THE DISTRIBUTION OF NUCLEIC ACIDS IN RAT EGGS IN FERTILIZATION AND EARLY SEGMENTATION

I. STUDIES ON LIVING EGGS BY ULTRAVIOLET MICROSCOPY

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Summary

Observations have been made on the absorption of ultraviolet radiation at 260 mμ by living rat eggs, as full-grown oocytes and during fertilization and early cleavage. Precautions have been taken to permit the results to be interpreted in terms of nucleic acid concentrations.

High concentrations of nucleic acid have been observed around the nucleolus of the oocyte, in the sperm head during its metamorphosis in the egg, in the nucleoplasm of the early pronuclei, and in the nuclei of segmenting eggs, particularly in the region immediately about the nucleoli. Little or no nucleic acid was detected within the nucleoli at any stage.

A moderate concentration of nucleic acid has been observed in the cytoplasm of the oocyte and of the egg during fertilization; in the course of early cleavage the concentration decreases progressively. There appears to be some association between nucleic acid and the granular elements of the cytoplasm.

Quantitative data are presented on the nuclear and nucleolar volumes, and nucleolar surface areas, in living 2-cell, 4-cell, and 8-cell eggs. In general these functions decrease in the proportions 4:2:1 with successive segmentations.

The observations made are considered to provide general support for the following concept: The egg contains a store of nucleic acid in its cytoplasm, and certain specifically limited amounts of nucleic acid, contributed by the sperm head and the egg chromosomes, are contained within the pronuclei. During the pronuclear phase the function of nucleic acids is obscure, but in the early segmentation nuclei a structure disposed about the nucleolus, and containing a high concentration of nucleic acid, is progressively developed at the expense, in part, of the cytoplasmic store of nucleic acid. This structure may well be analogous to Caspersson's "nucleolus-associated chromatin" and be involved in protein synthesis during embryonic development.

I. INTRODUCTION

In previous communications the cytology of fertilization, as seen in the living egg of the rat by phase-contrast microscopy, has been described (Austin 1951, 1952). It was recognized, however, that though the phase-contrast microscope is unexcelled for the study of structure in living cells, no specific information of a chemical nature could be obtained by this means alone. For a better understanding of fertilization, some knowledge is needed of the chemical processes which accompany or underlie the visible structural changes.

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In recent years, much attention has been given to the nucleic acids and to the important role they appear to play in cell function (Brachet 1950; Caspersson 1950). Moreover, many of the cytochemical techniques employed may readily be applied to mammalian eggs. For these reasons investigations have now been made on the distribution of nucleic acids in the rat egg during fertilization and early segmentation. Two methods have been used: the observation of ultraviolet absorption at 260 mμ in living eggs, and the histological study of fixed eggs with the aid of the Feulgen reaction. These methods were selected because they involve well-established procedures and seemed the most likely to yield useful results. The use of ultraviolet microscopy for the study of living cells is examined by Ludford, Smiles, and Welch (1948), who also employed phase-contrast microscopy as a collateral method. In the present paper observations on the ultraviolet absorption of living eggs are described and discussed; a second paper will be devoted to results obtained by histological methods involving principally the Feulgen reaction.

Microscopic observations on the ultraviolet absorption of cells were probably made first by Köhler (1904), but the method has been developed and exploited most effectively by Caspersson and his associates, whose work is summarized in a recent book (Caspersson 1950). Most of the work has been done on cells which were actively growing or secreting, such as cells from embryonic, tumour, or glandular tissues. Little attention has been paid to the ultraviolet absorption in the eggs of any species; there appear to be only the reports of Caspersson and Schultz (1940) on *Psammechinus*, of Harvey and Lavin (1944) on *Arbacia*, and a preliminary note by Flax (1951) on the mouse.

The stages of fertilization which will be referred to in this paper are based upon phases of development of the male pronucleus and have been described in a previous communication (Austin 1952). Briefly, the sperm, shortly after entering the vitellus, undergoes changes which include the detachment of the head and the metamorphosis of this into a faintly seen "grey zone," from which is formed the male pronucleus. The development of the male pronucleus passes through six stages, designated as follows: 1 and 2, early and late primary growth, in which the nucleoli appear, enlarge, and coalesce; 3, the single nucleolus stage; 4 and 5, early and late secondary growth; and 6, full development. In the last three stages secondary nucleoli appear, grow, and gather near the middle of the pronucleus, while the large nucleolus formed at stage 3 dwindles in size. After maintaining their maximum size for a period, the male and female pronuclei diminish in size and finally disappear, giving place to the chromosomes of the first segmentation spindle.

II. Methods

Adult female rats, killed at the appropriate times after mating, provided oocytes and also eggs in various stages of fertilization and early cleavage. The eggs were dissected from the ovary or from the fallopian tubes under normal saline solution and placed on a fused quartz slide. A coverslip of similar material, with a layer of vaseline applied to its edges, was placed over the

preparation and pressed down until the internal structure of the eggs could be clearly seen under the microscope, i.e. until the vertical depth of the egg had been reduced to about 20 μ (Austin 1950). After a preliminary examination with the phase-contrast microscope a photograph was taken at a selected focal point. For this purpose the 4-mm. objective was used as it had about the same depth of focus as the objective of the ultraviolet microscope. The slide was transferred to the ultraviolet microscope and approximately the same focal level found in visible light. A second photograph was taken at the desired wavelength in the ultraviolet.

The optical parts of the ultraviolet microscope consisted of spherical reflecting condenser and objective, both of N.A. 0.78 and focal length 3 mm.* Wavelength was isolated by means of the monochrometer from a Beckman model DU quartz spectrophotometer; for a 2-mm. slit width the range in the region of 260 mμ is $\pm$ 2.5 mμ with this instrument. The source of radiation was the standard hydrogen lamp used with the Beckman spectrophotometer.

Photographs in the ultraviolet were taken on Kodak "Ortho-X" plates, at exposures of 6-10 min. Tests showed that living rat eggs could tolerate at least 20 min. exposure at 260 mμ without any observable change in structure or absorption characteristics. Magnification at the plate was $\times$ 330; further enlargement was obtained photographically.

III. Observations

(*a*) *Ultraviolet Absorption in the Full-Grown Oocyte*

In the ovarian egg, obtained shortly before the time at which the final maturation changes occur, the distribution of ultraviolet absorbing material is clearly defined (Plate 1, Figs. 1*a*, 1*b*). Strongest absorption occurs in the region immediately surrounding the nucleolus and in small irregular bodies which can be seen in the nucleus often in the vicinity of the nucleolus. The nucleolus itself appears to absorb slightly, but this effect may well be due to the shell of strongly absorbing material just described. The nucleoplasm shows little or no absorption of ultraviolet radiation. In the cytoplasm there is quite strong absorption and this is distributed evenly throughout the egg.

(*b*) *Ultraviolet Absorption during Fertilization*

The structure which is formed from the sperm head soon after it has entered the egg cytoplasm, and which has been termed the "grey zone," is seen to absorb radiation strongly (Plate 1, Figs. 2*a*, 2*b*), so that it can be distinguished from the adjacent group of cytoplasmic granules more easily in the ultraviolet than the phase-contrast picture. Plate 1, Figures 2*a* and 2*b*, also shows the group of chromosomes extruded in a polar body, and these have an even greater absorption of ultraviolet radiation. The polar body appears to lie within the egg in these pictures but has in reality been pressed into the upper surface of the egg by the overlying coverslip.

* These parts were constructed by the Division of Physics, C.S.I.R.O., Sydney.

The early primary stage of growth of the male pronucleus is seen in Plate 1, Figures 3*a*, 3*b*, and 3*c*. When first observed, this egg showed a clearly defined "grey zone." Soon, early nucleoli began to appear (Fig. 3*a*) and, about 15 min. later a picture was taken at 260 mμ (Fig. 3*b*). Absorption of ultraviolet radiation is very distinct in the nucleoplasm, but there is an indication of weak absorption in the nucleoli. Pronuclear development continued, apparently quite unaffected by the ultraviolet irradiation, and, after a further 15 min., it reached the stage shown in Figure 3*c*. Another view of a male pronucleus in early primary growth, but seen "end-on," shows the strong ultraviolet absorption in the nucleoplasm and its relative absence from the nucleoli (Plate 2, Figs. 4*a*, 4*b*). In this picture the intensely absorbing mass of chromosomes which will shortly form the female pronucleus can also be seen. Plate 2, Figures 5*a* and 5*b*, shows a male pronucleus in the phase of late primary growth; absorption in the nucleoplasm is still evident but it is now appreciably weaker. At the single nucleolus stage (Plate 2, Figs. 6*a*, 6*b*) absorption has become vague in the male pronucleus although it is still quite distinct in the smaller female pronucleus. In subsequent pronuclear growth the absorption in the nucleoplasm of both pronuclei becomes so weak that it cannot be discerned with any certainty at the stages of late secondary growth and full development (Plate 2, Figs. 7*a*, 7*b*). The rings which can be seen outlining the nucleoli in the ultraviolet picture may well be due to refraction alone.

Throughout most of the fertilization stages there is a moderately strong, diffuse, cytoplasmic absorption. To a certain extent the absorption is associated with granular elements in the cytoplasm. This is particularly noticeable in the early stages of fertilization when the granular elements show some aggregation and are generally absent from the peripheral cytoplasm (Figs. 2 and 3). Nevertheless the cytoplasm, which is free of granules, also absorbs quite strongly.

(*c*) *Volume and Surface Area Changes in Early Segmentation*

To obtain information on the changes in nuclear and nucleolar volume, and in nucleolar surface area, during the process of early segmentation, measurements were made of the diameters of nuclei and nucleoli. The diameter of the nucleolus and the longest and shortest horizontal diameters of the nuclei were measured with an eyepiece micrometer. The vertical depth of the nucleus was estimated with the aid of the fine adjustment on the microscope.

When the nucleus re-forms, after cleavage, it is initially much smaller than a mature nucleus and contains a number of small, closely arranged, nucleoli. As the nucleus matures it enlarges, several of the nucleoli coalesce, and more free space appears within the nucleus. The attempt was made to select for measurement only those eggs in which the nuclei appeared to be mature. This, however, is open to subjective error and it is probable that some relatively immature nuclei have been included in the data at each of the segmentation stages.

From the data obtained, the volumes of nuclei and nucleoli, and the surface area of nucleoli, were calculated and are recorded in Table 1. Measure-

ments were made on 40 2-cell, 20 4-cell, and 10 8-cell, eggs, i.e. on 80 nuclei for each stage of segmentation.

The number of nucleoli per nucleus decreases from a mean of 5.4 in the 2-cell egg, to 4.0 in the 4-cell, and 1.4 in the 8-cell egg. The total volumes of nucleoli per nucleus are reduced from a mean of 324 cu. μ in the 2-cell egg, to 159 cu. μ in the 4-cell, and 75 cu. μ in the 8-cell egg. Corresponding values for total nucleolar surface area were 379, 211, and 92 sq. μ respectively. The figures for both nucleolar volume and surface area are thus approximately halved at each segmentation. Nuclear volumes showed a reduction from a mean of 2808 cu. μ in the 2-cell egg to 1206 cu. μ in the 4-cell, and 840 cu. μ in the 8-cell egg. These figures differ from the expected ratio of 4:2:1; the mean volumes of the 4-cell nuclei are smaller than expected, and those of the 8-cell nuclei a little larger.

TABLE 1

QUANTITATIVE CHANGES IN THE NUCLEI AND NUCLEOLI OF LIVING RAT EGGS DURING THE FIRST THREE STAGES OF CLEAVAGE

	No. of Eggs	Volume of Nuclei		Number of Nucleoli per Nucleus		Total Volume of Nucleoli per Nucleus		Total Surface Area of Nucleoli per Nucleus	
		Range (cu. μ)	Mean (cu. μ)	Range	Mean	Range (cu. μ)	Mean (cu. μ)	Range (sq. μ)	Mean (sq. μ)
2-Cell eggs	40	1867-3630	2808 $\pm$42*	2-11	5·4 $\pm$0·2*	241-616	324 $\pm$6·0*	236-509	376 $\pm$6·9*
4-Cell eggs	20	611-1886	1206 $\pm$32	2-9	4·0 $\pm$0·15	110-253	159 $\pm$3·1	149-376	211 $\pm$4·2
8-Cell eggs	10	540-1298	840 $\pm$16	1-3	1·4 $\pm$0·07	51-106	75 $\pm$1·5	74-133	92 $\pm$1·5

* Standard error.

(*d*) *Ultraviolet Absorption during Early Segmentation*

The distribution of ultraviolet absorption showed distinct and progressive changes during the early segmentation of the egg. Cytoplasmic absorption, which was noted to be distinct in the fertilization stages, was of about the same intensity in the 2-cell egg (Plate 3, Figs. 8*a*, 8*b*). In the 4-cell egg (Plate 3, Figs. 9*a*, 9*b*) there was an appreciable reduction in the intensity of absorption in the cytoplasm except in areas near the nuclei where it was about the same as in the 2-cell egg. This change was even more evident in the 8-cell egg (Plate 3, Figs. 10*a*, 10*b*) and in the 16-cell egg (Plate 3, Fig. 11); in these the absorption in the outlying parts of the cytoplasm was much less than in the 2-cell egg. The absorption near the nuclei was associated with the granular cytoplasmic elements which were gathered there. The cytoplasmic granules show a distinct

change in distribution during segmentation. In the 2-cell egg their arrangement is similar to that seen during the later part of fertilization. With succeeding stages of segmentation the cytoplasmic granules appear to undergo a steady reduction in number and tend to aggregate around the nuclei. Even where free of granules, however, the cytoplasm still shows definite absorption of radiation.

The most striking changes seen during segmentation occurred in the nuclei. In the 2-cell egg the rings about the nucleoli were darker than those seen in the later pronuclei. Peri-nucleolar absorption was more distinct in the 4-cell egg, and stronger still in the later segmentation stages. In these eggs the absorption about the nucleoli could be related to the presence of irregular peri-nucleolar masses which could be clearly discerned with the phase-contrast microscope (Plate 3, Figs. 12*a*, 12*b*). Throughout all these stages the nucleoli themselves showed little or no ultraviolet absorption.

IV. Interpretation

The interpretation of photographs obtained in the ultraviolet must be made with care. In general, the dark regions of the print may be considered to represent areas of absorption, provided the loss of radiation through refraction, particularly by small particles, is distinguished from the loss due to absorption. After true absorption has been shown to occur, it is then necessary to demonstrate that the absorption can be imputed to nucleic acids. Although full proof of this does not appear to be possible yet (Danielli 1947), good evidence for the presence of nucleic acids can be obtained by observing the absorption at 280 mμ and 300 mμ as well as at 260 mμ. From the evidence available (Pollister and Ris 1947; Caspersson 1950; Leuchtenberger *et al.* 1952), it can be concluded that, if the absorption is higher at 260 mμ than at 280 mμ, and is negligible at 300 mμ, nucleic acids are chiefly responsible.

To establish these points for rat eggs in the present study, the 8-cell egg shown in Plate 3, Figures 10*a* and 10*b*, was also photographed at 280 mμ and 300 mμ (Plate 3, Figs. 10*c* and 10*d*). It may be seen that most of the image obtained at 260 mμ and 280 mμ is caused by the absorption of radiation, for the image at 300 mμ is very much fainter. Indeed, the loss of radiation due to refraction is only appreciable in some of the granular elements in the cytoplasm, and, to a smaller degree, as a faint ring about each nucleolus, and just within the zona pellucida. It is, of course, possible that some of the cytoplasmic granules absorb specifically at about 300 mμ, but for the purposes of this investigation the picture obtained at 300 mμ is taken to represent the effects of refraction.

Comparison between the absorption evident at 260 mμ and at 280 mμ (Plate 3, Figs. 10*b*, 10*c*) shows that it is distinctly stronger at 260 mμ in the nucleus, particularly in the peri-nucleolar material, and somewhat stronger in the cytoplasm. The absorption in these regions may therefore be imputed principally to the nucleic acids.

Observations by Brumberg and Larionow (1946) suggest that nucleic acids may show little or no absorption of ultraviolet in living cells: that the cells must be killed before the characteristic absorption is displayed. In the present

investigations, however, absorption is found in cells which are manifestly still alive. It has already been mentioned that the eggs show no change in structure or in absorption characteristics even after irradiation for two or three times the period required for photography. Furthermore, pronuclear development in the egg shown in Plate 1, Figure 3*a*, continued in the normal manner, as illustrated in Plate 1, Figure 3*c*, even after the photograph of its ultraviolet absorption (Plate 1, Fig. 3*b*) had been taken.

V. Discussion

From the premises stated in the previous section, it seems sound to conclude that nucleic acids exist in the living, segmenting rat egg in concentrations which are approximately represented by the strength of absorption at 260 mμ. In the early stages of fertilization the nucleic acids of the sperm head may reasonably be supposed to enter into the composition of the "grey zone" and early male pronucleus, and to be responsible in large measure for the strong ultraviolet absorption noted in these structures. Similarly, the nucleic acids of the egg chromosomes may be held largely responsible for the strong absorption shown by the early female pronucleus.

During the early growth of the pronuclei the nucleic acid concentration of the nucleoplasm falls rapidly, so that, after the single nucleolus stage, detection of nucleic acid through ultraviolet absorption becomes doubtful. It can be calculated from data previously described (Austin 1952) that by the time the single nucleolus stage has been reached in the living rat egg, the nucleoplasm of both pronuclei has undergone an increase in volume of about five times, so that the drop in nucleic acid concentration may be regarded as a consequence of dilution alone. In the remaining stages of pronuclear development dilution would continue, since the volume of the nucleoplasm increases by a further five times approximately. The falling concentration with increasing nucleoplasmic volume is consistent with the idea that the amount of nucleic acid originally contributed to each pronucleus remains unchanged during pronuclear development.

The rings seen about the nucleoli of the late pronuclei may connote a peri-nucleolar distribution of nucleic acid, as in the oocyte and segmenting eggs, but this is hypothetical as the rings are faint and may well be due to the effects of refraction alone. It has been suggested that a function of the pronuclei may be some form of synthetic activity, such as the production of templates for future embryonic growth (Austin 1951). This may still be true, but evidently a protein synthesizing system such as that described by Caspersson and his associates is not involved, for the required high concentrations of nucleic acid about the nucleoli and pronuclei are lacking.

In the course of the early cleavage stages, the highest concentrations of nucleic acid in the living egg are found in the region immediately surrounding the nucleoli. This peri-nucleolar material is fairly certainly present in the 2-cell egg, but becomes progressively more evident in the 4-, 8-, and 16-cell stages, and assumes various irregular shapes in the process. The development of the peri-nucleolar structure is clearly related to changes in the surface area of the

nucleoli. In the 2-cell egg the total nucleolar surface area is about 379 sq. μ, compared with values of about 730 and 345 sq. μ reported (Austin 1952) for the fully formed male and female pronuclei respectively. During the early segmentation stages, the nucleolar surface area is approximately halved at each division. Changes of this order are sufficient to account for a large part of the apparent increase in the mass of the structure formed by the peri-nucleolar material.

If, as seems likely, the peri-nucleolar material has a high content of desoxyribonucleic acid (DNA), it may be regarded an analogous to Caspersson's "nucleolus-associated chromatin" (Austin 1953). The system as conceived by Caspersson is, however, incompletely represented in the segmenting egg, for the nucleoli contain little or no nucleic acid at any of the stages investigated, and the nucleic acid concentration in the cytoplasm is decreasing rather than increasing. It is therefore suggested that the peri-nucleolar structure, which is built up during the first few cleavage divisions, is an early form of the machinery involved in active protein synthesis later in embryonic growth.

The observation of increasing nucleic acid concentrations in the nuclei during cleavage is in general agreement both with the conclusions of Brachet (1950) on sea-urchin eggs, and with those of Alfert (1950) on mouse eggs, and Lison and Pasteels (1950) on sea-urchin eggs. These authors all studied the distribution of DNA in fixed eggs, but whereas Brachet described increasing *amounts* of DNA in cleavage nuclei, Alfert, and Lison and Pasteels considered that the amounts remained about the same, so that only the *concentrations* increased. On the other hand the present observations do not support Hertwig's (1939) theory that the amount of chromatin in each nucleus is halved at each division in early cleavage.

The cytoplasm of the full-grown oocyte and of the egg during fertilization shows evidence of a moderately high concentration of nucleic acid. The distribution throughout the egg is even, except that there is an indication of slightly higher concentrations associated with the groups of cytoplasmic granules. During early segmentation the concentration of nucleic acid falls off progressively; there is little observable change in the 2-cell egg, but distinct reductions in later stages. Here again, cytoplasmic nucleic acid shows a tendency to be associated with the granular elements. Brachet (1937, 1945) proposed that the increase in nuclear DNA during segmentation occurred at the expense of cytoplasmic ribonucleic acid (RNA), and the observations just described are therefore in agreement with this theory. Some agreement is also found with Dalcq (1951) who noted an association of RNA with granular elements in the cytoplasm of the rat egg. On the other hand, Harvey and Lavin (1944) found that, in *Arbacia,* ultraviolet absorption was chiefly evident in the hyaline part of the cytoplasm.

The general conclusions drawn from the present study on the ultraviolet absorption of living rat eggs may be stated as follows: A store of nucleic acid exists in the cytoplasm of the oocyte and the unfertilized egg, and limited contributions of nucleic acid, made by the sperm head and the egg chromosomes, are later contained within the pronuclei. During pronuclear development the

function of the nucleic acids is obscure, for the cytoplasmic form shows little change in concentration and the nuclear moiety seems merely to undergo a progressive dilution with pronuclear growth. In early segmentation more distinct changes occur; in the nucleus a peri-nucleolar structure is gradually developed, which contains a high concentration of nucleic acid and is probably analogous to the "nucleolus-associated chromatin" of Caspersson and his associates. This structure may well be involved in vigorous protein synthesis later in embryonic growth, but does not appear to be active during early cleavage. The additional nucleic acid required for the formation of peri-nucleolar material may be drawn from the cytoplasmic store, which clearly diminishes during early cleavage.

VI. References

Alfert, M. (1950).—*J. Cell. Comp. Physiol.* **36**: 381.

Austin, C. R. (1950).—*Nature* **166**: 407.

Austin, C. R. (1951).—*J. R. Micr. Soc.* **71**: 295.

Austin, C. R. (1952).—*Aust. J. Sci. Res.* B **5**: 354.

Austin, C. R. (1953).—*Exp. Cell. Res.* (in press).

Brachet, J. (1937).—*Arch. Biol.* **48**: 529.

Brachet, J. (1945).—*C. R. Soc. Biol., Paris* **139**: 193.

Brachet, J. (1950).—"Chemical Embryology." (Interscience Publ. Inc.: New York.)

Brumberg, E. M., and Larionow, L. T. (1946).—*Nature* **158**: 663.

Caspersson, T. O. (1950).—"Cell Growth and Cell Function." (W. W. Norton & Co. Inc.: New York.)

Caspersson, T. O., and Schultz, J. (1940).—*Proc. Nat. Acad. Sci., Wash.* **26**: 507.

Dalcq, A. M. (1951).—*Proc. Acad. Sci. Amst.* C **54**: 351.

Danielli, J. F. (1947).—Nucleic Acid. *Symp. Soc. Exp. Biol.* No. 1.

Flax, M. H. (1941).—*Anat. Rec.* **111**: 465 (proc.).

Harvey, E. B., and Lavin, G. I. (1944).—*Biol. Bull.* **86**: 163.

Hertwig, G. (1939).—*Z. mikr.-anat. Forsch.* **45**: 37.

Köhler, A. (1904).—*Z. wiss. Mikr.* **21**: 129, 273.

Leuchtenberger, C., Leuchtenberger, R., Vendrely, C., and Vendrely, R. (1952).—*Exp. Cell Res.* **3**: 240.

Lison, L., and Pasteels, J. (1950).—*Arch. Biol.* **62**: 1.

Ludford, R. J., Smiles, J., and Welch, F. V. (1948).—*J. R. Micr. Soc.* **68**: 1.

Pollister, A. W., and Ris, H. (1947).—*Cold Spr. Harb. Symp. Quant. Biol.* **12**: 147.

Explanation of Plates 1-3

Plate 1

Stages of fertilization

Each egg was photographed in visible light with phase contrast (*a*), and in the ultraviolet at 260 mμ (*b*). × 1000.

Fig. 1.—A fully grown oocyte, showing strong ultraviolet absorption immediately around the nucleolus, and moderate absorption in the cytoplasm.

Fig. 2.—Strong absorption is evident in the "grey zone" (g.z.) and in the group of chromosomes (chr.) in the polar body.

Fig. 3.—The early primary stage of development of the male pronucleus. There was an interval of about 15 min. between each picture. In this egg and that shown in Figure 2 the absorption associated with the cytoplasmic granules can be seen.

PLATE 2

Stages of fertilization (*continued*)

Conditions were the same as for Plate 1.

Fig. 4.—Another early primary male pronucleus, this time disposed "end-on" to the observer. Strong absorption is apparent in the nucleoplasm and also in the group of chromosomes (chr.) from which the female pronucleus is to be formed. The nucleoli are relatively free of absorbing material.

Fig. 5.—A male pronucleus in the stage of late primary growth. Ultraviolet absorption in the nucleoplasm is weaker than in the early primary stage of development.

Fig. 6.—Male and female pronuclei at the single nucleolus stage. Nucleoplasmic absorption is now not very distinct in the male pronucleus but is still quite strong in the smaller female pronucleus.

Fig. 7.—Male and female pronuclei at the stage of full development. The nucleoplasm does not appear to absorb the ultraviolet radiation. The rings outlining the nucleoli may be due to refraction alone.

PLATE 3

Stages of early cleavage

Fig. 8.—Nuclei of 2-cell egg photographed with phase contrast (*a*) and in ultraviolet at 260 mμ (*b*). × 660. There are distinct rings of absorption around the nucleoli. Cytoplasmic absorption is still fairly uniform.

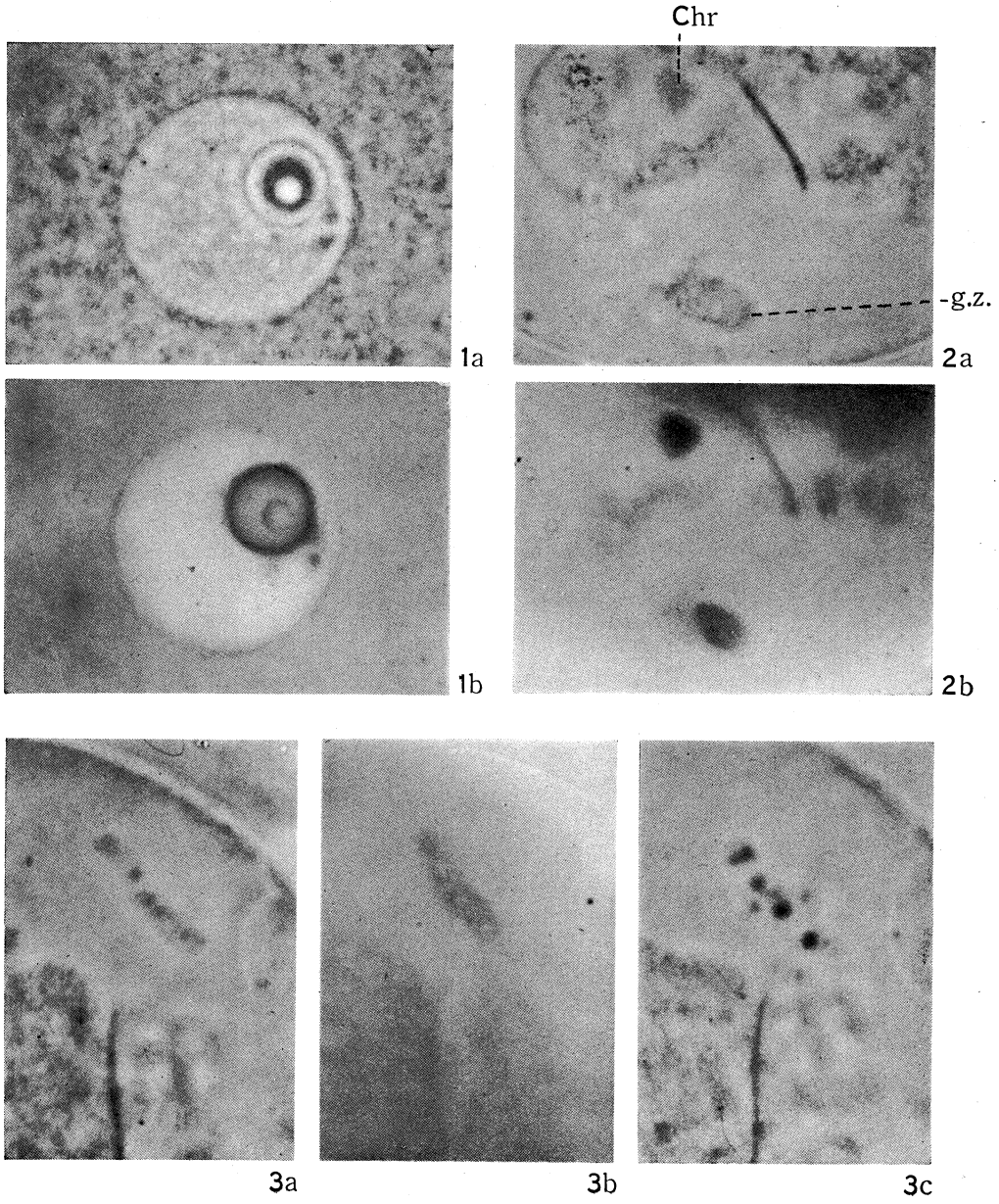
Fig. 9.—Two nuclei of a 4-cell egg photographed with phase contrast (*a*) and in ultraviolet at 260 mμ (*b*). × 660. Peri-nucleolar absorption is stronger than in the 2-cell egg, and the weaker absorption of the peripheral cytoplasm is more evident.

Fig. 10.—Three nuclei of an 8-cell egg photographed with phase contrast (*a*), and in ultraviolet at 260 mμ (*b*), 280 mμ (*c*), and 300 mμ (*d*). × 660. Absorption generally is stronger at 260 mμ than at 280 mμ, and particularly in the peri-nucleolar material. The picture taken at 300 mμ is considered to represent the effects of refraction alone. Compared with the 4-cell egg, peri-nucleolar absorption is stronger and absorption in the peripheral cytoplasm is weaker.

Fig. 11.—Portion of a 16-cell egg photographed at 260 mμ. × 660. Strong absorption can be seen in the nuclei and particularly about the nucleoli. The peripheral cytoplasm shows very weak absorption.

Fig. 12.—Nucleus of an 8-cell egg photographed with phase contrast (*a*) and in ultraviolet at 260 mμ (*b*). × 1300. The peri-nucleolar material is clearly seen and this has strong ultraviolet absorption whereas the nucleolus does not absorb the radiation appreciably.

DISTRIBUTION OF NUCLEIC ACIDS IN RAT EGGS. I



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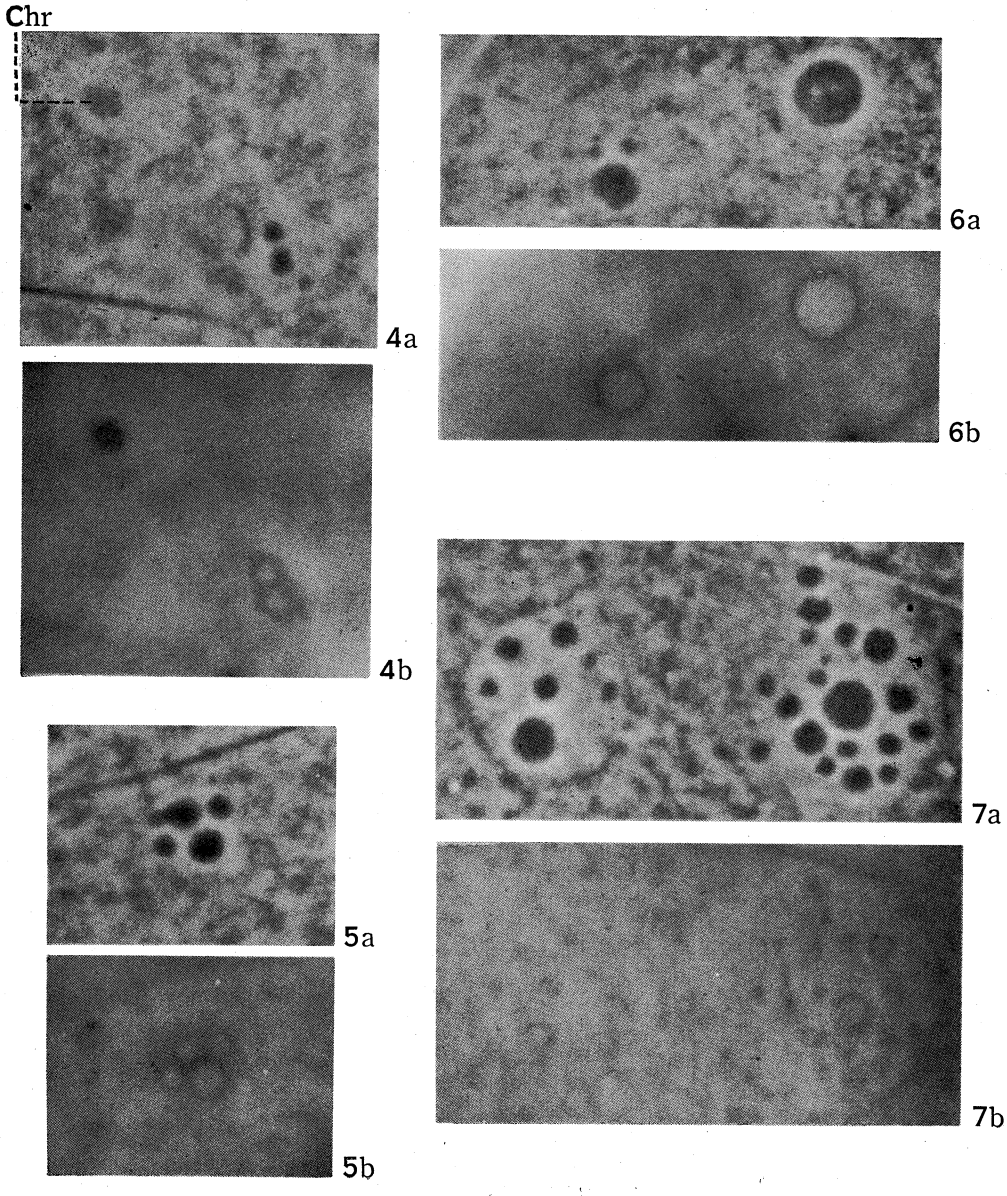

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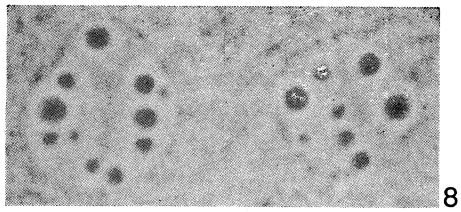

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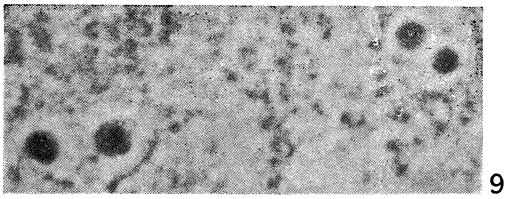

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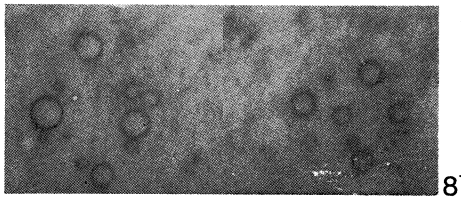

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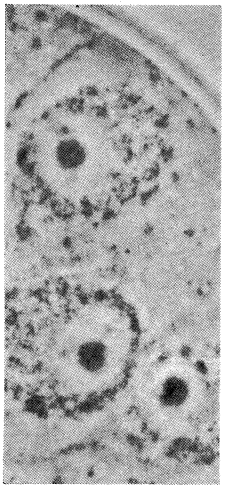

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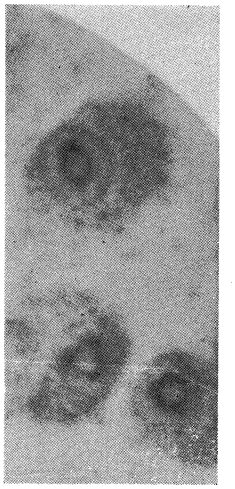
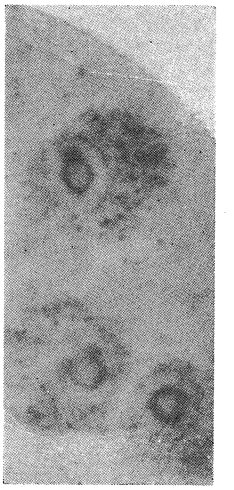
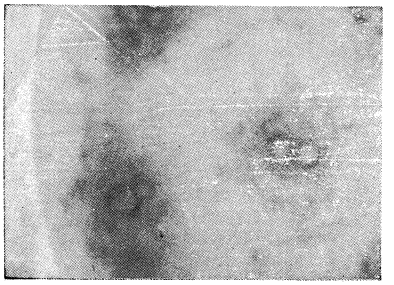


10b

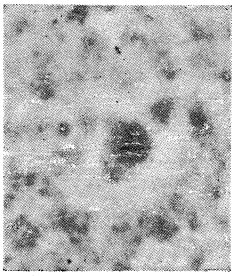


10c

10d



11



12a

12b