Resumen por el autor, A. Franklin Shull

Volumen nuclear relativo y el ciclo vital de Hydatina senta.

El volumen relativo de los núcleos de la glándula vitelina aumentó durante diecinueve generaciones; el de los núcleos del estómago-intestino permaneció constante durante la primera mitad de este periodo disminuyendo ligeramente después. Ninguno de estos cambios está relacionado con la alternancia de la reproducción partenogenética y sexual. El volumen relativo de los núcleos de la glándula vitelina aumentó marcadamente con la edad del animal, mientras que el de los del estómago-intestino aumentó ligeramente o no aumentó en absoluto. El núcleo de los ovocitos disminuyó en volumen relativo durante el crecimiento de la célula, pero no cambió en relación con la edad del individuo o durante una serie de generaciones. No existe diferencia entre los productores de machos y los productores de hembras en lo referente al volumen nuclear relativo.

Los cambios relativos observados se deben a cambios en el volumen nuclear en algunos casos, en el volumen citosómico en algunos, y en el volumen nuclear y citosómico en otros. Estos hechos indican que la teoría de Herteig y otros que suponen que la alternancia de reproducción sexual y partenogenética depende de cambios en el volumen nuclear relativo no es aplicable al caso de Hydatina, porque dicha teoría requeriría un aumento del volumen nuclear relativo durante o antes de cada epidemia de productores de machos, un volumen nuclear mayor en los adultos de edad media que en los jóvenes y viejos, un volumen nuclear mayor en los rotíferos criados en agua de manantial que en los cultivados en solución de estiércol y un volumen mayor en los productores de machos que en los productores de hembras.

Translation by José F. Nonidez
Cornell Medical Collgee, New York

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RELATIVE NUCLEAR VOLUME AND THE LIFE-CYCLE OF HYDATINA SENTA[1]

A. FRANKLIN SHULL

Zoölogical Laboratory, University of Michigan

ONE FIGURE

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[1] Contribution from the Zoölogical Laboratory of the University of Michigan.

The many thousands of computations made in the preparation of this paper were mostly done on a calculating machine furnished by the trustees of the Elizabeth Thompson Science Fund, to whom I desire to express my gratitude.

For aid in providing much of the routine labor involved in the study I am glad to acknowledge my indebtedness to a grant from the Bache Fund.

ORIGIN OF THE IDEA OF SIGNIFICANCE OF NUCLEAR VOLUME

The idea that the relative volume of the nucleus, as compared with the volume of the cytosome, is a causal factor in metabolic processes appears to have had its origin in the early observation that large cells have large nuclei, small cells small nuclei. The extent of the surface of contact between the nucleus and the surrounding protoplasm offered a possible explanation of the degree of interaction of these two parts of the cell—an interaction which most authors have regarded as highly important in cell physiology. When it was found that under certain circumstances the relative volumes of nucleus and cytosome vary, the discoverers were not slow to seize upon these variations as possible physiological factors.

Hertwig ('03), to whom is due the chief development of the idea, found, for example, that the relative size of the nucleus in the Protozoa varies in relation to changes in their life-cycle. Actinosphaerium reared under favorable conditions exhibits a gradual increase of nuclear size through a series of generations, at the end of which the former relative size of the nucleus is restored by extrusion of part of the excess chromatin into the cytoplasm. Similar observations were made by Popoff ('08, '09 a) on Frontonia, Stylonychia, and Paramecium. Supporting evidence was found in the work of Gerassimow ('02, '05) upon several species of Spirogyra, in which he was able to show that a change in the size of the nucleus leads to a corresponding change in the size of the cell, and that, under favorable conditions, when the relative volume of the nucleus reaches a certain value cell division ensues. Boveri ('03, '05) extended the general idea of a normal size relation between nucleus and cytosome to the metazoa by discovering that in sea-urchin larvae, in which multipolar mitoses produce nuclei containing various numbers of chromosomes, the size of both the nucleus and the cell depends upon the number of chromosomes. His measurements indicated that the surface of the nucleus and the volume of the cell are proportional to the number of chromosomes.

From some of these investigations and others mentioned below Hertwig formulated his idea of the significance of relative nuclear size as follows. Under normal conditions the quotient obtained by dividing nuclear volume by cytosomal volume (the Kernplasmarelation, K/P) has a certain value (the Kernplasmanorm). Any disturbance of this ratio, due to a change in either nuclear or cytosomal volume, leads to a condition of tension (Kernplasmaspannung). This tension is a factor in the cell physiology, and leads to certain consequences, some of which Hertwig pointed out.

APPLICATIONS OF THE IDEA

Other investigators have applied the conception to a variety of fields. A fair notion of the importance attached to changes of the Kernplasmarelation can only be gained by contemplating the wide range of phenomena which biologists have sought to explain on this basis or which have a bearing on the ratio. The list which follows is incomplete.

1. Size of cell. The work of Gerassimow and of Boveri has already been cited. Godlewski ('08) attributes giant cells in echinoderms to an increase of nuclear material. Hegner ('19, '20) finds nuclear volume in Arcella related both as cause and as effect, to cell size, and Popoff ('09 a) explains changes of size in Paramecium by changes in the value of K/P.

2. Physiology of cell. Hertwig ('03) conceived the idea that increase of cell activity is associated with (causes?) an increase of relative nuclear volume. A decrease of nuclear ratio has been held responsible for senescence (Minot, '08). The nucleus enlarges in the degeneration of Amoeba (Prandtl, '07).

3. Cell division. Changes of the ratio K/P may cause or postpone cell division, according to Hertwig ('08), and it makes no difference whether the change in the ratio is due to changes in nuclear volume or to changes in cytosomal volume. Gerassimow's similar conclusion has already been mentioned. Popoff ('08, '09) discovered a regular decrease[2] of relative nuclear volume

[2] Some confusion has been introduced into the literature by the fact that Popoff, Godlewski, Rautmann, Eycleshymer, and Metcalf express the nucleoplasma ratio as the quotient of the cytosomal volume divided by the nuclear volume (P/K), whereas all other authors follow Hertwig in placing the nuclear

for a few hours after division in Paramecium and Frontonia, followed by an increase up to the time of the next division, and held that cell division occurred when the ratio K/P had increased to a certain value.

4. Embryonic development. Godlewski ('08) found that in Echinus the nucleoplasma ratio increases greatly up to the 64-cell stage, but changes little after that, and Koehler ('12) reports a similar increase in Strongylocentrotus. In Crepidula Conklin states that this increase during segmentation is less than has been supposed. Eycleshymer ('04) demonstrated a decrease of K/P during embryonic development of Necturus, and Child ('15) points out that as a rule differentiated cells have relatively smaller nuclei than embryonic cells. Herlant ('17) found that in the artificially activated sea-urchin egg there are two periods of increase of K/P, separated by a period of decrease, and that the accessory treatment must be applied during one of the periods of increase if segmentation is to be caused.

5. Regeneration. Godlewski ('10), Metcalf ('15), and Sutherland ('15) describe an increase of the Kernplasmarelation in regenerating tissue, though in some cases the increase was preceded by a short period of decrease or of fluctuation.

6. Sex. Hertwig ('03) calls attention to the striking difference between the low nucleoplasma ratio in eggs and the high value of that ratio in spermatozoa, and sees in this difference a cardinal distinction between the sexes. Sexual dimorphism is attributed by him to the same difference. From the statements of Oehninger ('13) concerning the absolute sizes of the nuclei in the

volume in the numerator of the fraction (K/P). Rautmann explains that it is more convenient to reverse the terms, since on Hertwig's plan the value is nearly always a fraction. The other four authors offer no explanation of the reversal, nor do they indicate that they are aware that they have reversed the terms. Decision as to the relative value of these two formulas for the Kernplasmarelation should depend on whether it is the nucleus or cytoplasm that is most active in changing the value of the ratio. If, as most investigators who attach any importance to this ratio of volumes hold, the active changes are chiefly if not exclusively due to changes of the nucleus, the formula of the ratio should be such that the value would increase as the nuclear volume increases. This can be done only by placing the nuclear volume in the numerator, thus, following Hertwig, K/P. In this paper the confusion resulting from the two opposed practices is obviated by translating all conclusions in terms of the formula K/P.

male and the female honey-bee I infer that the Kernplasmarelation is about the same in both sexes, notwithstanding that the male is presumably haploid. Woltereck ('11) is frankly critical of the applicability of the Kernplasma theory to the problem of sex determination, particularly, however, in parthenogenetic species.

7. Age. The relation of nuclear volume to the age of the egg (Herlant) and to the age of the embryo (Eycleshymer) is mentioned above under 4. Berezowski ('10) describes a decrease of the Kernplasmaratio in the mouse during the first five months after birth, and Minot ('08) bases an important part of his theory of age and death on the conclusion that a decrease of the ratio K/P not only accompanies but causes senescence.

8. External conditions. The relative size of the nucleus in various organisms is found to be greater at low temperature than at high, by Erdmann ('08), Hartmann ('19 a), Koehler ('12), Popoff ('08), and Rautmann ('09), and, by inference, Papanicolau ('10), though Rautmann observed that increase of temperature above 20° reversed the change of the ratio in Paramecium, making the nucleus larger, instead of smaller as in the case of increases of temperature up to 20°. With high nutrition Hertwig ('03) observed an increase of relative nuclear size in Actinosphaerium and Paramecium, while Morgulis ('11) obtained an increase of K/P with starvation in the guinea-pig, with some discordant results in the rat. Popoff ('09 b) increased the size of the nucleus in Paramecium by means of chemical substances in the water. Absorption of water decreases the K/P relation in the pluteus of Strongylocentrotus (Erdmann, '08).

9. Depression periods in Protozoa. The periods of depression, common in Protozoa, were found by Popoff ('07) to be accompanied or preceded by enlargement of the nuclei, and subjecting these Protozoa to certain chemical solutions produced both depression and nuclear increase (Popoff, '09b). A similar conclusion is reached by Hartmann ('19a) for certain Cladocera, in which the periods of depression are said to be characterized by a high K/P ratio. Hertwig ('08) discusses with approval the suggested connection between depression and relative nuclear size.

10. Parthenogenesis. Issakowitsch ('07) holds that long-continued parthenogenesis results in an increase of the nucleoplasma ratio in daphnians—a conclusion also reached by Popoff ('07) and Hartmann ('19 a).

11. Alternating life-cycles. In Cladocera Issakowitsch ('07) infers from degeneration phenomena that the alternating phases of parthenogenesis and sexual reproduction are governed by changes of the Kernplasmarelation—a conclusion supported by Popoff ('07), who generalizes from a study of the Protozoa. Papanicolau ('10), after a brief study of intestinal cells in Cladocera, also concludes that the alternating modes of reproduction are ushered in by changes of the nucleoplasma ratio—a view concurred in by Hartmann ('19 a). Strohl ('08), however, attacks this view on theoretical grounds, and Woltereck ('11) offers various reasons why the K/P relation can be only secondarily related to the mode of reproduction, if it has anything to do with the cycle at all.

The above citations are not a complete list, but are probably representative of the types of phenomena which have been held to be explained by, or to cause, changes of relative nuclear volume. They indicate that, among certain biologists, the relative volume of nucleus and cytoplasm is regarded as an important factor in physiology. No further general discussion of the question is contemplated in this paper. Since, however, the observations and experiments here reported bear particularly upon items 10 and 11 in the above list, it is deemed necessary to enlarge upon the application of the Kernplasmarelation theory to parthenogenesis and its alternation with bisexual reproduction.

RELATIVE NUCLEAR VOLUME IN THE LIFE-CYCLES OF PARTHENOGENETIC ANIMALS

The first discoveries of the relation of the relative nuclear volume to cyclical phenomena involving a series of generations were made in the Protozoa by Hertwig, Popoff, and others, as related above. Hertwig concludes, from his own studies and those of other workers on Paramecium, Actinosphaerium, Frontonia, and Dileptus, that the size of the nucleus increases during continued vegetative reproduction until such a size is reached as incapacitates the cell for further activity by preventing the usual metabolic processes. In this condition the cell remains until it either, 1) dies or, 2) conjugates or, 3) reorganizes itself by eliminating some of the nuclear matter into the cytoplasm. By either of the last two processes the 'normal' relative volume of the nucleus to cytosome ('Kernplasmanorm,' Hertwig, '08, p. 9) is restored, and the cell rendered again capable of a series of vegetative divisions.

Extension of this theory to other cyclical phenomena was no doubt hastened by the discovery that the two sexes of certain animals, or at least their germ cells, are distinguished by different nucleoplasma ratios. Hertwig ('05) conceived that any increase of the quotient K/P would lead to the production of males, decrease of that quotient favoring females. Inasmuch as the alternation of parthenogenesis and sexual reproduction was then regarded, and by many biologists is still regarded, as a phenomenon of sex determination, those who were working with such alternating cycles were quick to suggest that volumetric changes of the nucleus are responsible for the change from one mode of reproduction to the other. On the basis of experiments performed by Issakowitsch on Cladocera, Hertwig ('05) concluded that the change from the parthenogenetic to the sexual mode of reproduction is a direct result of an increase of the Kernplasmarelation; that is, of an increase of nuclear volume relative to cytosomal volume. This view was adopted by Issakowitsch ('07). It does not appear that any measurements of nuclear and cytosomal volumes in the daphnids were made by Issakowitsch. The evidence obtained from the daphnids was indirect. Long-continued parthenogenesis led to a 'physiological degeneration,' one sign of which was the disintegration of eggs in the brood chamber at the end of such a series. Since in Protozoa these periods of depression ('physiological degeneration') were preceded or accompanied by an enlargement of the nucleus, it was assumed by Hertwig and Issakowitsch that a similar increase of the nucleus probably occurred also in the daphnians. Furthermore, the effect of temperature upon Protozoa and the daphnians, while not then specifically employed as an argument by either of these authors, may have led them unconsciously to the same conclusion. Low temperature caused a relative increase of the nucleus in the Protozoa, low temperature hastened the advent of sexual forms in daphnians; if low temperature also increases the relative nuclear volume in the daphnids, the Kernplasma theory is again supported.

In accordance with the above facts, Hertwig and Issakowitsch conceive the ratio K/P to change during the life-cycle as follows. It is least in the early generations descended from the fertilized egg. With continued parthenogenesis and favorable conditions of nutrition and temperature, the ratio increases, the volume of the nucleus increasing relatively more than the volume of the cytosome. Low temperature and deficient nutrition hasten the increase. When a given high value of the ratio K/P is reached, males are produced. With a further slight increase in the ratio, sexual eggs are formed by the fusion of groups of oögonia, and in this fusion the Kernplasmarelation is reduced.

Issakowitsch ('07) specifically states, and Hertwig's writings imply, that in these changes of the life-cycle it is the Kernplasmarelation of the egg that is concerned. Other tissues, however, were studied by Papanicolau ('10 b) with a view to testing the Kernplasma theory. In conjunction with a number of experiments with daphnians, many of the animals from different phases of the life-cycle and from different environmental conditions were fixed. Preparations of the intestinal epithelium, composed of flat polygonal cells, were made. In the paper cited are given figures of these intestinal cells, and in some of them the cells and nuclei are both distinctly larger than in others. While Papanicolau nowhere specifically states that the relative volume of the nucleus is greater in any of these cells than in others, he nevertheless concludes that they support the Kernplasmarelation theory of Hertwig and Issakowitsch.

The portion of Papanicolau's paper dealing with the Kernplasmarelation was admittedly a preliminary report. No more extensive investigations have been published, however. I have therefore had photographic enlargements of his figures made, and have measured the nuclei and cytoplasm with a planimeter in accordance with the method described below, in order to discover more precisely the grounds for this conclusion. The results, stated in simple averages for all of the cells in each figure, are as follows.

A parthenogenetic female Moina, taken from an early generation after the fertilized egg and from a culture at high temperature (Papanicolau's fig. 1), which should for both of these reasons have a small Kernplasmarelation, shows a ratio of nuclear area to cytosomal area of 0.229. In his figure 2 are similar cells from a parthenogenetic female likewise from an early generation, for which reason the Kernplasmarelation should be small; but this female was reared at room temperature, hence the nuclear volume should be relatively somewhat greater than in figure 1. The ratio of nuclear area to cytosomal area in this figure is 0.231. Papanicolau's figures 3 and 4 represent, respectively, a parthenogenetic and a sexual female from a middle period of the life-cycle. Presumably both of these should show a larger Kernplasmarelation than either figure 1 or figure 2 and that of figure 4 should be greater than that of figure 3. The measurements show the relative nuclear area to be 0.354 in figure 3 and 0.351 in figure 4.

In his figure 5, which is from an early generation, but from a starved animal at room temperature, and which should have relatively larger nuclei than figures 1 and 2, though perhaps not greater than figures 3 and 4, the ratio K/P is found to be 0.312.

His figures 6 and 7 are from intestinal cells of Simocephalus, the two specimens differing in that the former is from a somewhat earlier period in the life-cycle than the latter, and the first was reared at room temperature, the second at low temperature. On both these counts figure 7 should show a larger Kernplasmarelation than figure 6. The actual ratios indicated by measurement are 0.175 for figure 6, 0.209 for figure 7.

With one exception the differences in these figures are of the kind assumed by Papanicolau, though in one instance the difference is very small. Whether any of the differences are significant cannot be determined statistically, since none of the figures represent more than thirteen cells, and one of them includes only five. In the absence of any knowledge of the variability of the cells of the intestine, of the variability of the individual animals reared under like conditions, and of the precautions taken to prevent unconscious selection in making the drawings, no criterion of the value of the above figures is possible. At least it cannot be said that Papanicolau's conclusions are incorrect.

Hartmann ('19a) studied other species of Cladocera, employing the hypodermis and certain ganglia as well as the intestine, and in general confirms Papanicolau's conclusions. His observations on changes associated with differences of temperature and differences of position in the cycle appear to have been made on animals collected from nature, rather than on experimental animals, so that one may question what other differences in the environment were involved; but his conclusion is that low temperature (also Hartmann, '19b) and long-continued parthenogenesis increase the ratio K/P. An attempt to change this ratio by chemical substances gave results which, according to the author, are plainly seen; but he exhibits these effects by drawings rather than measurements, and I find it difficult to convince myself that there is any difference in the K/P ratio. Hartman also tests the ratio of volume of the nucleolus to that of the nucleus (N/K), and finds that on the whole it changes in the same manner as does K/P. The whole question of the significance of the relative nuclear volume is discussed in considerable detail, in terms of nuclear volume and surface and cell volume and surface, with many formulas, not so much in relation to discovered facts as in relation to facts that may conceivably sometime be discovered.

Hertwig does not apparently express any opinion of the extension of the idea that the relative nuclear volume is of physiological significance to somatic tissues, but he does expressly sanction its application to parthenogenetic species. Nor was the idea a mere passing fancy with Hertwig, for it appears in a series of papers over a period of years. Furthermore, it is clear that he regarded the relative volume of the nucleus as a causal factor, not a mere accompaniment.

In view of this strong support of the Kernplasmarelation theory from responsible quarters and its widespread application to a variety of phenomena, it seemed desirable, when opportunity was offered to test it in one of its phases in an animal that afforded at least a chance of separating causes from effects or inconsequential events, that such opportunity be embraced. Hydatina seems to present the requisite material, for reasons stated below.

HYDATINA AS MATERIAL FOR THE STUDY OF THE KERNPLASMA-RELATION IN A PARTHENOGENETIC ANIMAL

The life-cycle of Hydatina senta is more accurately known than that of any other animal exhibiting its type of parthenogenesis. There is no longer any disagreement among recent investigators regarding the effectiveness of external conditions in altering the life-cycle. Moreover, the precise point in the cycle at which the external agents are effective is known. It has been shown (Shull, '12) that artificial modifications of the cycle must occur in the growth or maturation period of the egg.

There are, moreover, at least three independent ways in which the Kernplasmarelation may be connected with changes in the life-cycle if Hertwig's views are correct. First, many lines of rotifers show a marked periodicity (Shull, '15) in the production of males, periods of many male-producers alternating with (usually) longer periods of few male-producers. The ratio K/P should change with these alternating periods.

Second, male-production is related to the age of the parents (Shull, '10). The first eggs laid by a female yield relatively few male-producers; the number of male-producers in the family increases gradually up to the middle of the family, at which time they are three or four times as common as at the beginning, and then they become gradually less frequent to the end of the family. There should be a corresponding change of the Kernplasmarelation during the lifetime of the parent.

Third, external agents profoundly alter the number of male-producers. Manure solution, one of the most effective of the agents that repress the sexual phase, may easily exclude male-producers altogether. Animals reared in manure solution should, therefore, show a different Kernplasmarelation from those reared in spring water.

Hydatina has also the advantage of being free from certain objections which pertain to other material. First, the cells do not divide after the adult condition is reached. Martini ('12) showed that the number of cells was (usually) the same in all individuals. Since this is as true of young adults as of old ones, it follows that there are no cell divisions in the adult (except, of course, the maturation division of the oöcyte). Although it has since been pointed out (Shull, '18) that the cell number is not as constant as Martini supposed, there is nothing in the new discoveries to indicate that cells divide in the adult. The absence of cell division eliminates one source of error in the measurement of the Kernplasmarelation in other tissues than the oöcytes, since there is no danger of measuring cells just prior to division, at which time the nuclei become rapidly larger ('divisional growth' of Hertwig).

Second, there is no chromidial apparatus in the cytoplasm which might interfere with an accurate determination of nuclear volume—a difficulty which Hertwig found in certain Protozoa. The yolk gland of Hydatina occasionally contains deeply staining masses which are probably not related to chromatin, but no such specimens were used in this work.

Third, differences in environment need not introduce any discrepancies in K/P, the Kernplasmarelation, since the animals are small and their life short. Except in those experiments in which differences of environment were intentional, the conditions under which animals to be compared were reared were alike and highly uniform.

Even the foregoing advantages of Hydatina for the study of the Kernplasmarelation might not have led me into so laborious an investigation, but for the further circumstance that a difference in the quantity of chromatin between the male-producing and female-producing individuals might thereby be detected. From a cytological study no difference in chromosome number had been detected (Shull, '21), but it was suggested to me by several biologists that the quantity of chromatin might nevertheless be greater in one kind of female than the other. An investigation of the nuclear volume offered a test of this suggestion.

TESTS EMPLOYED

Periodicity of male-production. One of the lines of rotifers used for this study showed to a marked degree the periodicity of male-production which I have described (Shull, '15) as being common in other lines. This line was reared in a way which would show the periodicity more sharply than the usual complete family-pedigree method. A dozen young female-producing females of about the same age were placed together in a dish, where they were kept until about twenty-four hours after egg-laying began. They were then removed to another dish and given fresh food. The young hatching from the first eggs laid after this transfer were isolated and reared to maturity, the number of male-producers and female-producers being recorded. From the female-producers a number were selected and, about twenty-four hours after their egg-laying began, put into a dish with fresh food. The earliest-hatching young in this dish, up to as high a number as could conveniently be handled, were isolated as before and reared to maturity, the number of male-producers and female-producers being again recorded. By this means a series of generations was obtained in which the confusion arising from the overlapping of late members of one generation and the early members of the next is avoided. The families are not complete, each generation being represented only by members from the middle of the family, at which time they are most likely (Shull, '10) to be male-producers.

The periodicity of male-production in this line is quite evident from the records which are given in table 1. Two distinct periods of male-production occurred, each preceded and followed by a period of few male-producers.

From each of these generations a number of female-producers and sometimes also male-producers, were fixed in Bouin's fluid. They were sectioned 5μ in thickness and stained with iron hematoxylin followed by eosin.

At least one generation in advance of each of the periods of male-producers there should, according to Hertwig's Kernplasma-relation theory, be an increase in the volume of the nucleus relative to the volume of the cytosome. The results bearing on this question are described in a later section of this paper.

Age of individual. A number of young females, each in a dish by herself, were divided into three lots. In one lot the females were removed as soon as they began to lay eggs. If the eggs were large enough to be probably female eggs, the mothers were

TABLE 1

Showing the number of male-producing (♂♀) and female-producing (♀♀) individuals of Hydatina in random lots taken from the middle of the families of a 'semi-pedigreed' line for nineteen generations. In this time there were two periods of relatively high male-production

DATE OF ISOLATION	NUMBER OF ♂♀	NUMBER OF ♀♀	DATE OF ISOLATION	NUMBER OF ♂♀	NUMBER OF ♀♀
January 16	2	24	February 15	0	47
19	0	24	18	0	48
22	1	35	21	0	13
25	3	33	24	0	35
28	19	46	27	17	21
30	14	46	March 2	13	27
February 2	0	56	5	6	29
5	0	40	8	2	36
9	2	38	11	0	42
12	0	43			

fixed, all together in one vessel in Bouin's fluid. The offspring hatching from their eggs were subsequently examined to ascertain that the mothers had all been female-producers. If any of them had turned out to be male-producers, the whole lot would have been discarded after fixation, but this precaution proved to be unnecessary. The females of the second lot were removed from thirty-six to forty-eight hours after egg-laying began, at which time they could be certainly recognized as female- or male-producing. Only the female-producers were fixed. The female-producers of the third lot were fixed just before the end of the egg-laying period, three or four days after they became adult.

All that appeared unhealthy, even if they still had eggs in the oviduct, were rejected.

The three lots of female-producers, young, middle-aged, and old, were sectioned 5μ in thickness and stained with iron hematoxylin and eosin.

Since the middle-aged females are more likely to have male-producing offspring than either the young or old ones, their Kernplasmarelation should, according to Hertwig's theory, be greater than that of the latter two. The results obtained are described later.

Effect of manure solution. A number of female-producers of approximately the same age, taken at twelve to twenty-four hours after egg-laying began, were divided into two lots. One lot was continued in spring water, the other was put into strong manure solution. Twelve to twenty-four hours later, both lots were fixed in Bouin's fluid. They were sectioned 5μ in thickness and stained with iron hematoxylin and eosin.

Since manure solution almost immediately reduces the number of male-producers, the females treated with manure solution should, on Hertwig's theory, have a smaller Kernplasmarelation than those reared in spring water.

Tissues examined for Kernplasmarelation. Hertwig and Issakowitsch appear to have considered only the Kernplasmarelation of the germ cells as important in determining the life-cycle. Papanicolau, as pointed out above, employed intestinal cells, and Hartmann used also hypodermis and ganglia. In the rotifers I was encouraged to use other tissues than the oöcytes for two reasons. First, the occurrence of males is determined a whole generation in advance of the actual production of male eggs, so that any change in the Kernplasmarelation might be detectible in the somatic cells of at least the male-producing parents. Second, since the alteration of the Kernplasmarelation is supposed to be a metabolic (essentially a nutritive) phenomenon, it seemed not unlikely that organs concerned with nutrition would show similar alterations. Two such organs are the yolk gland and the stomach-intestine. The egg, in passing down the oviduct, is lodged in the angle between the yolk gland and stomach-intestine, and is believed to absorb or ingest the material for its growth from one or both of these organs. If any somatic cells exhibit changes in the Kernplasmarelation in relation to the life-cycle, it seems likely that they are the cells of the yolk gland and stomach-intestine.

METHOD OF MEASUREMENT AND PRECAUTIONS IN STUDY OF KERNPLASMA RELATION

The general method of determining the Kernplasmarelation was to make drawings of the sections of the cells, measure the areas of the nucleus and cytosome in these drawings, and divide the nuclear area by the cytosomal area. This method does not, of course, determine the actual volume of either nucleus or cytosome. It does not determine even the relative volume of nucleus and cytosome. If, however, in two cells of the same shape, one has a relatively greater nucleus than the other, the drawing of a section of the former will also have greater relative nuclear area than will the latter. That is, any increase in the actual Kernplasmarelation will be shown by an increase in the relative nuclear area in the drawing of a section; but the two increases will not be equal. Since we are nowhere interested in the absolute value of the Kernplasmarelation, but only in whether there is an increase or decrease; since we require only to know whether the Kernplasmarelation in one lot of animals is greater or smaller than in another lot, but not how much greater or smaller, the method described seems adequate.

Oöcytes. Drawings of the oöcytes were made with the aid of a camera lucida and were then enlarged photographically. Only those oöcytes were used which had undergone sufficient growth to be easily drawn with accuracy. None that had reached full size and were about to produce the first maturation spindle were drawn, for at that time the nucleus grows rapidly, so that great variability in the Kernplasmarelation would have been introduced. The stage during which this nuclear growth occurs can be easily recognized, since the egg is nearly fixed in shape, and appears in the sections in the form of a regular ellipse or circle, whereas the younger oöcytes, which are still elastic, are distorted into various shapes by the surrounding cells and organs. The staining reaction of the full-grown oöcytes also differs from the younger ones, the sections of the oöcytes during the period of 'divisional growth' of the nucelus being decidedly pink, the younger stages blue.

Of the sections passing through the nucleus of a given oöcyte, the first and last of the series were rejected. Since each nucleus was usually cut three or four times, only one or two drawings were obtained from each. Other precautions necessary in the case of oöcytes are discussed in a later section in relation to the results obtained.

Stomach-intestine. The wall of the stomach-intestine is composed of a single layer of cells, polygonal in form, flattened, and set edge to edge. It therefore makes a difference which way the sections are cut. The Kernplasmarelation shown in tangential sections is much smaller than in transverse sections. To have used both kinds of sections would have introduced a great variability which would undoubtedly have increased the probable error of the mean, notwithstanding the larger total number of cells that could have been used in that way. Only transverse sections, therefore, were used. Either transverse or longitudinal sections of the stomach-intestine were used, since in either case the cells were cut transversely. Inasmuch as the diameters of the cells parallel, respectively, to the long axis and the transverse axes of the stomach-intestine are about equal, it made no difference whether the organ was cut longitudinally or crosswise. The gradual change, in the series of sections, from transverse to tangential sections of the cells could be detected partly by the shape of the sections, but better by the increasing indefiniteness of the cell boundaries, or by the position of the section in the series. Only those with clear boundaries were drawn. Care was taken to use only sections passing near the middle of the nucleus.

The drawings of the cells of the stomach-intestine were made with a camera lucida, and then enlarged photographically for measurement.

Yolk gland. The yolk gland is a syncytium in the form of a thick saucer or a baseball catcher's mitt. Inasmuch as the organ is relatively large and stains deeply, the drawings could be made with a micro-projection lantern. Because it is a syncytium, it was necessary to draw sections of the entire organ, not individual cells. The nuclei are not appreciably longer in one direction than another, so that sections in all planes are equally good. The only requirement of a section to be drawn was that the boundaries of both the organ as a whole and of its nuclei be sharp and distinct. This requirement could be met only in case the section passed near the middle of the nuclei. The first two or three sections through a given nucleus were invariably rejected because they were too nearly tangential, while frequently more than three were rejected.

Although the yolk gland usually contains eight nuclei, and as many as five (rarely more) nuclei might appear in a single section, it was seldom that a section passing through many nuclei passed near the middle of all of them. The drawings were necessarily made, therefore, mostly from sections passing through one, two, or three nuclei, the smaller numbers more frequently than the larger.

Measurement of the drawings. All measurements were made by means of a polar planimeter. Since absolute measurements were not desired, no tests of the absolute accuracy of the planimeter have been made. The value of the planimeter in this work depends on the uniformity of its measurements. Several tests of the instrument were made by measuring a single figure a number of times, always in the hands of the same operator. One such test resulted as follows:

1 time, 4.55 square inches
3 times, 4.56 square inches
4 times, 4.57 square inches
3 times, 4.58 square inches
3 times, 4.59 square inches
1 time, 4.60 square inches

While there is some irregularity, there are no large deviations from the mean. A single measurement of each figure was therefore regarded as adequate.

The personal equation. While the drawings and measurements were made by several persons, precautions were taken to prevent error from this source. The drawings to be made were listed by the author by means of stage readings and other suitable designations, but in such a way that no one else could know the source of the animal whose cells were to be drawn. The drawings and measurements were therefore made by persons wholly ignorant of the bearing which any given cell would have on the correctness of Hertwig's theory.

Furthermore, all drawings and measurements which were to be compared with one another in any way whatever were made by the same person. Whatever idiosyncrasies this person possessed must have affected both sides of the comparison equally and in the same direction.

ACKNOWLEDGMENTS

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NUCLEAR VOLUME AND PERIODICITY OF MALE-PRODUCTION

The periodicity in the production of males exhibited by the line of rotifers used for this study has been shown in table 1. The relative nuclear volumes in animals from different parts of this line are here recorded for the three tissues separately.

Yolk gland. Over a thousand measurements, each involving one to three nuclei, are available for determining the relative nuclear volume of the yolk gland in the nineteen generations through which the line was bred. These have been averaged for each generation separately, with the result shown in table 2. The averages are plotted in the second curve of figure 1, in the first curve of which is given the percentage of male-producers in these nineteen generations.

There is an irregular but pronounced increase in the relative nuclear volume through the series of generations. The differences between the early generations and the late ones are large as compared with their probable errors. To determine whether this increase is due to absolute increase of nuclear volume or to decrease of cytoplasm, or to both, the absolute sizes of all nuclei included in this series, totaling about 1900, were collected. This comparison is possible, since all drawings were made to the same scale. The sizes given are square inches of area in the drawings. To reduce the probable errors involved, the nineteen generations were divided into an early, middle, and late period of six, seven, and six generations, respectively, and the nuclear sizes averaged for the three groups separately. The results are as follows:

Size of nuclei of yolk gland in first six generations.......0.443 ± 0.0052
Size of nuclei in seventh to thirteenth generations.......0.469 ± 0.0065
Size of nuclei in last six generations.......................0.532 ± 0.0054

TABLE 2

Relative nuclear volume of the yolk gland of Hydatina senta for each of nineteen generations, including two well-marked periods of male-production. Compare with table 1

GENERATION	RELATIVE NUCLEAR VOLUME	GENERATION	RELATIVE NUCLEAR VOLUME
1	0.408 ± 0.016	11	0.559 ± 0.012
2	0.454 ± 0.017	12	0.621 ± 0.021
3	0.442 ± 0.007	13	0.536 ± 0.017
4	0.496 ± 0.006	14	0.599 ± 0.011
5	0.477 ± 0.010	15	0.649 ± 0.018
6	0.517 ± 0.010	16	0.562 ± 0.008
7	0.509 ± 0.009	17	0.594 ± 0.014
8	0.487 ± 0.015	18	0.559 ± 0.010
9	0.498 ± 0.009	19	0.598 ± 0.011
10	0.483 ± 0.010		

The increase in absolute nuclear volume is nearly proportional to the corresponding increase of relative nuclear size shown in table 2, indicating that the relative increase is due to enlargement of the nuclei, while the volume of the cytoplasm remained nearly stationary through the nineteen generations.

It is difficult to see any relation between nuclear volume and the number of male-producers (upper curve). Although the curve of nuclear volume has a slight appearance of bimodality, the second hump begins rather too early to be held responsible for the second 'wave' of male-producers. In this connection it should be remembered that those external conditions known to affect the

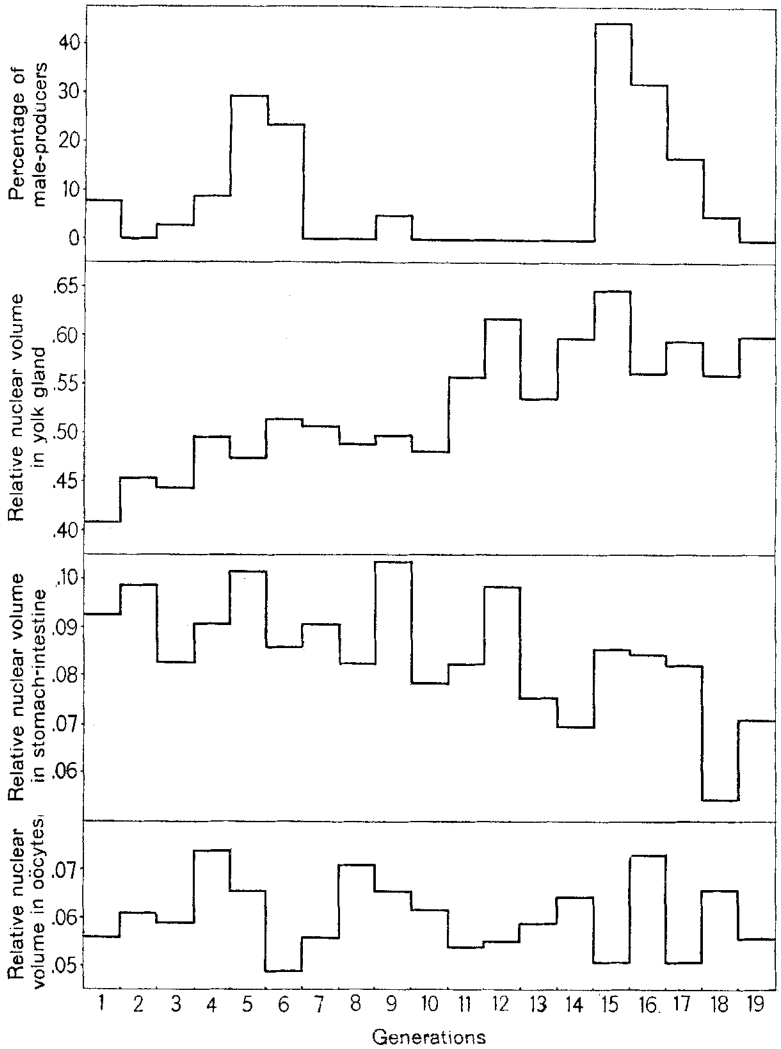

Fig. 1 Curves representing the relative nuclear volume in three tissues of the rotifer Hydatina senta, in relation to periods of male-production. First curve, the percentage of male-producers in each of nineteen successive generations, showing two distinct periods of male-production. Second curve, the relative volume of nuclei in the yolk gland during the same nineteen generations. Third curve, relative nuclear volume in the stomach-intestine during the same nineteen generations. Fourth curve, relative nuclear volume in the oöcytes.

life-cycle exert their influence almost immediately (Shull, '12), and one would suppose that an internal agency would operate with similar promptness.

Stomach-intestine. The average relative nuclear volume of the cells of the stomach-intestine for the nineteen generations in which periods of male-production were well marked is shown in table 3. The data are graphically shown in figure 1, third curve, together with the curve of male-production (first curve) for the purpose of comparison. The curve of nuclear volume shows a small but evident decline in the latter part of the period studied. If the nineteen generations be divided into three approximately equal parts, the average relative nuclear volume in the first two of these is practically the same; but in the third division (the last six generations) the relative size of the nucleus appears to be significantly less. To determine whether this relative decrease is due to an absolute decrease in the size of the nucleus or to an absolute increase of the volume of cytoplasm, or to both, the absolute sizes of all intestinal nuclei of the nineteen generations have been collected. To reduce the probable errors, these generations have been divided into three periods, the first six generations, the seventh to thirteenth, and the last six generations, respectively. The mean absolute nuclear sizes (square inches in the drawings) of the nuclei in these three periods were found to be as follows:

Size of intestinal nuclei, first six generations	0.428 ± 0.0047
Size in seventh to thirteenth generations	0.432 ± 0.0083
Size in last six generations	0.400 ± 0.0059

The first two of these periods of time were marked by intestinal nuclei of practically the same size, but the nuclei in the third period appear to be significantly smaller. This decrease of absolute nuclear size is approximately what is required to explain the decrease of relative nuclear size shown in table 3 in the same six generations, indicating that the mean volume of the cells as a whole remained nearly constant.

The variation in nuclear volumes in the stomach-intestine shows no relation to the 'waves' of male-producers shown in the upper curve of figure 1. Even if one might attribute the second period of high male-production to the decrease in volume of nuclei in the intestine, there would be no similar decrease of nuclear volume coincident with the first period of high male-production.

Oöcytes. Drawing conclusions from the nuclear volumes of these cells must be done with caution for the reason that smaller numbers of cells are available for measurement and that the cells are of very different sizes correlated with stages in the growth period. The former condition obviously causes high probable

TABLE 3

The relative nuclear volume of the cells of the stomach-intestine of Hydatina senta for each of nineteen generations of the same line as is represented in table 2

GENERATION	RELATIVE NUCLEAR VOLUME	GENERATION	RELATIVE NUCLEAR VOLUME
1	0.093 ± 0.009	11	0.083 ± 0.003
2	0.099 ± 0.005	12	0.099 ± 0.010
3	0.083 ± 0.004	13	0.076 ± 0.006
4	0.091 ± 0.002	14	0.070 ± 0.002
5	0.102 ± 0.003	15	0.086 ± 0.004
6	0.086 ± 0.003	16	0.085 ± 0.005
7	0.091 ± 0.004	17	0.083 ± 0.007
8	0.083 ± 0.001	18	0.055 ± 0.003
9	0.104 ± 0.006	19	0.071 ± 0.003
10	0.079 ± 0.009		

errors of mean nuclear size, and, as pointed out below, the second condition has a like effect. Disregarding the differences in size and grouping all cells together, one obtains the relative nuclear sizes shown in table 4. The graph of these sizes is given in figure 1, bottom curve.

One might be tempted to see in the lower curve of this figure three modes which correspond to the periods of high male-production in generations 5 and 6 and 15 and 16, and to the much less marked but isolated male-production in the ninth generation. The rise of relative nuclear volume in the eighth and two succeeding generations seems out of all proportion to the percentage of male-producers occurring at that time, but were there no other means of weighing the evidence, the two phenomena might doubtfully be referred one to the other.

Since oöcytes of all stages of growth are included in table 4, it is important to know whether relative nuclear volume varies with the size of the cell. To determine this point, all drawings of oöcytes were sorted into groups, all cells of one group being of approximately the same size. The relative nuclear volumes were then averaged for each size group separately, with the results shown in table 5.

There is a distinct decrease of relative nuclear volume during the growth of the cell. The growth of the nucleus does not keep

TABLE 4

The relative nuclear volume of the oöcytes of Hydatina senta for each of nineteen generations of the same line as is represented in tables 2 and 3

GENERATION	RELATIVE NUCLEAR VOLUME	GENERATION	RELATIVE NUCLEAR VOLUME
1	0.056 ± 0.006	11	0.054 ± 0.006
2	0.061 ± 0.005	12	0.055 ± 0.004
3	0.059 ± 0.005	13	0.059 ± ?
4	0.074 ± 0.008	14	0.065 ± 0.006
5	0.066 ± 0.008	15	0.051 ± 0.003
6	0.049 ± 0.003	16	0.073 ± 0.005
7	0.056 ± 0.003	17	0.051 ± 0.004
8	0.071 ± 0.006	18	0.066 ± 0.006
9	0.066 ± 0.008	19	0.056 ± 0.008
10	0.062 ± 0.005		

pace with that of the cytoplasm. It is obvious that, if any generations, or groups of generations, in the parthenogenetic line which formed the basis of this study had furnished for measurement an undue percentage of oöcytes of any given stage of development, the relative nuclear volume determined from the measurements might have departed considerably from the true mean. There was no reason why the data should have happened to be 'loaded' in this manner, but to make sure that there was no such source of error the measurements were collected into groups, with respect to both cell size and the generation from which the cells came. The results need not be given in detail; it seemed very plain that there was no one-sided grouping of the data, the cells of a given size being spread rather evenly over the generations, and the cells of one generation being distributed fairly uniformly with respect to their size. Hence, although the inclusion of cells of various sizes greatly increased the probable error of the mean, it could not have altered the mean itself very much.

A further test of the significance of the relative nuclear size with relation to the life-cycle was obtained in the following manner. It is well known (Shull, '12) that whether a rotifer is to be

TABLE 5

Variation in the relative nuclear volume of oöcytes of Hydatina senta, with reference to size (stage of growth) of the cells

SIZE OF CELL (SQUARE INCHES IN DRAWING)	RELATIVE NUCLEAR VOLUME	SIZE OF CELL (SQUARE INCHES IN DRAWING)	RELATIVE NUCLEAR VOLUME
0.0–0.5	0.124	6.0– 6.5	0.047
0.5–1.0	0.105	6.5– 7.0	0.033
1.0–1.5	0.083	7.0– 7.5	0.039
1.5–2.0	0.069	7.5– 8.0	0.031
2.0–2.5	0.073	8.0– 8.5	0.033
2.5–3.0	0.061	8.5– 9.0	0.035
3.0–3.5	0.053	9.0– 9.5	0.029
3.5–4.0	0.045	9.5–10.0	0.033
4.0–4.5	0.044	10.0–10.5	0.027
4.5–5.0	0.048	10.5–11.0	0.029
5.0–5.5	0.038	11.0–11.5	0.028
5.5–6.0	0.039	12.0–12.5	0.039
		13.0–13.5	0.023

a male-producer or a female-producer is decided in the preceding generation, that is, while the egg from which the rotifer develops is still in the body of the mother. If, therefore, relative nuclear volume determines the proportion of male-producers, that volume in generations preceding many male-producers should be different from the corresponding relative volume in generations preceding no male-producers. In table 1 it is shown that there were in the line studied nine generations preceding generations that included male-producers. The measurements of oöcytes from these two groups of generations have been collected separately and averaged. The comparison of the two groups, with respect to nuclear size, is briefly stated as follows:

Relative nuclear volume of oöcytes in generations preceding male-producers	0.0606 ± 0.0018
Relative nuclear volume of oöcytes in generations preceding no male-producers	0.0580 ± 0.0020
Difference of mean sizes	0.0026 ± 0.0026

The difference is equaled by its probable error, which is usually interpreted to mean that the difference is insignificant. A large probable error does not, however, prove a result insignificant; it merely does not prove it significant. To avoid any possible error in the conclusion due to a probable error that is large because cells of very different sizes are included, a somewhat similar comparison has been made involving only cells of approximately the same size, namely, those whose drawings were from 2 to 3 square inches in area. To increase the number of cells in each group, the generations were not in this case studied separately. Instead, there were distinguished two periods of many male-producers and three periods of few or no male-producers (table 1). The small percentage of male-producers in generation 9 was ignored. The oöcytes (of the size mentioned above) from these periods were combined into two groups which were averaged separately. The following comparison was obtained:

Relative nuclear volume of oöcytes in periods of many male-producers	0.0584 ± 0.0024
Relative nuclear volume of oöcytes in periods of few male-producers	0.0599 ± 0.0020
Difference of mean sizes	0.0015 ± 0.0031

Here the difference is less than its probable error. It is worthy of note, furthermore, that while in the earlier comparison the mean relative nuclear size was a trifle greater in periods of male-production than in periods of no male-producers, in the second comparison the relative nuclear volume is less in periods of many male-producers. The conclusion to be drawn, therefore, is just what would have been drawn from a simple comparison of differences of mean and probable error in any of the tests applied, namely, that there is no relation between relative nuclear volume of the oöcytes and the number of male-producers.

NUCLEAR VOLUME AND AGE OF INDIVIDUAL

From the rotifers fixed in early life, at middle age, and just before the end of the reproductive period, as described in the introduction, were obtained the measurements described in this section. The cells from the three tissues are described separately.

Yolk gland. Three lots of rotifers were fixed for the study of cells in relation to age, each lot being divided into three groups of different ages. The relative nuclear volumes of all cells in each group have been averaged, and the means given in table 6. In every experiment the nuclear volume is greater the older the animal. The combined results shown in the last line of the table show differences that are many times their probable error, and

TABLE 6

Relative nuclear volume in the yolk gland of Hydatina senta in young, middle-aged, and old specimens. The data are derived from three similar experiments

EXPERIMENT	RELATIVE NUCLEAR VOLUME		
	In young individuals	In middle-aged individuals	In old individuals
1	0.533±0.010	0.682±0.017	1.140±0.047
2	0.564±0.016	0.586±0.019	0.677±0.021
3	0.517±0.010	0.607±0.025	0.661±0.023
All combined.......	0.538±0.0088	0.644±0.0198	0.805±0.0227

there seems no question that they are significant. The relative nuclear volume in the yolk gland increases with the age of the individual.

The manner in which this increase of relative nuclear volume is brought about, whether by increase of the absolute nuclear volume or decrease of the volume of the cytoplasm, or both, is of importance. All drawings of the yolk gland were made on the same scale, so that absolute sizes can be determined (table 7). The mean nuclear sizes, expressed in square inches on the drawings, for the three age-groups are very much larger at middle age than in early adult life, but decrease later without, however, being reduced to the size of nuclei in young adults. The smallest difference shown in this comparison, that between middle-aged and old females, is four times its probable error, and appears certainly significant.

Changes in the size of the cytosome in the yolk gland are also shown by the measurements made. Since the yolk gland is a syncytium, the cytosome of single 'cells' can be measured only when the section passes through but one nucleus. Safe comparisons can be made so long as sections of approximately the same size are used. Probably the most satisfactory criterion of size, for this purpose, is the number of nuclei cut in the section, since the nuclear content of the section would be much less variable with this criterion than with the criterion of equal absolute area of the figure. Sections containing two nuclei are, on the whole,

TABLE 7

Mean absolute sizes of nuclei in yolk gland of Hydatina senta females of different ages, expressed in square inches on drawings. Also size of cytosomal portion of gland, as shown by sections containing two nuclei

	SIZE OF NUCLEUS	σ OF NUCLEUS	SIZE OF CYTOSOME	σ OF CYTOSOME
Young adult females.......	0.358±0.0087	0.146±0.0062	1.43±0.042	0.272±0.030
Middle - aged females.......	0.569±0.0163	0.234±0.0115	1.77±0.053	0.161±0.037
Old females.....	0.486±0.0127	0.211±0.0089	1.27±0.046	0.429±0.033

more numerous in my material than any other size, and these alone are used in the comparison. The areas of the cytosomal part of these sections are added to table 7, in the last two columns. The figures show that the cytoplasm is more abundant at middle age than earlier, but is later reduced to an amount less than that in the young adults.

From the data given in table 7, therefore, it must be concluded that the rather steady increase of the relative nuclear volume of the yolk gland during the lifetime of an individual is accomplished in the following manner. The increase from early adult life to middle age is the result of absolute increases in both nuclear and cytosomal volume, the percentage of nuclear increase being much the greater. The increase of relative nuclear volume from middle age to old age is due to decreases in both nuclear and cytosomal volume, the percentage of cytosomal decrease being much the greater.

The variability of nuclear and cytosomal volumes, as indicated by the standard deviation (σ), is perhaps worth noting. The nuclear volumes are much more uniform in the early adults than later, while the cytosomal volumes are more constant in middle life. The probable errors pertaining to the values of the standard deviation appear to show that this conclusion is well founded.

Stomach-intestine. The relative volumes of the nuclei of the cells in this organ are summarized as follows:

Relative nuclear volume in stomach-intestine of young adult females	0.071 ± 0.003
In middle-aged females	0.072 ± 0.002
In old females	0.077 ± 0.004

Although there is a slight progressive increase in the relative nuclear volume through life, the size of the probable errors indicates that no significance of the differences is proved. However, the comparative uniformity of the relative nuclear volume seems not to be due to stationary size of both nucleus and cytosome during the lifetime. For, when the absolute nuclear sizes are averaged, the following results are obtained:

Size of intestinal nuclei in young adults	0.325 ± 0.0088
Size of intestinal nuclei in middle-aged females	0.357 ± 0.0086
Size of intestinal nuclei in old females	0.384 ± 0.0130

While one would hesitate to ascribe significance to the difference between young and middle-aged, or between middle-aged and old females, the difference between young and old fulfills the usual statistical requirement for significance, indicating that the smaller differences between middle-aged individuals on the one hand and young and old females on the other are also significant. There is an increase, therefore, in the size of the cytosome from early adult life to old age, to maintain the comparative constancy of the relative nuclear volume shown above.

Oöcytes. The mean relative sizes of the nuclei in the oöcytes of females of different ages were found to be as follows:

Relative volume of nuclei in oöcytes of young adult females	0.065 ± 0.0072
In middle-aged females	0.051 ± 0.0033
In old females	0.057 ± 0.0042

These means and high probable errors seem to indicate that there is no relation between age and relative nuclear volume in the oöcytes. The largest difference of means, that between young and middle-aged females, is less than twice as great as its probable error.

It is not possible to determine whether the absolute sizes of the cells are different at different ages, for the reason that these cells change so much in size during their growth stages. I incline to

TABLE 8

Relative nuclear volumes of the yolk gland, stomach-intestine and oöcytes of Hydatina senta reared in spring water and in manure solutions

TISSUE	RELATIVE NUCLEAR VOLUME	
	Spring water	Manure solution
Yolk gland	0.718±0.0105	0.709±0.0119
Stomach-intestine	0.069±0.0029	0.079±0.0028
Oöcytes	0.070±0.0048	0.066±0.0039

the opinion that all of the irregularity of the results shown above are due to this growth, rather than to any fundamental changes which the statistics are incapable of revealing.

EFFECT OF MANURE SOLUTION

The relative nuclear volumes of cells in animals of the same age, some of them reared in spring water, others in manure soution, as described in the introduction, are given in table 8. The results from all three of the tissues studied are combined in one table.

The differences in the yolk gland and oöcytes are negligible. The values for the relative nuclear volume in the stomach-intestine are, however, such that, by the usual statistical standards, they must probably be regarded as significant. To determine the route by which the relative increase of the nuclei in manure solution was attained, the absolute measurements of the nuclei in both halves of the experiment have been collected and averaged, with the following result:

Mean area of intestinal nuclei in spring water.......... 0.379 ± 0.0087
Mean area of intestinal nuclei in manure solution........ 0.391 ± 0.0088

The nuclei are of practically the same size in both media, hence the increase in relative nuclear volume in manure solution is almost solely due to a decrease in the size of the cytoplasm.

COMPARISON OF MALE-PRODUCERS AND FEMALE-PRODUCERS

Female-producing and male-producing individuals from the same families, reared under the same conditions, are compared, with respect to relative nuclear volume, in table 9. Nothing in the measurements indicates that the nuclei of one of these types of female are significantly larger than those of the other type in any of the tissues studied. The differences disclosed are not large enough, relative to their probable errors, to prove a difference between male- and female-producers.

DISCUSSION

The major result of the experiments described in this paper is that no change of relative nuclear volume in oöcytes, yolk gland or stomach-intestine appears to have any relation to the type of reproduction that occurs. This conclusion, while opposed to certain phases of a widely held theory, is hardly a contradiction of previously observed facts in any animal with alternating parthenogenesis and sexual reproduction. It must be remembered that the relation of changes in nuclear volume to cyclical phenomena was first observed in Protozoa, and the idea was extended to parthenogenetic animals at first on purely theoretical grounds. Facts in support of that extension were later sought, but came haltingly. Papanicolau's observations, described in the early pages of this paper, were based on a very small number of individuals, showed differences that were mostly very slight, and were in part contradictory to the theory. Hartmann's work was more successful. Larger numbers of individuals were studied, and he was able to demonstrate changes of relative nuclear volume related to the seasons and certain environmental factors (temperature, chemical substances). One misses in his paper, however, the accurate relation of these changes to the life-cycle. It

TABLE 9

Relative size of nuclei in three tissues of male-producing and female-producing rotifers of the same origin, reared under the same conditions

TISSUE	EXPERIMENT	MALE-PRODUCERS	FEMALE-PRODUCERS
Yolk gland	1	0.571±0.015	0.477±0.010
	2	0.635±0.019	0.649±0.018
	3	0.651±0.073	0.562±0.008
	4	0.558±0.040	0.524±0.014
	5	0.643±0.012	0.682±0.017
	6	0.645±0.012	0.684±0.031
	All	0.635±0.0143	0.576±0.0152
Stomach-intestine	1	0.101±0.005	0.102±0.003
	2	0.084±0.002	0.096±0.004
	3	0.085±0.006	0.085±0.005
	4	0.063±0.003	0.083±0.007
	5	0.081±0.002	0.074±0.003
	6	0.079±0.003	0.080±0.005
	All	0.084±0.0034	0.092±0.0042
Oöcytes	1	0.079±0.001	0.066±0.008
	2	0.062±0.003	0.051±0.003
	3	0.056±0.002	0.073±0.005
	4	0.065±0.007	0.051±0.004
	5	0.064±0.002	0.059±0.006
	6	0.059±0.005	0.083±0.008
	All	0.064±0.0033	0.060±0.0040

is not clear what changes in the mode of reproduction accompanied the nuclear changes. One is left to infer, on the basis of work by earlier authors, that changes in temperature and of the chemistry of the water bring on the changes of reproduction. The weakness of such argument is mainly that the nuclear changes might be incidental, might be other effects of the same cause, and might therefore be prevented or modified by unknown agents which would not similarly prevent or modify the changes of reproduction.

The observations on Hydatina are subject to the same dangers. But owing to the many unrelated ways in which the theory can be tested in this organism, it should be possible to separate fundamental from incidental events. Thus, if the investigation had involved only the yolk gland, and only the changes in the yolk gland during successive generations, it might have been supposed that the increase of the nucleoplasma ratio in the yolk gland is the cause of the gradual reduction in the amount of sexual reproduction which has been observed to take place during long-continued parthenogenesis. However, the relative nuclear changes of the yolk gland do not coincide with the other changes in the type of reproduction; for example, not with the changes occurring during the lifetime of the individual, nor with the changes due to different media in which the animals are reared. It must be regarded as accidental, therefore, that the nuclei of the yolk gland increase in volume simultaneously with the diminution of sexual reproduction during a series of generations.

Notwithstanding the positive nature of the conclusions of Hartmann, these conclusions appear to have been reached partly by inference. The work of Papanicolau was based on too small numbers to be conclusive, and he recognized that it was only suggestive. Now that Hydatina is shown to offer no support to the theory that changes of nuclear size determine the changes of reproduction, continued application of the theory to parthenogenetic animals must for the present rest mainly on what are believed to be analogous phenomena in the Protozoa. A note of caution should be sounded with reference to this supposed analogy. Periods of depression in Protozoa, in which nuclear enlargement occurs, are also commonly succeeded or terminated by periods of conjugation. Perhaps it may be assumed that the Cladocera will show similar phenomena, for periods of depression toward the end of a series of parthenogenetic generations have been shown by Papanicolau ('10 a) to be also periods of sexual reproduction. In Hydatina, however, sexual reproduction does not occur chiefly in periods of depression. Sexual females occur most abundantly in times of rapid growth, rapid development, and large families. What phenomena stand in the relation of cause and effect at these times is uncertain, but the heightened speed of metabolism is usually recognizable before the abundance of sexual females exists. Furthermore, those lines which under a given set of conditions are producing the largest proportions of sexual females are, under like external conditions, the most vigorous lines. This has been the experience, I believe, of all who have worked extensively with Hydatina.

If depression is not, in parthenogenetic animals, universally associated with the initiation of sexual reproduction, great risk attaches to the assignment of causal significance to other phenomena (nuclear enlargement, for example) which accompany depression. The question whether the nuclear changes are merely accompaniments of, or accidentally associated with, reproductive changes, or possibly effects of the agents that also cause changes in reproduction, is comparable to the question already often raised concerning the validity of the Kernplasma-relation theory in other fields. Although Minot ('08) plainly regards a decrease of the nucleoplasma ratio as the cause, or at least a cause, of senescence, and though Hertwig ('08) expressly states that changes of K/P cause or postpone cell division, other authors are either non-commital or regard the nucleoplasma ratio as incidental or even as an effect of the phenomena which it is supposed to govern. Child ('15), for example, considers the relative size of the nucleus incidental as regards senescence, and Conklin ('12, '13) concludes that so far as the relation of nuclear size to cell division is concerned, nuclear size is an effect instead of a cause. The implication of Issakowitsch ('07), therefore, that the supposed relation of nuclear changes to the mode of reproduction in parthenogenetic animals might be merely incidental, and the expressly stated views of Strohl ('08) and Woltereck ('11) that volumetric changes of the nucleus can have nothing to do with the alternation of parthenogenesis and sexual reproduction, find a counterpart in the ideas of workers in other fields. In view of the results with Hydatina described above, it may be necessary eventually to conclude that the relation between the nucleoplasma ratio and the alternation of parthenogenesis and sexual reproduction is not merely incidental, but that it does not exist.

It is not my purpose to discuss the possible physiological modus operandi of changes of relative size of nucleus and cytosome. That has been done repeatedly, in general terms, by others. Some of the discussions of the general significance of this size relation have already gone so far that it may now be desirable to wait for the observed facts to overtake them. Certain of the minor conclusions with regard to Hydatina may, however, profitably be mentioned in their physiological relations.

It is found that the nucleoplasma ratio of the yolk gland increases during a series of generations. Had only the values of K/P been obtained or studied, this might have been held to indicate a reduction in the amount of cytoplasmic material produced or at least of the contained yolk, while the nucleus remained stationary in size or at least lagged behind the cytoplasm in a decrease. However, the fact that the increase of K/P in the yolk gland is entirely due to increase of the nucleus while the volume of the cytosomal portion remains nearly stationary, shows that an active nuclear change is involved.

This increase of relative nuclear volume in the yolk gland during a series of generations confirms the statements made for Cladocera on which has been based the conclusion that the increase is caused by long-continued parthenogenesis. The stomach-intestine, however, changes only slightly and in the opposite direction. No change at all has been demonstrated in the oöcytes. It appears to be too early to generalize about the effects of parthenogenesis on nuclear volume.

There is also an increase in the value of K/P in the yolk gland during the life of the individual. In this case, again, if only the values of the ratio were known, it might be held that the increase is due to a consumption of the cytoplasmic material or of the contained yolk. It will be remembered that Lenssen ('98) was of the opinion that the growing oöcytes of Hydatina actually ingest fragments of the yolk gland. The absolute measurements of nucleus and cytosome show, however, that both nucleus and cytosome increase up to middle age, the nucleus enlarging somewhat the faster, while both of them decrease in size in later life, the decrease of the nucleus lagging behind that of the cytosome. These facts suggest that in early adult life there may be an active production of yolk material in which the nucleus takes a leading rôle, but that in later life the nutritive processes of the animal cannot keep up with the demands of the growing oöcytes for yolk so that there is an actual diminution of the size of the yolk gland. The adjustment of the nucleus to the reduced volume of the cytosome—a phenomenon observed in other cases of reduction of cell size—would, whatever its cause, naturally follow rather than precede the excess consumption of yolk material. Such an explanation would not lead one to expect a similar increase of the relative nuclear volume in other tissues, and, as a matter of fact, there is little or no change of this relative volume in either the stomach-intestine or oöcytes.

Further need of caution against generalizing concerning the nucleoplasma ratio in different tissues is shown by the fact that manure solution causes an increase of K/P in the stomach-intestine, but no change in the yolk glands nor, so far as can be ascertained, in the oöcytes. The nature of the effect of the medium on the cells of the stomach-intestine is problematical.

Not the least valuable result obtained is the demonstration that the nuclei of parthenogenetic and sexual females are of equal relative size. It has lately been shown (Shull '21) that the number of chromosomes is the same in both types of female, and now it seems likely that these females are also equal in their chromatin content. It had been suggested that a difference in the quantity of chromatin might exist without involving a difference in chromosome number.

SUMMARY

1 None of the changes in the relative nuclear volume in the stomach-intestine, yolk gland, or oöcytes of Hydatina appear to have any relation to the mode of reproduction, whether parthenogenetic or sexual. Individual phenomena, if observed alone,

might lead one to assign to them a significance in relation to the life-cycle, but in each case tests of the same phenomena in other ways have negatived such an assumption.

2. The relative volume of nuclei in the yolk gland increased steadily through a series of nineteen generations. This increase was due almost solely to an absolute increase of the nucleus, while the volume of the cytosome remained nearly stationary. The significance of this nuclear enlargement is discussed.

3. The relative nuclear volume of cells in the stomach-intestine remained on the whole nearly constant through the first ten or twelve generations of the line on which this study was based, and then decreased somewhat to the end of the series (nineteen generations). This decrease was apparently due to absolute decrease of the size of the nuclei, not to a change of the cytosome.

4. The relative volume of the nuclei of the oöcytes appeared not to change during nineteen generations. Determination of 'long range' changes in the nuclei of the oöcytes is made difficult, however, by a marked change in the relative nuclear volume during the growth of each cell. The ratio of nuclear volume to cytosomal volume is about five times as great in a moderately early stage of growth as it is when growth is nearly completed.

5. The ratio of nuclear volume to cytosomal volume in the yolk gland increases considerably during the lifetime of the individual. This increase is brought about by an increase of the absolute size of both nuclei and cytosome during early adult life, and a decrease of the absolute size of both nuclei and cytosome in later life. The absolute increase referred to is relatively more rapid in the nuclei than in the cytosome, while in later life the decrease is less rapid in the nuclei than in the cytosome. The net result is a steady increase of the relative nuclear volume throughout life.

6. The absolute size of both nuclei and cytosome in the stomach-intestine increases with age, the nuclear increase being perhaps slightly the more rapid. Hence the relative nuclear volume is nearly stationary throughout life, or perhaps increases slightly with increased age.

7. The relative nuclear volume of the oöcytes probably does not change with the age of the female.

8. Rearing the rotifers in manure solution increases the relative nuclear volume in the stomach-intestine, but does not change that of the yolk gland or oöcytes. The increase in the stomach-intestine is brought about by a decrease of the cytosome while the nuclei remain practically unaltered in size.

9. Relative nuclear volume in the yolk gland, stomach-intestine, and oöcytes is probably the same in male-producers as in female-producers.

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