., when it has produced 16 progeny.

My aim was to find an easy method of eliminating the micronucleus from the animal's body. For that ultra-violet rays brought about the desired result. The procedure was as follows: The animals being in about a 5 ml. food solution in a Boveri cup, with a Hanau quartz lamp I directed a just less than lethal dose of u. v. rays upon them. The animals reacted variously to the rays. A relatively small percent of them were utilisable for our purposes. The effect of the u. v. rays on a small percentage of them was to create twin-figures, joined at the back. These twin forms made possible the creation of animals containing only the macronucleus. (I refer you to the work of my colleague F. ÖRDÖGH (1941), to whom I gave this problem for detailed study, while for myself I kept only the part dealing with the role of the somatic and germ nuclei.)

The real cause for the formation of the twin figures it was not possible to establish. According to our observations they could be formed in the following way: A small percentage of the animals were submitted to the effect of the u. v. rays as they moved about on the bottom of the vessel, which meant that the back or the ventral surface of the animal was more strongly exposed to the rays, as our animal has a dorsi-ventral flattened body. The reversibility of the coagulation brought about by the u.-v. rays showed the latest in that part

of the body which was directly touched by the rays. Thus could arise the interesting situation that the organisms, more quickly overcoming the effect of the u.-v. rays in the vicinity of the back or ventral surface, could there begin locally and gradually to grow. As effect of this one-sided growth the animal takes a V-form, as can be seen from drawing. (Figure I. a.) This state occurs 3—4 hours after the u. v. radiation. The two sides of the V in reality each represent an animal which appears as an abnormal division. Mouths develop at the two ends of this V. Then, as result of constant one-sided laming, the two individuals which were disturbed in the process of division close almost together. The division following this state definitively created the twin figures joined at the back. It is possible that the 2 individuals originating from the front part can again take a V-shape, but those originating from the back already have a form as if they were complete mirror-pictures of one another. In movement the two behave as one animal, but from the standpoint of organization as two, having 2 adoral zones, 2 motile organs, 2 mouths, 2 cytopyge, 2 contractile vacuoles, and double sets of nuclei. ÖRDÖGH and I found that this twin development is only a lasting modification which could be maintained only with optimum cultivation and nutrition for any length of time. If the cultures, kept at room temperature, were not transferred every 3—4 days then the separation of the animals took place. If over a longer time the culture was not refreshed all the animals separated. This phenomenon could be used in the elimination of the germ or micronucleus. That is to say, in the longitudinal separation — as could be seen from the coloured preparations — the micronuclei did not disperse regularly so that they necessarily arrived in both halves. If now we cultivated the separated individuals separately we formed of them the so-called clon, and could shortly see in the coloured preparations from which clon the micronucleus was missing.

OTHER TECHNICAL PROCEDURES.

I made good use of a variety of aceto-carmine staining of the nucleus which I had earlier worked out (1940). The microphotograms were also made from preparations staining in the same way. I used FEULGEN'S nuclear reaction as standard preparation.

THE VEGETATIVE FUNCTION OF AMICRONUCLEATE ORGANISMS.

In general the amicronucleate organism does not differ in its exterior morphology or appearance from the normal animal. From the

standpoint of motion, nutrition, digestion and excretion, as well as osmotic conditions, they also seem identical. Similarly, the progeny produced in a given time in the course of propagation by division in 2 is also numerically the same as in normal animals. To this extent it corresponds with the findings of others (DAWSON, PATTEN, WOODRUFF). But there is a difference from the standpoint of life-span. After a certain number of generations, according to previous experiments, the amicronucleate Ciliata die out.

In my experiments this dying-out does not occur. I have cultivated the animals through 1800 generations up to now and left the cultures encysted, but I can excyst them at any time. In itself the circumstance that they are capable of normal cysts makes possible the continued existence of these amicronucleate animals.

PARTHENOGENESIS or endomixis occurs from time to time (on an average of every 8—10 days) in our animals too. The dimensions of the animal's body diminish, the somatic or macronucleus breaks up into pieces. On the basis of WOODRUFF's experiments we should expect that the somatic nucleus would be absorbed and, there being no germ nucleus and therefore nothing from which to build up anew, that the animal would die. After the disintegration of the nucleus which is associated with such a diminution of body-dimensions our amicronucleate animal begins again to form the macronucleus out of the stumps of the old one if we transfer it to a new culture. The individuals which have gone through parthenogenesis and which have been transferred to fresh ground regain their normal capacity to divide in 48 hours. We can also bring about parthenogenesis artificially by giving them a greater quantity of bacteria as nutriment. If we give the same amount of bacteria immediately after parthenogenesis they do not react as quickly with repeated parthenogenesis (small body-size, disintegrated nucleus) as before. On the contrary, the body remains big for 72 hours. Shortly their bodies conform to those of animals with normal nuclei living under optimal conditions, in spite of the effect of the great amount of bacterial metabolites. This resistance may be attributed to the regeneration due to parthenogenesis.

There is however a fairly rare phenomenon in regard to the somatic nucleus, first mentioned by HERTWIG, which seems to be catastrophic for the animal. That is, the body of the animal becomes smaller in this case too, but this is not followed by the disintegration of the macronucleus. First both parts of the macronucleus grow excessively in comparison to the size of the animal's body. The growth can be such that, not finding sufficient room within the body, its ends roll up.

In that phase the large nucleus is still rich in chromatin granules and does not differ in its inner structure from the macronucleus in normal animals. 50—60 hours after this phenomenon takes place the large nucleus begins gradually to swell up, becomes hollow, then falls to pieces. At the same time the body becomes round, and the animal dies in 7—8 days.

CONJUGATION.

The amicronucleate Kahlias conjugate relatively easily if the temperature of the culture is elevated to 30—35°. As can be seen from the first microphotogram, conjugation begins by the first conjugating pair adhering at their frontal parts. (Fig. 1.) The 2 individuals of the conjugating pair are always of different size; that is, they couple by anizogamic conjugation. At the very beginning of conjugation the macronuclei lose their regular oval shape and become irregular in form. In the next phase, as can be seen from microphotograms 2 and 3, the macronuclei become elongated in fronto-caudal direction and develop a long ribbon-like shape. The macronuclei of normal animals show in this stage of conjugation a more advanced development, as is to be seen in the 4th microphotogram: The macronuclei begin to fall to pieces in inequal divisions and at the same time the chromotin granules begin to be absorbed in the plasma.

A further stage of conjugation is shown in microphotogram 5: The conjugating pair become fused in their whole length, so that the tails of the 2 animals can be seen only in the caudal part; the macronuclei fall by division into several parts. As is shown in the 6th microphotogram, the smaller of the conjugating pair gets fused with the body of the bigger one to such an extent that it is seen only as a swelling. At the same time the macronuclei multiply by further divisions, but simultaneously some of them begin to disappear in the plasma.

The total fusion of the conjugating pairs is not characteristic for normal animals.

In other Ciliata and in normal *Kahlia* the next conjugating phase can be seen only after the coupling of the germ nuclei and after the separation of the pairs. In our amicronucleate animals, however, there are no ex-conjugants, and therefore this technical term cannot be used; we are obliged to continue to speak of conjugation. This state stops only, not alone when the 2 conjugants look to be one from the outside, but when their nuclei are reduced to the set of nuclei of a single animal. Our amicronucleate animal is shown at this stage in microphotogram No 7:

The macronucleus is doubled, but this is not yet a vegetative form even though it may seem to be, for one of the macronuclei is in process of absorption. Its borders are corroded, bubbles appear on its body, these two things being indications of absorption. That equilibrium is not reached at that stage is proved by the 8th microphotogram: Though the 2 greater macronuclei are in the process of absorption, as is shown by the corrosion of their edges, 2 new somatic nuclei of the size of a micronucleus appear at their sides. In the next phase there are no more big somatic nuclei but in their place we find 2 or 3 nuclei of the size of the micronucleus (9th microphotogram). Following this the 2 permanent macronuclei are formed (10th and 11th microphotograms). The successful end of the conjugation is shown on the 12th diagram, when the animal begins to divide. The nuclei are fused and begin to close in at the middle, so as to be able to divide into the 2 successor cells.

Conjugation takes place on an average in 2—3 days. During this time our animals take no nourishment, and so the symptom occasionally occurs that the body diminishes decidedly. At such times the animal loses its roundness and, though it develops again in the final macronucleus form, it cannot reorganize its body, and dies. The changes which such a fate after conjugation cause in the ratio of nucleus to cytoplasm can be seen in the 13th and 14th microphotograms. The resistance of the conjugating individuals varies enormously and for that reason it is never possible to give averages for the mortality arising in consequence of conjugation. I have had experiments where of 10 pairs not one died, while there were others with a death rate of 50%.

DISCUSSION.

Our findings coincide with those of former experiments in this, that amicronucleate Ciliata are capable of vegetative functioning, including also division. (In my earlier experiments (1939) the deaths were probably due to the operating technic not being suitable.) Here the question also arises as to what age the Ciliata propagating only vegetatively can attain. According to previous experiments (Dawson, Patten, Woodruff, *et al.),* after certain varying numbers of generations the extinction of the culture takes place. In my experiments there is no question of such extinction, under normal cultivating conditions, but there are cases if the large quantity of bacteria affects the protozoa. But at such times not only the amicronucleate individuals die out but also the control Kahlia. So this can be taken as a

pathological condition, and does not agree with JOLLOS' ideas on the length of life of the amicronucleate animals, which establish that they must die out because the macronucleus is absorbed during parthenogenesis and, there being no micronucleus, the animal cannot replace the loss. In our experiments it is proved that during parthenogenesis the macronucleus is capable of regenerating of itself. This gives the means of judging the results which can be expected from sexual reproduction (conjugation) too. (Presuming that in respect to the macronucleus parthenogenesis (endomixis) has the same value and role as in conjugation). Those authors (SCHWARTZ, TZE-TUAN CHEN, KIMBALL) who could make the amicronucleate Ciliata conjugate all of them report that conjugation is a catastrophe in the life of the amicronucleate animal, because the macronucleus is absorbed (as in endomixis) and the animal without nucleus dies.

In my experiments I proved that the macronucleus regenerates after conjugation. My observations, made during the conjugation of the amicronucleate animals, was that the macronucleus regenerates from the parts of the macronuclei which have the same size and structure as the micronucleus; and this led to the supposition that in normal conditions the macronucleus does not derive from the micronucleus, but should regenerate independently, of itself. If this were the case then we should have to speak, not of nuclear dimorphism but of double nuclei. Beyond the proof of change of form which can be seen in the microphotograms we know from our experiments with the macronucleus too that it assures itself the functions of reproduction and survival. The contrary of this experiment, that is, that only micronuclei should be present in the animal, would decide whether the micronucleus were equivalent to the macronucleus in the vegetative life-span also. As I did not make such experiments it can be taken as only hypothetical that the somatic role of the 2 nuclei are identical. Experiments in polyploidizing the micronucleus (MACDOUGAL, 1931) prove that the micronucleus is the germ nucleus, and my experiment proves the identical qualities of the micronucleus with the macronucleus in sexual reproduction.

How do we reconcile the differing findings of previous experimenters with our own?

We might suppose that the earlier experimenters had worked under an error to which could be ascribed the differences in results. The conjugation of our experimental animals is a peculiar conjugation, differing so much from the characteristics of the object in the similar experiments of others that one is inclined to say that there is no analogy

between my experimental object, the *Kahlia simplex,* and experimental objects of the others, but that they differ.

If the others' experimental error is the cause of the discrepancy in our experimental findings, then the previous theory of somatic and germ nuclei division is entirely overthrown. If in our experiments we have to deal with an extraordinary object then there is no ground for generalizations about rules, for most probably our experimental object is not a unique exception. Thus the previous conceptions of nuclear dimorphism can be refuted ,as the nucleus called somatic can perform the same functions as the germ nucleus. It can even be included in WEISMANN's conception of immortality. Therefore clearly not nuclear dimorphism but double nuclei are in question.

The entire experimental findings cause serious difficulties only insofar as we do not know where in the somatic nucleus to place the genes of heredity. In his studies of conjugation KITZKE (1916) saw chromosomes in the growing macronucleus which were covered as they developed by increasing amounts of chromatin granules. IVANIC (1933) and PIEKARSKI (1941) also reported sorts of Ciliata in which there might be chromosomes in the macronucleus. But we did not find this in our animals. On the other hand, the fundamental beginning of reorganization of the somatic nucleus after the conjugation of amicronucleate animals is formally identical with the macronucleus in a resting state. According to WEISMANN we should take this as the germ part existing in the somatic nucleus, because it regenerates the somatic nucleus. It is to be supposed that the genes of heredity are also therein.

SUMMARY.

1. From the *Kahlia simplex* HORVÁTH soil protozoön the micronucleus was eliminated by u. v. radiation.

2. From the amicronucleate *Kahlia* were established:
   a) The vegetative life-function capacities — digestion, selection, excretion, regeneration, division, encysting and excysting.
   b) That the life-span under optimum conditions was interminable.
   c) That the animals are capable of endomixis (parthenogenesis) and of conjugation.

3. In the amicronucleate *Kahlia* during parthenogenesis the macronucleus at first breaks up but under optimum conditions regenerates anew.

4. If during the conjugation of amicronucleate *Kahlia* the macronucleus breaks up and is absorbed to such an extent that only micronucleus-sized pieces are left, it can still reorganize from these.

5. Under points 2, 3 and 4 we can declare that the macronucleus is equal in value to the germ nucleus.

6. The genes of heredity are probably present at the inception of the macronucleus.

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EXPLANATION OF THE FIGURES.

I. a) V-shaped double figure formed by ultra violet radiation.

I. b) Twin-form united at the back, derived from the V-shaped form. The double sets of nuclei distinctly visible. (Sketches after carminacetic acid staining.)

1., 2. and 3. Conjugation of amicronucleate animals. The different figures show the progress of conjugation: the micronucleus gradually prolonged. Microphotogram after carminocetic acid staining. The same method applies to the other, following, Figures.

Magnified: 240 x, 330 x, 210 x.

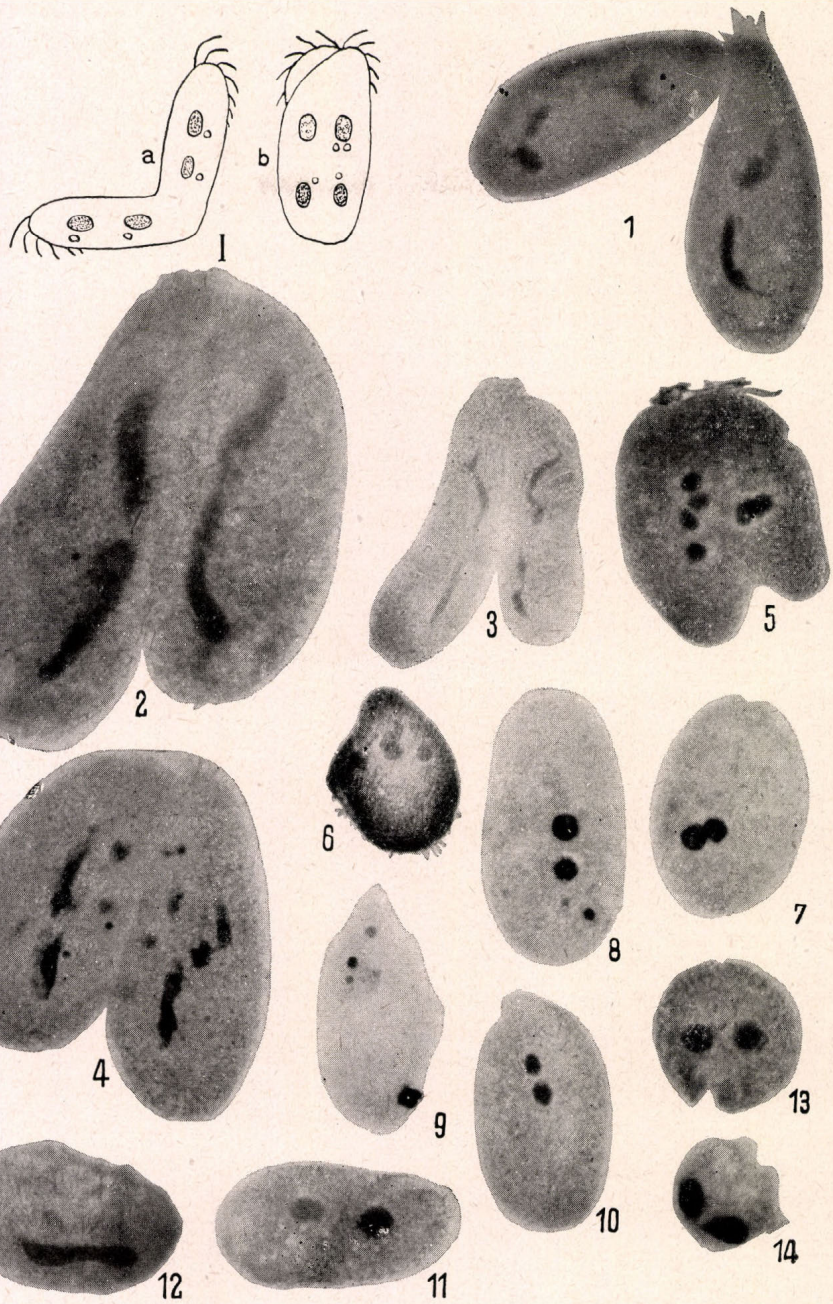
4. Conjugation of animals with normal nuclei Magnified 330 x

5. and 6. Further progress of amicronucleate animals' conjugation. Gradual fusion of the 2 bodies and the disintegration of macronucleus. Magnified: 240 x, 180 x.

7, 8, and 9. The more important phases of final formation of macronuclei. Magnified: 240 x, 250 x, 210 x.

10, 11 and 12. The definitive form of normal macronuclei and their first division. Magnified: 210 x, 240 x, and 180 x.

13 and 14. The new-formed macronucleus not followed by body growth. Magnified: 210 x.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