STATISTICAL AND PHYSIOLOGICAL STUDIES ON THE INTERPHASIC GROWTH OF THE NUCLEUS

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1. Introduction

1. (*a*) *The "rhythmic" growth of nuclear size*

We define as "rhythmic growth" that growth of the cell nucleus in which periods of quick development alternate with others of rest or slower growth. The volumes attained in each step of this growth are generally in a simple mathematical ratio.

The simplest rhythmic growth is that in which nuclear size is doubled at every step (Jacobj, 1926). By measuring the nuclear sizes (volumes) of a homogeneous tissue, such as, for instance, the mammalian liver, and distributing the values into classes of a frequency curve ("Statistiche-kariometrische Untersuchungen), Jacobj verified that there are many modal classes, and that these modes form a regular series with a ratio of 1 : 2.

Some studies following the discovery of Jacobj led to the explanation that rhythmic growth of the nucleus is due to the reduplication of the nuclear content, which establishes in the tissues a series of polyploid nuclei or nuclei with polytene chromosomes (D'Ancona, 1939–40–41 ; Biesele, Poyner and Painter, 1942). Biesele later published a series of papers dealing with the size of the chromosomes in different conditions, ages, and tissues. We cannot discuss these results here because they do not appear to be sufficiently defined in relation to nuclear volume and nuclear growth.

In the meantime, other studies revealed that the steps in rhythmic growth of the nucleus may occur at a ratio different from that of 1 : 2. To some authors this fact seems difficult to interpret in genetic terms (Wermel and co-workers). Several authors described a rhythmic growth with a ratio of 1 : 1, 5 (Wermel and Portu-

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galow, 1935; Bogojawlensky, 1935), and others found the ratio to be 1 : 1.41, which is the value of the square root of 2 (Brummelkamp, 1939 and G. Hertwig, 1938–39). The nature of these intermediate stages, which German-speaking authors call "Zwischenklassen," has been discussed from different points of view in a previous paper (Schreiber, 1943). Both Brummelkamp (1939) and Hertwig (1938–39) studied the problem from a physiological point of view without considering the genetic basis of the phenomena that form nuclear activity during the interphase.

The main object of the present paper is the study of the intermediate classes of nuclear size during the reduplication step.

For this purpose, statistical analysis of nuclear size has been used, as by previous authors, in actively reproducing tissues as well as contemporary physiological methods such as hormone stimulation. With this method we can arrest or increase the mitotic activity of the cells by specific stimulation, and thus define the limits of the interphasic variation of nuclear size.

For the statistical study of nuclear growth some basic facts must be ascertained. First we must find out whether there is any correlation between nuclear volume and multiple value of the genome,[2] and if so at what point in the nuclear cycle it occurs.

The problem as to whether the number of chromosomes is related to the volume or to the surface of the nucleus, or to some other quantitative value of the cell is still open to discussion.

The second fact to be established is the limit of the variation of nuclear size during an interphasic growth cycle, with reference to the volume of a nucleus with a genome of known quantitative value. The term "interphase" is here used to indicate in a general way the period between two somatic divisions. Although the term was originally used to signify the interval between the first and second meiotic divisions, it was subsequently used by various authors as a synonym for "resting" or "metabolic" stage.

Another important fact which must be established in these caryometric studies is whether modifications of the model value of nuclear size in a tissue are natural or experimentally induced. We must be sure that variations in mode represent true growth or reduction and are not due to the occurrence of various types of cells of different characteristic sizes (i.e. grade of ploidy) originally present in the tissue and assuming by differential proliferation, the role of the main cell under the new conditions (Schreiber and Romano-Schreiber, 1941; Paccagnella, 1944–45).

In previous papers we approached these different problems from different angles and with different materials, each offering characteristics that would answer one of the questions. Thus the problem of the relationship between nuclear size and

[2] The term "genome" is here used following the definition of Sharp (Introduction to cytology, 3d Ed., New York, pg. 121): "In any given kind of plant or animal each nucleus contain an outfit or complement of chromosomes composed of a certain number of members showing characteristic difference in form and function. As a general rule the nucleus of an egg before fertilisation contains a complement made up of one each of several kind of chromosomes, such a complement is called a *set* or *genome*"; and pg. 353: "The monoploid chromosome set *or genome* is a group of chromosomes differing among themselves in the number and kind of their component elements. Ordinarly all or nearly all of the genetic elements (genes) are probably necessary to the normal activity of the nucleus; in other terms the genom is a harmonious differentiated system of elements, the majority of which are essential parts of the system."

genome has been studied in a series of polyploid Coffea plants (Schreiber, 1946) and in the spermatogenesis of snakes (Schreiber, 1946–47). The problem of the nature of the steps in rhythmic growth, and the limits of the mitotic interphase has been studied in experiments on the uterine cells under different physiological conditions and on the granulosa layer and luteal cells of the mammalian ovary (Salvatore and Schreiber, 1947; Schreiber, Mello and Salvatore, 1948; Salvatore, 1948).

We shall here summarize those studies which contribute to the knowledge of nuclear growth during interphase.

2. The Study of the Nuclear Growth

2. (*a*). *Statistical methods*

Analysis of the statistical distribution of nuclear sizes in a homogeneous mass of cells of some tissues verifies the existence of various modal values. This indicates that the nuclei stop growing or grow at a slower rate when they attain the sizes corresponding to the modal values. Within a given time, therefore, these nuclei appear with greater frequency. This finding is confirmed by comparing rhythmic growth of the nucleus, as indicated by caryometric statistical research, with the rhythmic growth of the nucleus in cells in cultures, measured at regular intervals with motion pictures (Wermel and Portugalow, 1935). A somewhat similar comparison between a statistical study of a dynamic phenomenon and the direct study of the same in living cells has been made by Möllendorff and co-workers (1937) in order to determine the relative length of the mitotic phases (see also W. Thompson-D'Arcy, 1942).

Some technical precautions must be taken, the first being that of geometric determination of nuclear size. In all tissues with spherical nuclei the problem is of course easy, but when we deal with ellipsoid-shaped nuclei the problem of the orientation of the axes is of the greatest importance and needs careful previous control. For the first type of cells the liver, testicle, and corpus luteum offer very good material; for the second groups the root tip cells, the cubical or cylindrical epithelia, and the smooth muscular cells of the uterine wall are very suitable.

All studies summarized here were conducted by drawing the nuclear outline with a camera lucida and measuring its diameters. The frequency curves were drawn and the modal values calculated. Since the curves are influenced by the growth of the nuclei they are not of the true "normal" type; mean, standard deviation, and median have no biological significance. Only the modal value is of biological interest because it reveals the steps during the growth cycle. The modal value of the volume can be calculated directly from the modal value of the diameter, which is not the case for other statistical parameters.

2. (*b*) *Physiological methods*

In the statistical study of nuclear variability, frequency curves sometimes have more than one modal value. Sometimes these modes are represented by very different frequencies, one mode appearing as principal and the others as secondary. If observed on isolated histograms the secondary modes may sometimes be considered statistically doubtful because they are determined from very scant data. However, if we consider the histograms of the same tissue under different physiological

conditions, we can frequently observe that what is a secondary mode under one condition may became a fundamental one in another physiological status.

Considering the histograms of various physiological conditions as a whole, we can not only confirm the statistical consistency of the small secondary modes of each physiological status, but also interpret the cyto-physiological significance of the nuclei that constitute each mode. This system of studying the caryometric variability of a tissue physiologically enables us to give biological value to certain data, which the purely statistical study of a single tissue could not do.

The modes that correspond to the volumes of the prophase are particularly important. These volumes belong to nuclei whose genes—and therefore chromosomes or chromonemata also—have completed a duplicating cycle and effectively represent a basic stage of interphasic growth, even though the prophasic nucleus shows some conditions of variability that affect caryometric measurements more than the interphasic nucleus (ellipsoidic form not oriented, larger imbibition phenomena, etc.).

The fundamental observation of Jacobj that the values of the maximum of frequency of nuclear sizes are in the relation 1:2:4:8, etc., leaves no doubt that the material constituents of the nucleus reduplicate at each cycle of growth, and therefore the phenomenon is related to the process of reduplication of the genes which constitutes the basic occurrence of the interphasic period. The nuclei belonging to the higher multiples of the volume are polyploid or with polytene chromosomes (Biesele, Poyner and Painter, 1942).

In our studies we are attempting to extend knowledge of the phenomena in the following manner:

First we tried to find out whether the nuclei that have no interphasic growth and have differing numbers of chromosomes (such as the meiotic elements) have a corresponding volume for each number of chromosomes.

Secondly we tried to ascertain whether the interval of rhythmic growth during which the nucleus duplicates its volume corresponds to an interphasic growth period, which begins with the post-telophase of the preceding division and ends with the prophase of the subsequent one.

Thirdly we tried to use special physiological conditions which would arrest nuclear reproductive activity and then cause it to begin again simultaneously in all nuclei under new, experimentally controlled conditions.

The statistical variability of these nuclei suggests that the growth obeying these physiologically stimulating or arresting conditions is of the "rhythmic" type.

With these three elements—comparison between nuclei without interphasic growth and with different numbers of chromosomes, comparison between the stages of rhythmic growth and the prophases, and the induction of simultaneous interphasic growth in all the nuclei of a tissue by means of controlled physiological stimulation—we can give a true significance of rhythmic growth in terms related to the duplicating processes of the nuclear genes.

2. (*c*) *The caryometric analysis of spermatogenesis*

The first question to be settled is whether in cells which have no interphasic growth and have a known but different numbers of chromosomes, the nuclear volumes have a constant value in proportion to the number of chromosomes.

The spermatogenetic series gives favorable results here, since the spermatocyte of the first order, the spermatocyte of the scond order, and the spermatid generally have chromosomes (or genomes) that are in the ratio 4:2:1.

The corresponding volumes of their nuclei, as has been known since the first studies of Jacobj (Jacobj, 1926; Freerksen, 1933; Hertwig, 1933; Sauser, 1936; etc.), are strictly in the same relation 4:2:1.

In certain cases the relationship is not the same (Wermel: Lepidoptera; Hertwig, Schreiber: Vertebrata), and we must consider these cases separately because they probably have different stages of endomitotic growth or some phenomena of chromatin elimination.

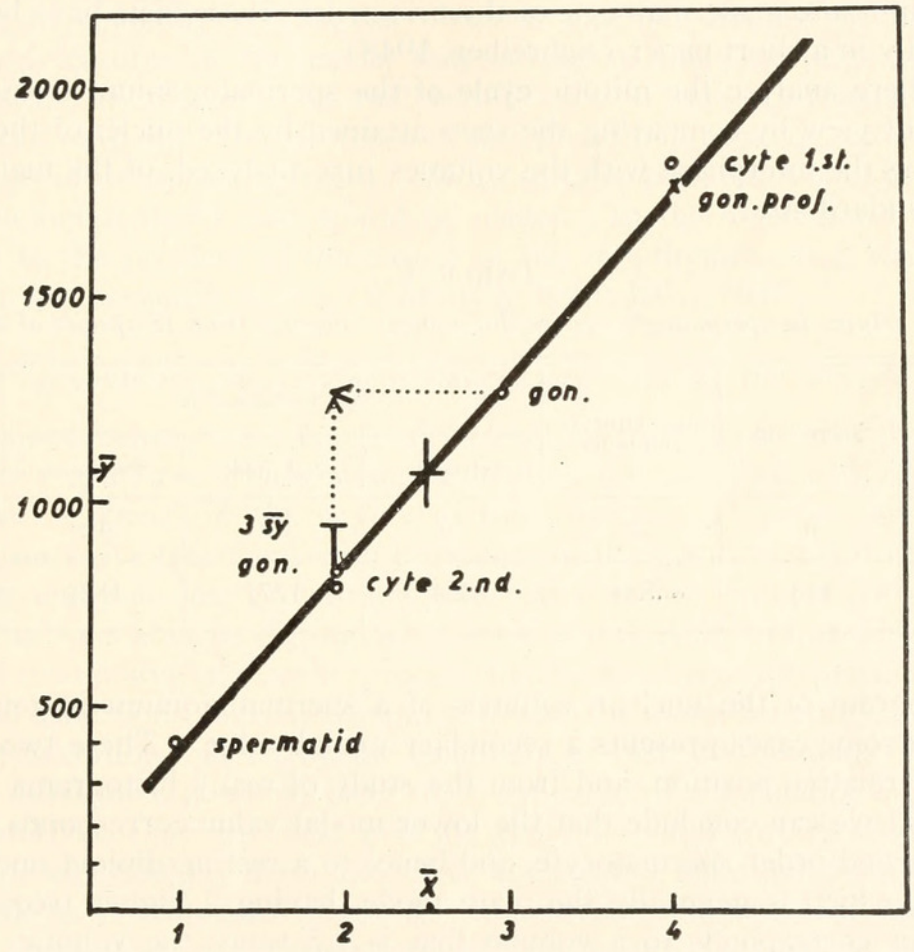

FIGURE 1. Scatter diagram and regression line between modal values of nuclear volume of spermatogenetic stages and the theoretical multiple values of the genome. From the average of 15 species of snakes.

It is important to stress here that the meiocytes ready for division represent a series of nuclei in which the multiple value of the genome is entirely proportional to the multiple value of the nuclear volume.

In the course of studies on meiocytes of snakes (Schreiber, 1946–48) we have verified this fact in 15 different species of neotropical Ophidia. The correlation between nuclear value and multiple value of the genome is always perfect, and the differences between the theoretical values and the actual ones are statistically insignificant.

We can therefore accept in testicular tissue the meiocyte series as a standard series of nuclear sizes that allows us to determine the quantitative value of the genome on the basis of nuclear volumes. This fact must be recognized as being limited to this tissue and no generalization made, for there are conditions in other tissues and organisms under which the correlation between genome and nuclear size does not follow the same rule (Wettstein, Dobzhansky, Barigozzi: see Schreiber 1946 c).

In the testicle, however, we find another category of cells, the spermatogonia, closely related to the meiocytes, but presenting a mitotic reproductive cycle with normal interphasic growth before the beginning of the meiotic process.

It is not our present task to analyze the growth phase which transforms the spermatogonium into a spermatocyte of the first order; these facts have been studied caryometrically in a short paper (Schreiber, 1948).

We will here analyze the mitotic cycle of the spermatogonium from the caryometric point of view by comparing the sizes attained by the nuclei of the spermatogonium during the interphase with the volumes just analyzed, of the meiocytes considered as standard size.

TABLE I

Nuclear volume in spermatogenesis (modal values)—average from 15 species of snakes

Cell	Spermatid	Spermatocyte 2nd order	Spermatogonium			Spermatocyte 1st order
			1st mode	2nd mode	Prophase	
Genome	n	2n	2n	(3n)	4n	4n
Volume	414	884	824	1271	1824	1765

The histogram of the nuclear volumes of a spermatogonium is generally unimodal, but in some cases presents a secondary modal value. These two modes are in a constant relative position, and from the study of many histograms (of 15 different species) we can conclude that the lower modal value corresponds to the volume of the second order spermatocyte, and hence to a resting diploid nucleus. The higher mode, which is generally the main mode, having a higher frequency value than the other corresponds to a volume that is 1.5 times the volume of the first mode, i.e. half way between the mode of the spermatocyte of the second order and the spermatocyte of the first order.

In the regression line between the modal values and the number of genomes (Fig. 1), the main mode of the spermatogonium corresponds to a genome value of 3. The difference between the volume of the main mode of the spermatogonium and the mode of the resting diploid nucleus (secondary spermatocyte) is statistically significant (more than 3 Sy.).

The volume of the spermatogonial prophase, which represents the final stage of interphasic growth in the mitotic cycle, is very nearly the same as that of the first order of the spermatocyte.

With these indications we can try to consider the entire interphasic growth of the spermatogonium (from the caryometric point of view) as follows: the diploid

nucleus grows during the interphase and reduplicates its volume when it reaches the prophase. During growth, however, it stops when it reaches a volume that is about one and a half times the initial one.

This relation (1 : 1.5) between the two modal values of the spermatogonial growth cycle corresponds to the "Zwischenklassen" which some authors (Brummelkamp,, Hertwig) describe in comparing the nuclear volumes of different cells, tissues, or species. In the case of the spermatogonium we can be sure that these intermediate classes in the statistical analysis belong to the growth phases of a single category of cells, as shown in the growth curves studied by Wermel and Portugalow in the motion pictures of cells cultivated in vitro. In our preceding papers we called this intermediate stage of interphasic growth—"sesquiphase."

The rise of common hystological methods has not enabled us to detect any morphological features in the nuclei that belong to this "sesquiphasic" size and would allow us to interpret the real significance of this intermediate step. At present we can only infer the existence of this phase by means of statistical analysis of the volumes of interphasic growth. More suitable material would perhaps reveal some morphological detail that would be useful. In the discussion of results, we shall return to the problem of the nature of the sesquiphasic step, which has been discussed in our previous papers (Schreiber, 1943, 1946, 1947).

2. (*d*) *The caryometric analysis of uterine tissues during the sexual cycle*

As explained before, we have tried to analyze interphasic growth of nuclei in tissues where a specific morphogenetic stimulation was obtained with a suitable dose of hormones. Cutting off the supply of these hormones (as in castration), and followed by intensive treatment with hormones of the gland that had been removed, enabled us to study nuclear growth and to arrest it at both ends of the growth cycle.

The uterus in mammals during the normal estral cycle and pregnancy provides a medium for studying the same process of synchronous cell proliferation under physiological conditions, and for comparison with the above mentioned experimental conditions (castration and estrogenic treatment of castrated animals).

In the course of our work, facts have appeared which facilitate the statistical study of interphasic growth and give us the full picture of its rhythm. Many problems in endocrinology arise and these are treated separately (Salvatore, 1948, a, b, c). We have limited ourselves here to analyzing the statistical and cytological side of the phenomena, correlating this with the results obtained in other fields in which interphasic growth has been studied (Schreiber, 1943, 1946 a, b, and c, and 1948).

The studies reported here were performed by measuring nuclear volume and analyzing its statistical variability using the same general methods as those mentioned above. The first layer of the uterine epithelium, the glandular cells, and the muscular fiber of the meiometrium were examined during the estral cycle and pregmancy in white rats, mice and humans. In the castrated animal, we studied nuclear volumes in the untreated female, and during experimental estrus induced by injection of estrogenics.

In all cases the nucleus was considered as a rotating ellipsoid, and only those parts of the tissue with the nuclei well oriented for measurement were studied. We give here some typical cases representing respectively an estral cycle, a castration,

an experimentally induced estrus, and a pregnancy, which give us the clearest picture of rhythmic nuclear variation. A complete description of all cases, with histograms and numerical tables, is recorded in the papers by Salvatore and Schreiber (1947), Salvatore (1947–48), and Schreiber, Mello and Salvatore (1949).

The following facts were observed: During the period of diestrus all the nuclei are simultaneously at rest, at a basic volume which we conventionally call "1." During the period of increase in hormonic activity, the nuclei begin to grow, showing a rhythmic pattern (polymodal frequency curves) reaching double size after having stopped temporarily at the intermediate stage of 1.5 times initial size. The phenomenon is exactly the same in all categories of cells studied, and we believe that muscle cells are of special interest, since their cyclic growth has hitherto been completely unknown.

When the hormone reaches its maximum of concentration at estrus, the nuclei seem to stop simultaneously after reaching size 2, as though they were waiting for some new conditions which would allow them to begin mitosis. Some nuclei undergo further volumetric rhythmic growth and reach sizes 3 and 4.

During estrus and the succeeding short transitional stage (estrus-metaestrus) mitosis appears in many cells by a change in the hormonic conditions (the nature of which is still under discussion by physiologists, i.e. quantitative or qualitative), and statistical analysis of the nuclear sizes reveals the reappearance of lower volume categories. Some cells degenerate and are probably phagocytized; others begin a new growth cycle. At the end of this period, no mitosis is present (metestrus) and all the nuclei are once more resting at size 1, just as they were during the diestrus stage at which the cycle was begun (Fig. 2).

The morphological features of the nuclei during the phases of the cycle are slightly different. The nuclei of the initial stages are more likely stained and of more compact aspect than those of double size, which have one or two clearly visible nucleoli. These facts appear in both epithelial and muscular cells. An interesting modification in the morphological aspect of the nuclei has been reported by Pfeiffer and Hooker (1944) in the stromal tissue of the uterus of mice under different hormonic conditions, some of which can be compared with ours. It does not seem impossible that the features described by these authors belong to the phases of a typical "endomitotic cycle" (endoprophase, endometaphase, etc.), but the authors do not attempt any interpretation and do not give any detailed statistical record of the volumes.

During pregnancy there is a situation identical to that of the estrus stage, complicated in the rat by an intermediate cycle of cell division on the 13th and 14th days. Of special interest was the muscular layer, in which we found a number of cells continuing rhythmic growth and reaching high multiples of the basic volume. This condition is identical in the uterine segment bearing the foetus which is mechanically enlarged, as well as in the intermediate segment without foetus. This eliminated the idea of hypertrophy, of a mechanical origin, of the muscle cells, which some authors believe.

These studies elucidate from a quantitative point of view, the nature of uterine hypertrophy and hyperplasy. Rhythmic growth of the nucleus appears to provide a new explanation for the hypertrophy of cells during pregnancy and estrus growth, i.e., as being due to interphasic growth and subsequent division (hyperplasy), or

interphasic growth without subsequent division but followed by many successive endomitotic cycles (true hypertrophy) (Salvatore, 1948 c).

In the untreated castrated animal the caryometric picture is exactly the same as that during diestrus and metaestrus; i.e., an absolute rest of all the nuclei at the basic volume 1. The experimentally induced estrus and the successive estrus-

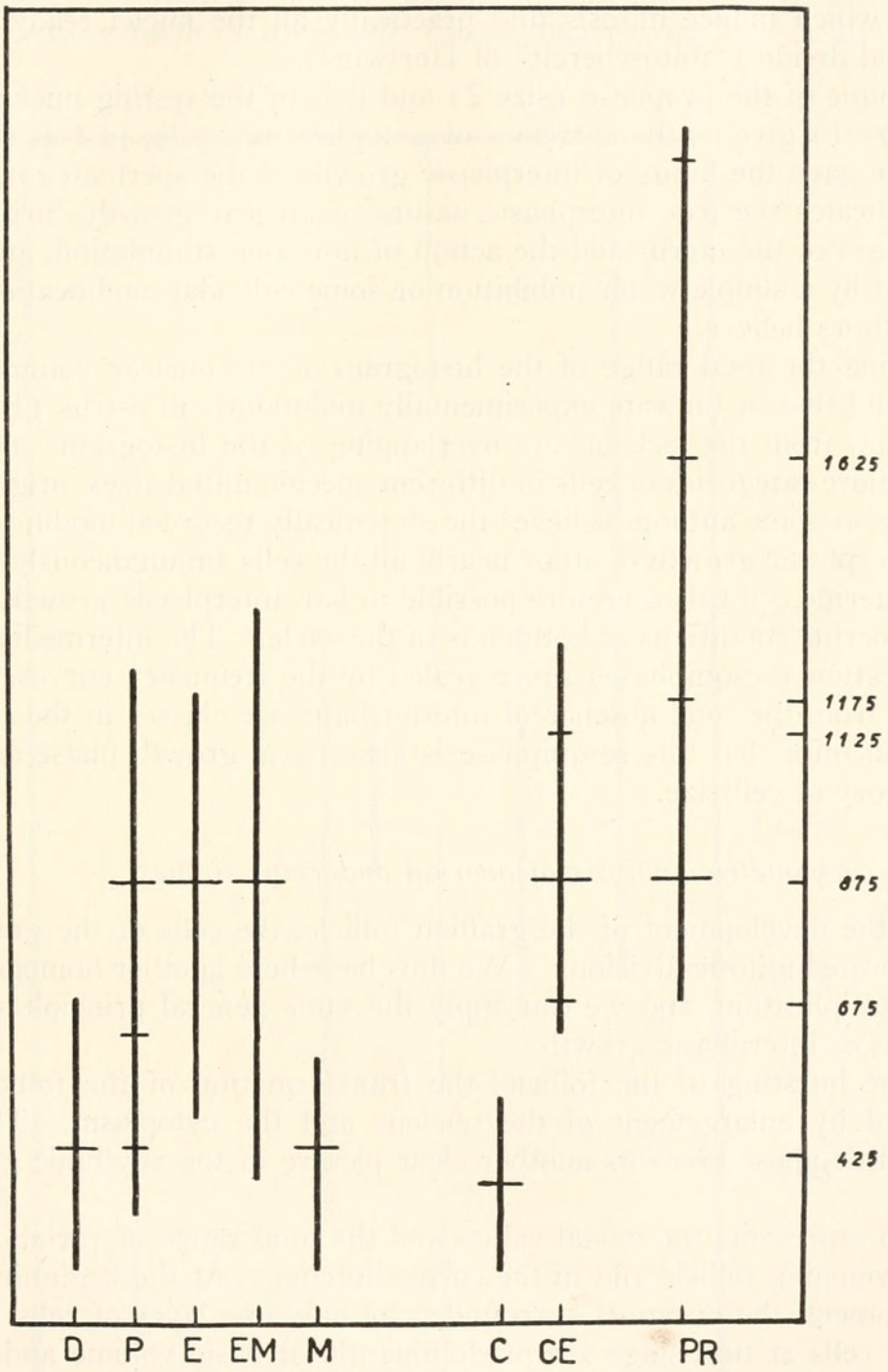

FIGURE 2. Nuclear sizes of rat miometrium during the stages of the estral cycle, castration, experimentally induced estrus, and pregnancy.

The vertical lines represent the total range of variation of nuclear volumes. The longer transverse lines represent the main modal values; shorter transverse lines represent the secondary modes. Values on the right side of the diagram give the average of the modal values of all stages. D = diestrus; P = proestrus; E = estrus; EM = estrus-metestrus; M = metestrus; C = castrate; CE = experimental estrus in castrated animal; PR = pregnancy.

metaestrus stage obtained by the interruption of the hormone supply are identical, however, and clearer than the corresponding physiological stages.

We can infer from the above facts that the increase in hormonic concentration results in the volumetric increase of the uterine cell nuclei, and this fact manifests itself by a doubling of the volume one or more times. During physiological estrus and during the interruption of the hormonic treatment, mitotic activity appears, and the factors which induce mitosis find practically all the nuclei ready to begin the prophase and divide ("mitosebereit" of Hertwig).

The volume of the prophase (size 2) and that of the resting nuclei in castrated animals (size 1) give us the extremes of a duplicating cycle, just as the volume of the meiocyte gave the limits of interphasic growth of the spermatogonia.

This indicates the true interphasic nature of nuclear growth during the physiological phases of the uterus and the action of hormone stimulation, and can hardly be explained by a simple water imbibition or some colloidal modification of the cell, as some authors believe.

Comparing the total range of the histogram of the nuclear volume of the castrated rat and that of the rats experimentally maintained in estrus (Fig. 2), it appears evident, from the lack of any overlapping of the histograms, that there are not two or more categories of cells of different specific initial sizes, originally present in the tissue, as some authors believe; the statistically recorded modifications consist of a real interphasic growth of all or nearly all the cells simultaneously in the tissue.

In the uterine cell it is therefore possible to halt interphasic growth by means of specific endocrine conditions at both ends of the cycle. The intermediate steps during reduplication (sesquiphase) are revealed by the frequency curves with unusual clarity, and from the total absence of intermediate size classes in the castrated animals, we can infer that this sesquiphase is also a real growth phase and not a different category of cell size.

2. (*e*) *The caryometric analysis of ovarian endocrine tissues*

During the development of the graffian follicle, the cells of the granulosa layer undergo repeated mitotic divisions. We thus have here another homogeneous tissue in active multiplication, and we can apply the same general principle of the statistical analysis of interphasic growth.

After the bursting of the follicle, the transformation of the follicular cells is accomplished by enlargement of the nucleus and the cytoplasm. The statistical analysis of this phase gives us another clear picture of the rhythmic growth of the nucleus.

Figure 3 represents the modal values and the total range of variation of nuclear size in a developing follicle and in the corpus luteum. At the beginning of the follicle development, the oocyte is surrounded by only one layer of cells. The nuclei of follicular cells at that stage are predominantly at basic volume and some nuclei are at 1.5 times greater. In a more developed follicle the histogram shows three distinct modes: volumes 1, 1.5, 2 respectively. The prophases are all at size 2. We have here the same condition as previously stated in the uterus layers and in the spermatogonia. The growth cycle of the cell consists of a duplication of the nuclear volume showing the intermediate step (sesquiphase) and ending with the prophase.

The granulosa cells have a mitotic index of about 10 per cent during the period

of the growth of the follicle, which drops to 0 per cent at the moment of luteal transformation.

The luteal cell of a transitory corpus luteum has a very regular statistical distribution of nuclear volumes with only one mode.

This mode corresponds exactly to the volume of the prophase (size 2) of the granulosa cells of the follicle. In the corpus luteum during pregnancy there is a further volumetric growth of some cells whose nuclei reach size 3, and probably 4 and 6.

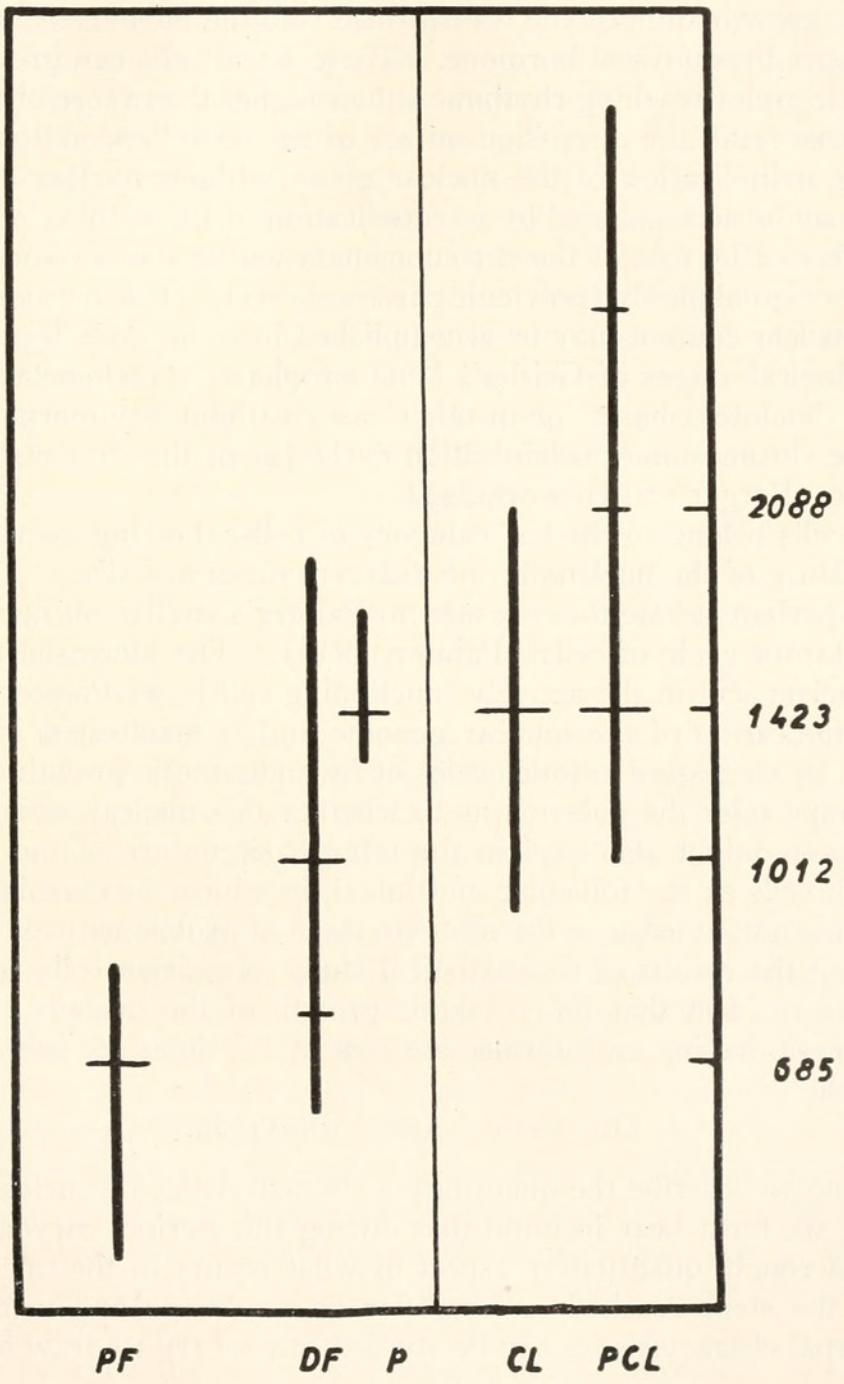

FIGURE 3. Nuclear sizes of the cells of graffian follicle and corpus luteus in the rat. Same explanation as Figure 2. PF = primary follicle; DF = developing follicle; P = prophases of developing follicle cells; CL = corpus luteus (cyclic); PCL = corpus luteus in pregnancy.

These higher multiple values of the phasic one are more difficult to establish owing to their rarity and the greater variability of the larger nuclei. There are no apparent differences in the morphology of these different classes of nuclei.

It is generally assumed that the luteal cells originate from the granulosa cells, although some authors believe that the cells of the "theca interna" of the follicle also contribute to their formation. The nuclear sizes of the cells of the granulosa layer and those of the luteal cells form a series of rhythmic values (Table II). This indicates the probable origin of the luteal cells from the granulosa; these reach the end of interphasic growth, and instead of beginning the prophase and dividing, continue the endomitotic growth and become transformed into the luteal cells under the influence of the proper hypophyseal hormone. These luteal cells can grow still further, with endomitotic cycles reaching rhythmic values higher than those of the initial size.

We must here recall the discussion on use of the term "endomitosis" in a broad sense, meaning reduplication of the nuclear genes without nuclear division. This reduplication may be accompanied by a reduplication of the number of chromosomes (true polyploids) or by that of the chromonemata within the chromosomes without variation of the original ploidy (polytenic chromosomes). Furthermore this reduplication of the nuclear content may be accomplished in some cases by the appearance of the morphological stages of Geitler's "endoprophase," "endometaphase," "endoanaphase" and "endotelophase," or in other cases without any morphological manifestation of the chromosome nucleinization cycle [as in the case of the ileum cells of the mosquito (Berger and co-workers)].

The luteal cells belong to the last category of cells, showing no visible variation of the inner feature of the nucleus in the different classes of size.

We should perhaps relate these results to Painter's studies on nuclear participation in the secretory cycle of cells (Painter, 1945). The increased need for cytoplasm ribosenucleic acid in the actively functioning cell is, as Painter thought, supplied by a reduplication of the nuclear genome and is manifested in the different types of glands by successive mitotic cycles or by endomitotic growth of the nucleus. We could perhaps raise the question as to whether this nuclear activity during the secretive processes might also explain the interphasic nature of the observed phenomena in such cells as the follicular and luteal, in which the chemical constitution of the secretion is not, at least, in the ultimate stage of proteic nature.

Summarizing the results of the statistical study of ovarian cells we can furthermore emphasize the fact that the rhythmic growth of the nucleus represents true interphasic growth, having an intermediate step at 1.5 times the initial size of each duplicating cycle.

3. Discussion and Conclusion

Before trying to describe the quantitative characteristics of nuclear growth during interphase, we must bear in mind that during this period, caryometric analysis can give only a rough, quantitative aspect of what occurs in the nucleus, and only by comparing the steps reached under different conditions and using material in which the internal characteristics can be studied can we try to draw some definitive conclusions.

It should not be forgotten that nuclear size is the result of a number of physical, physico-chemical, and chemical phenomena acting during the period in which the genes reduplicate and probably during which they perform their specific action in

the cells. It is not definitely ascertained at what moment of the nuclear cycle reduplication takes place, although the interphase seems to be the most probable. We cannot affirm either whether gene reduplication and chromosome splitting are simultaneous, because they occur at different levels of molecular and morphological organization. Furthermore, we must limit our analysis to the simplest case of the interphase of somatic cells, and not extend it to the most complicated cases of auxocytic growth, post-meiotic divisions or segmentation of the blastomeres, in which other conditions (i.e., multiple strand constitution of the chromosomes) would complicate the analysis.

Caryometric studies with statistical analysis of the prepared tissue conducted at the same time as the motion pictures of the living nucleus (Wermel and Portugalow, 1935) confirm that rhythmic growth, as deduced from the modal values of the frequency curves of nuclear volume, corresponds to a real discontinuous growth. The modal values correspond to the steps reached after each growth period is ended. The studies conducted in the regenerating liver by use of colchicine methods (D'Ancona, 1939–41–42) and in the neoplastic tissues by statistical methods (Biesele, Poyner and Painter, 1941) confirm the close correlation between multiple modal values and polyploid or politenic status of the chromosome complement.

The experiments we have carried out in the spermatogenetic stages and in the uterine and ovarian cells might give a more complete picture of rhythmic growth, because of material and physiological conditions that permit the recognition of the true interphasic nature of that discontinuous growth.

We can summarize the facts as follows:

(1) Comparison of the volumes attained at the successive steps of the spermatogonium mitotic cycle, with the volumes at the stages of the meiotic elements (in the vertebrate testicle), allow us to measure variations in volume in terms of quantitative values of the genome.

(2) During the mitotic cycle the prophase represents the end of a growth cycle of the nucleus and corresponds physiologically to the completion of a reduplication cycle of the nuclear genomes. In all cases the volume attained during prophase corresponds to volume 2 of the rhythmic growth series, thus indicating the interphasic nature of the rhythmic steps.

(3) Using the physiological conditions of the sexual cycle in mammals we can study interphasic growth in the nuclei of a specific tissue sensitive to the stimulus of corresponding specific endocrine conditions. These conditions permit growth to be stopped at both extremes of the nuclear cycle. Here too the prophasic volume gives us the limits of a reduplication cycle of the chromosomes, and allows us to consider rhythmic growth as truly interphasic.

(4) The whole series of rhythmic values of nuclear size in all the tissues studied indicates that this growth is accomplished by a succession of reduplicating cycles, but is complicated by the existence in each reduplicating cycle of an intermediate phase ("Zwischenklasse") in which the nuclear volume is one and a half times the initial volume of each cycle ("Sesquiphase").

The ratios between the steps thus appear to be alternately 1 : 1.5 and 1 : 1.33, and the whole series of values should be 1 :(1.5) :2 :(3) :4 :(6) :8 :(12) :16 etc., the steps in parenthesis being the so-called sesquiphases.

These facts clearly appear from the scatter diagram of Figure 4. Table II shows the regression of the modal volumes in the theoretical rhythmic growth series with

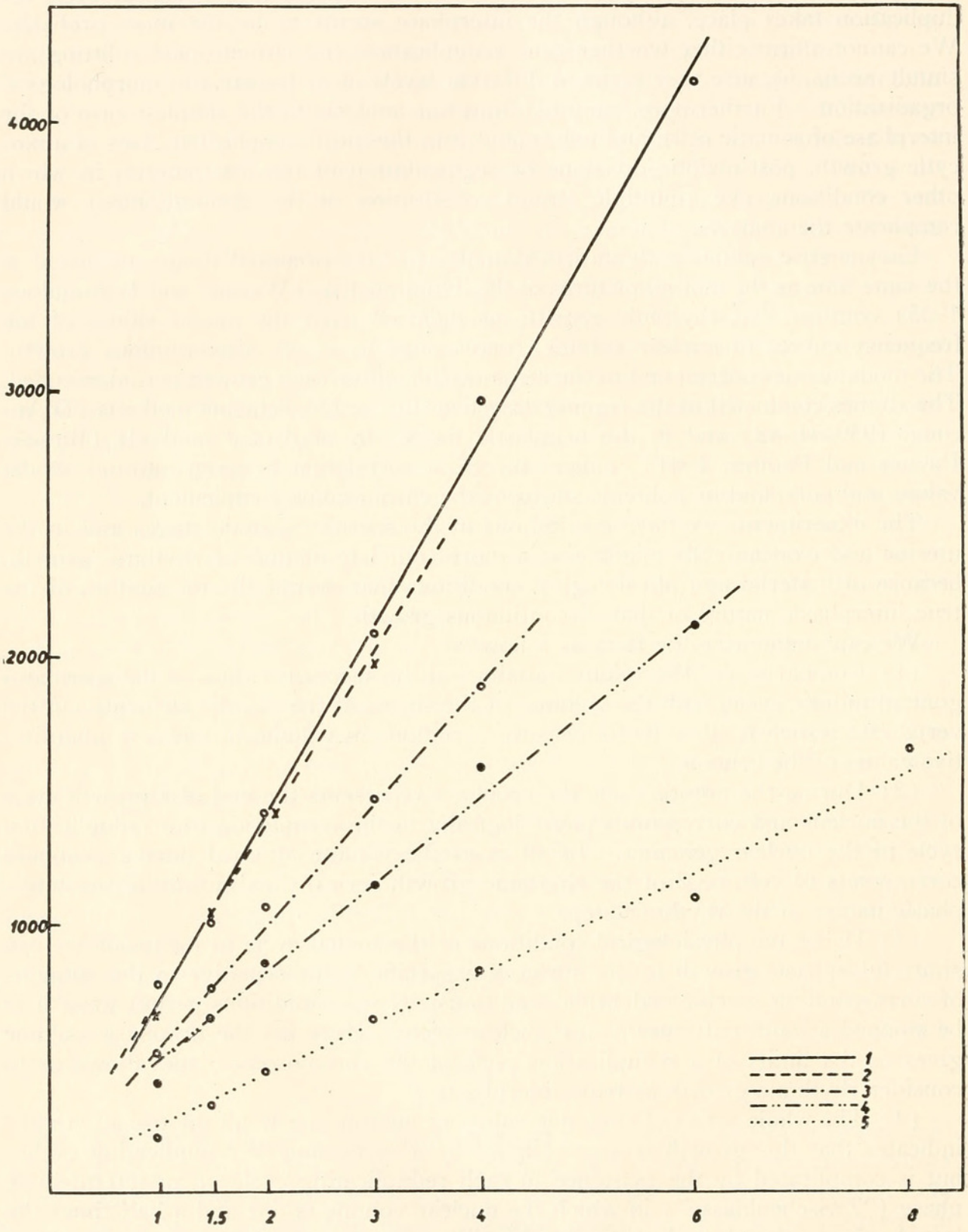

FIGURE 4. Scatter diagram and regression lines between modal values of nuclear volumes of uterine and ovarian cells and the theoretical series of the rhythmic growth stages with the intermediate step (sesquiphase).

(1) Graafian follicle and luteal cells in the rat. (2) Endometrium of the rat. (3) Human miometrium. (4) Miometrium of the rat. (5) Miometrium of the mouse.

the intermediate steps of the sesquiphase. We cannot discuss here the problem of th absolute values of nuclear volume in various tissues of an organism, which was considered in the original research of Jacobj, but which we believe should be further analyzed.

Rhythmic growth of the cell nucleus thus appears to be related to interphase activity. The size of the nucleus increases with a period of active growth, alternated with periods of rest. After reduplication of genic material is accomplished, generally the nucleus begins the prophase stage and divides. Sometimes mitosis is suppressed and a new reduplicating cycle (endomitosis) begins.

From the relationships between modal volumes and the multiple value of the chromosomes or chromonemata we must infer that at each step after a growth cycle, the nucleus consists of the genes and the material accompanying them which form the chromosomes, the nucleolus, and nuclear sap, the quantity and physico-

TABLE II

Nuclear volume (modal values) of the uterine and ovarian tissues (P = prophase)

	Series of modal values of the rhythmic growth of the nucleus						
Theoretical series	1	1.5	2	3	4	6	8
Rat endometrium	665	1037	1460 1428 P.	2100 1983 P.			
Rat follicle and lutheal cell	685	1013	1423 1436 P.	2088	2961	4157	
Rat miometrium	420	651	863	1152	1592	2108	
Mice miometrium	216	337	444	642	830	1100	1650
Human miometrium	522	767	1078	1473	1891		

chemical status of which determine, in regularly shaped nuclei, a nuclear size proportional to the number of genomes present in the nucleus.

This principle makes it possible for us to understand the whole mass of facts revealed by caryometric analysis. We must consider as a fundamental fact the constancy of the ratio between the genes and the accessory materials that accompany them in the morphological constitution of the nucleus, both in quantity and in physico-chemical status, during the true "resting" condition between two successive reduplication cycles.

During the "metabolic" period (interphasic growth period) this ratio is obviously altered by the phenomena of water and material changes between the nucleus and the cytoplasm; but the ratio goes back to its initial value at the end of each cycle.

The nature of the intermediate step during the reduplication cycle, which we call "sesquiphase," is more difficult to understand. We do not know of any cytological feature of the nucleus specific for that phase; its evistence is only inferred from statistical analysis. We note that these steps do not appear in all tissues or in all

species. The classical series of Jacobj and of many others give us a clear picture of a purely reduplication series.

Notwithstanding, in many other cases recorded by previous authors and in the ones here studied, as well as in the nuclear diminution series studied by Schreiber and Romano-Schreiber (1941) the existence of these intermediate steps is evident. It is not possible to call upon factors involving the geometric form of the nucleus in determining those steps, because they appear in both perfectly spherical nuclei (spermatogonium and luteal cell) and elliptical ones (endometrium and miometrium).

If we compare the histograms of the same tissue under different physiological conditions, we see that the same modal value always appears at the same position, and often what is the main mode in one physiological condition can be a secondary modal value in another. This fact allows us to consider the "sesquiphase," which statistics indicate to be a true biological phenomenon.

The theoretical explanation of the sesquiphase may be attempted by different methods and we can try to analyze some of them here. We might first look for a physiological explanation as G. Hertwig and Brummelkamp did. We can imagine some reasons for a stoppage or slowing down of the growth when approximately 50 per cent of the initial volume is attained. We cannot analyze here the complicated and perhaps artificial theories of G. Hertwig, based upon a hypothetical factor acting in different categories of nuclei, and correlating the chromosome number in some cases with the nuclear volume and in other cases with the nuclear surface. The mathematical explanations of Brummelkamp appear even more fantastic. Both authors consider the ratio between the steps as being 1 : 1.41, i.e., the square root of 2, which is very close to the ratio 1 : 1.5 considered correct by other authors. Wermel and his co-workers also consider the ratio to be 1 : 1.5. According to these authors the series should be 1 : 1.5 : 2 : 2.25. . . . For that reason these authors believe that the "Verdoppelungsgesätz" of Haidenhain and Jacobj must be rejected, but they do not give any new explanation of the nature of the rhythmic step of the growing nucleus.

We might also try to explain the "sesquiphase" in some other way, for example, by relating a different velocity of the splitting of euchromatic and heterochromatic regions of the chromosomes. Or we can take into consideration the different effects upon nuclear and nucleolar size of the two different types of chromatin (Fernandes and Serra, 1944). All this, however, would not explain the constancy of the ratio 1 : 1.5 between the steps in many categories of cells in which the sesequiphase can be detected, and which have a great variety of ratios of metero- and euchromatin.

Here it is interesting to note several facts found by Biesele (1940) relating to the 50 per cent increase in volume of the metaphasic chromosomes under certain physiological conditions without an apparent increase in the number of chromonemata. We cannot at present imagine what relation this may eventually have to the phenomena analyzed by us here, but we presume that these phenomena may eventually be taken into consideration.

Many other explanations could be offered in more purely speculative fields, such as for instance, different mathematical laws relating nuclear volume to the chromosome content in the different periods of the interphase, but here too, the constancy of the 1 : 1.5 ratio limits the possibilities of the hypothesis.

From a more genetic point of view, Heidenhain, since the very early studies of Jacobj, admitted that the intermediate values which in some cases appear as an

exception to the "Verdoppelungsgesätz" could be explained by admitting that the two halves of the nuclear content derived respectively from maternal and paternal origin, reduplicate independently (Jacobj, 1931).

In the case in which only one reduplicates, the duplication ratio is not maintained. Hertwig (1937) admitted a similar point of view but subsequently rejected it without any justification, preferring the above mentioned theory of the two different factors acting on the surface or on the volume of the nucleus.

On the basis of the facts analyzed in this paper we can perhaps try to support more clearly this genetic point of view. We can admit (Schreiber, 1943) that in a diploid nucleus each chromosome set of different gametic origin represents a physiological entity during the reduplication process, and one set may be more precocious than another.

The influence of gametic origin on the behavior of an entire set or on some special chromosomes also manifests itself in other phenomena of the cell cycle (White, Schrader, etc.): for instance, the precocious condensation of one haploid set in the scale insect, or the elimination of the paternal chromosomes in Sclara. A difference in the initial rhythm of reduplication between maternal and paternal chromosomes of each original pair is also invoked by Holt (1917) to explain the existence in Culex of the "six series" or "nine series" of chromosome numbers, in intestinal cells.

We could thus represent in an hypothetical way the so-called "sesquiphase" as a transitory stage in which one haploid set (or its multiples) has reduplicated and the other has not yet done so. The "quantum" of the simultaneous reduplication of the genes would in that case be the haploid set, or "genome."

All this is merely speculation which might perhaps lead to new research in a purely cytological field. As stated above we have at present no cytological evidence of the sesquiphase stage, which can only be detected by the statistical analysis of the growing nucleus.

As a general conclusion we can state that caryometric methods, when strengthened by the physiological conditions which specifically influence interphasic growth and mitosis, can help the cytologist to make a closer study of the growing nucleus and to formulate some suggestions on its quantitative aspect and on the dynamics of gene reproduction.

4. Summary

The author analyzes present knowledge on the problem of "rhythmic growth" of the nucleus as it appears from the point of view of statistical caryometric research. This analysis is carried out especially with regard to the problem of the intermediate steps during reduplication of nuclear volume to about 1.5 times the initial size.

The nature of these steps is analyzed experimentally in three different fields: (1) Interphasic growth of spermatogonia, whose nuclear size is compared with a meiocyte with a known number of chromosomes. (2) Interphasic growth of the uterine cells under different physiological conditions. (3) Interphasic growth of the granulosa cells of the ovary, and the transformation into luteal cells.

The interphasic nature of rhythmic growth is considered also as a possible explanation for the intermediate step during reduplication of nuclear size that is called sesquiphase.

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