THE CHROMATIN IN THE LIVING ARBACIA PUNCTULATA EGG, AND THE CYTOPLASM OF THE CENTRIFUGED EGG AS PHOTOGRAPHED BY ULTRA-VIOLET LIGHT

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Chromatin

In the living egg of *Arbacia punctulata,* as observed with high magnification with the best optical equipment for visible light, the mature nucleus in the unfertilized and recently fertilized egg appears clear and homogeneous. This is true in the normal egg where the red pigment somewhat obscures the picture, in the centrifuged whole egg where the pigment has been thrown down and the nucleus lies in the clear layer, and also in the white "half" (containing no pigment) obtained by centrifugal force. Nor can the chromosomes of the mitotic figure be seen in the living egg of Arbacia though the asters are very conspicuous. In sections of eggs, however, which have been fixed in Bouin's fluid and stained with Heidenhain's haematoxylin, the chromatin material is darkly stained and shows a characteristic structure, a network in the nucleus of the unfertilized and recently fertilized egg, and discreet bodies, chromosomes, during the mitotic divisions (Harvey, 1940).

A study of the chromatin material in the living egg of Arbacia as it appears with ultra-violet light has been made by the use of the quartz microscope devised by Köhler (1904), and modified by Lavin (1943). This is a microscope with quartz oculars and objectives so arranged that an accurate focus can be obtained on a fluorescent plate. The light source is a quartz spiral mercury resonance lamp (Hanovia Chemical Co.) from which the visible light is taken out by a liquid filter, so that only light of the wave length of 2537 A° is transmitted. The eggs were mounted on a quartz slide and covered with a quartz coverslip and partially compressed to make the eggs thin enough for the light to penetrate. Photographs of a selected field were taken with an exposure of three minutes. That the eggs were still living and coagulation had not set in was shown by progressive changes in serial photographs taken at intervals, of the same field of fertilized eggs.

We have arranged on Plate I photographs of similar stages of the egg of Arbacia: (1) Living eggs taken with ordinary visible light (Photographs 1, 4, 7). (2) Sections of eggs fixed in Bouin and stained with Heidenhain's haematoxylin, photographed with visible light (Photographs 2, 5, 8). (3) Living eggs photographed with ultra-violet light (Photographs 3, 6, 9). Three stages are shown, one of the immature egg with large germinal vesicle and nucleolus, a second of the mature egg with small nucleus, and a third of a late prophase or early metaphase. It will be seen that the chromatin material which does not show at all with visible light in the living egg is quite plain with the ultra-violet light and similar in appearance to the stained preparations with visible light.

The dark areas in the prints are, of course, the areas where the ultra-violet light is absorbed. The absorption is probably due to the presence of nucleic acid compounds since the purine and pyrimidine constituents of nucleic acid have an absorption maximum in this region of the spectrum. Of course this does not rule out the absorption due to the possible presence of proteins, but since the absorption of nucleic acid is much greater than that of the proteins, and until it is possible to differentiate between the two, the absorbing material will be referred to as nucleic acid compounds. It is not possible from the photographs to tell whether the absorbing materials are nucleic acids of the desoxyribose type (thymonucleic) or of the ribose type (yeast nucleic). The absorbing material in the immature egg (Photograph 3) is the nucleolus and a coarse network throughout the germinal vesicle; the bulk of the material in the germinal vesicle is non-absorbing, much less absorbing than the cytoplasm. In the mature nucleus (Photograph 6) the absorbing material is the chromatin network, and the rest of the material is less absorbing than the cytoplasm. In the dividing cell (Photograph 9) the chromosomes absorb and there is a considerable amount of non-absorbing material around them. There is no evidence of spindle or asters, such as one sees with visible light (Photographs 7, 8). There is the bare possibility that this may be due to the cell being somewhat compressed in order to allow the light to penetrate. The nucleic acid compounds of the nucleus, therefore, seem to be restricted to the nucleolus and network of the germinal vesicle of the immature egg, the chromatin threads of the mature nucleus and the chromosomes of the dividing egg.

It is generally agreed that the nucleic acid of the nucleus is of the desoxyribose type (thymonucleic) and that of the cytoplasm of the ribose type (yeast nucleic acid). The nucleolus is believed to be of the ribose type (Caspersson and Schultz,

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PLATE I

Photomicrographs, with approximate magnifications, as indicated, to bring comparative photographs to about the same size. Allowance is thus made for shrinking in fixation and expansion due to pressure of the coverslip for the ultra-violet.

PHOTOGRAPH 1. Living immature egg with visible light. 500 ×.

PHOTOGRAPH 2. Section of immature egg, fixed in Bouin and stained with Heidenhain's haematoxylin. Visible light. 660 ×.

PHOTOGRAPH 3. Living immature egg with ultra-violet light. 500 ×.

PHOTOGRAPH 4. Living mature unfertilized egg with visible light. 500 ×.

PHOTOGRAPH 5. Section of mature unfertilized egg, fixed in Bouin and stained with Heidenhain's haematoxylin. Visible light. 660 ×.

PHOTOGRAPH 6. Living mature unfertilized egg with ultra-violet light. 500 ×.

PHOTOGRAPH 7. Living fertilized egg at metaphase. Visible light. 500 ×.

PHOTOGRAPH 8. Section of fertilized egg at metaphase (early), fixed in Bouin and stained with Heidenhain's haematoxylin. 660 ×.

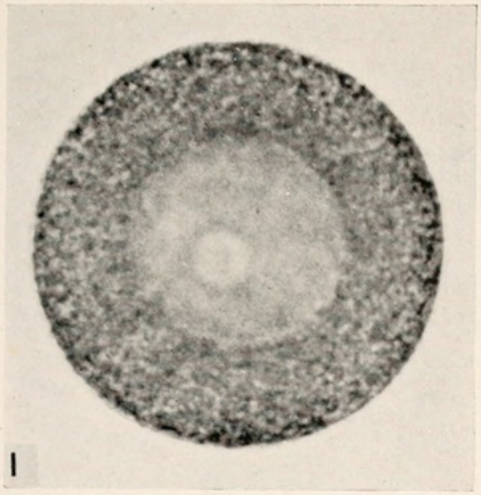
PHOTOGRAPH 9. Living fertilized egg at metaphase (early) with ultra-violet light. Egg somewhat squashed. 500 ×.

PHOTOGRAPH 10. Living egg centrifuged, 10,000 × g. for two minutes. Oil cap, clear layer, mitochondrial band, yolk, pigment. Nucleus under the oil cap. Visible light. 280 ×.

PHOTOGRAPH 11. Section of centrifuged egg, fixed in formalin and unstained. Visible light. 400 ×.

PHOTOGRAPH 12. Section of centrifuged eggs, fixed in formalin and stained with Heidenhain's haematoxylin. Visible light. 400 ×.

PHOTOGRAPH 13. Section of centrifuged eggs, fixed in formalin, and unstained. Ultra-violet light. 400 ×.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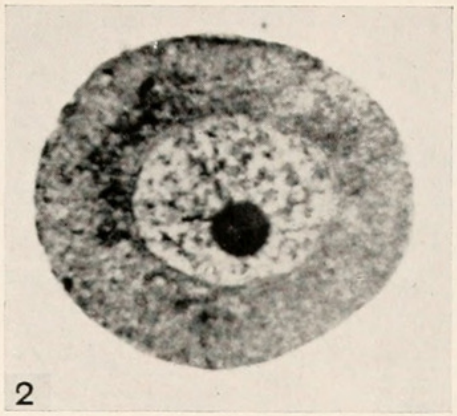
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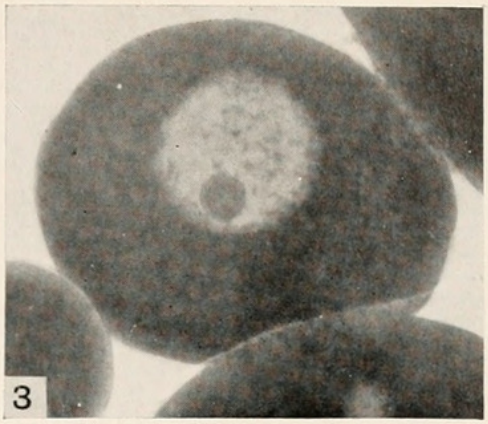
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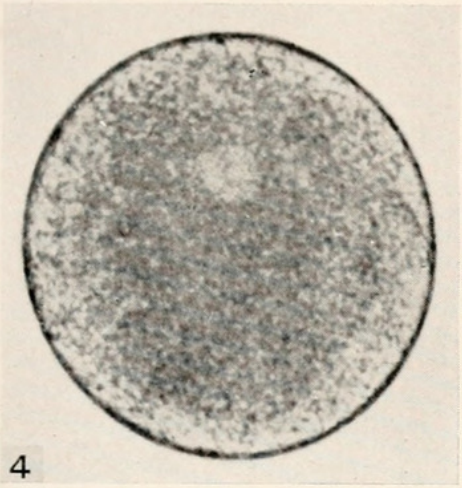
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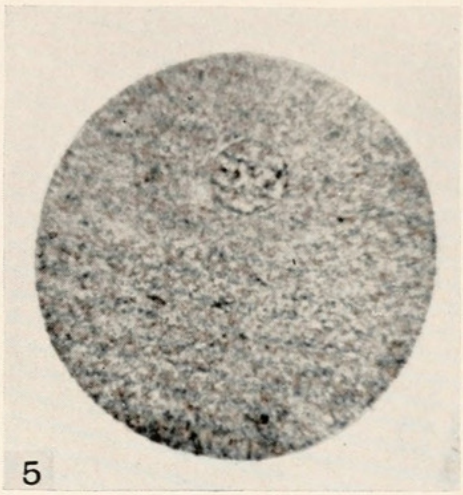
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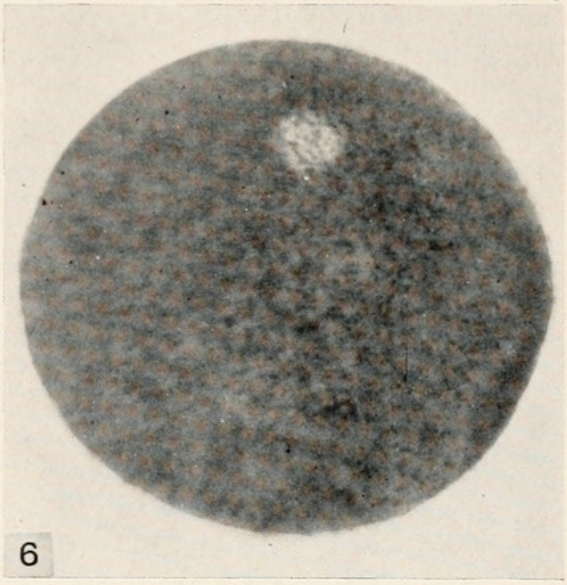
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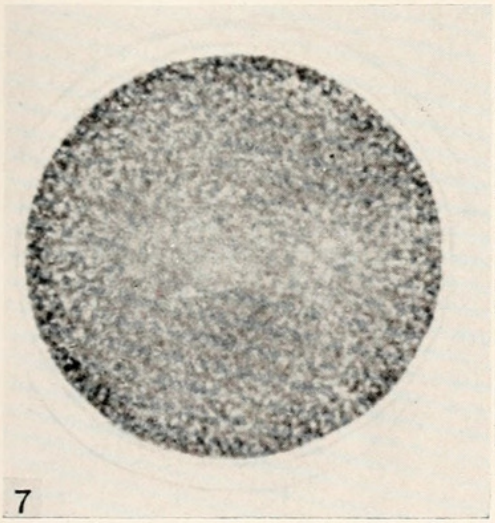
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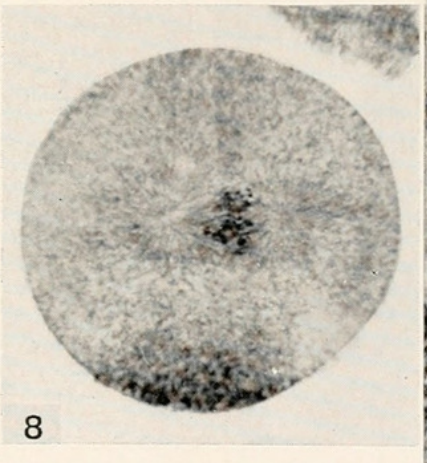
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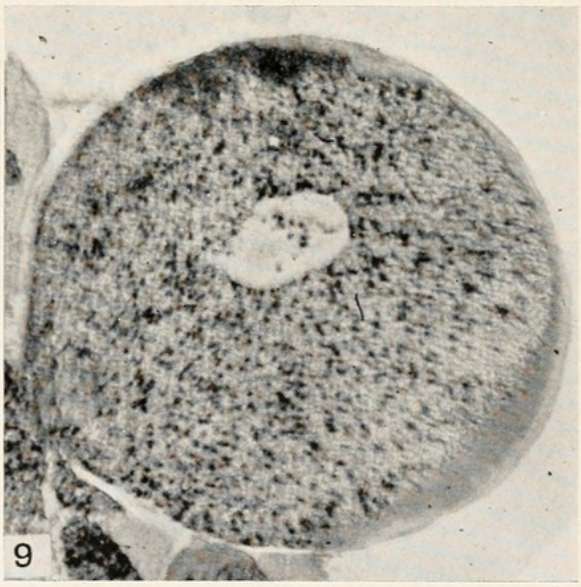
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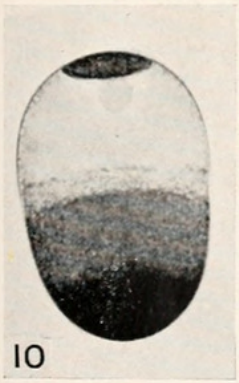
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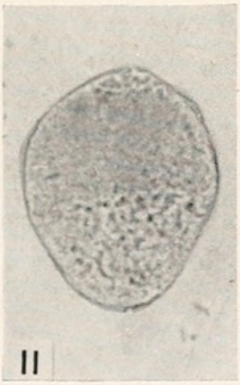
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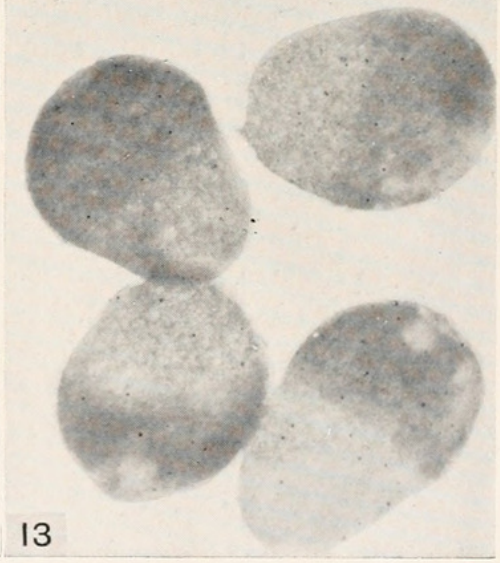
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PLATE I

1940). The spindle of the dividing cell probably does not contain nucleic acids of either type, though there is some disagreement about this (Stedman and Stedman, 1943a, b, c vs. Callan, 1943; Barber and Callan, 1944).

Blanchard (1935) extracted from masses of unfertilized mature Arbacia eggs nucleic acid of the desoxyribose type and also a pentose derivative whose properties resembled those of a ribose nucleic acid, both in approximately the same amount. Brachet (1933, 1937) found little of the desoxyribose type in unfertilized eggs of another sea urchin, *Paracentrotus lividus,* and thought that Blanchard's results were due to the presence of ovarian tissue, other than the eggs themselves, rich in the desoxyribose type. Brachet found that the desoxyribose nucleic acid increased in amount and the ribose type decreased after fertilization. According to Caspersson and Schultz (1940), there is an accumulation of ribonucleic acid in the cytoplasm close to the nuclear membrane in the ovarian (immature) egg of *Psammechinus miliaris,* another sea urchin; this they think indicates a "synthesis of nuclear products influencing cytoplasmic activity." In our ultra-violet photographs of Arbacia, one cannot distinguish between the ribose and desoxyribose nucleic acids since they absorb equally. In some of the photographs there is a distinct massing of strongly absorbing material, either as a clump or as a thick ring, on the outside of the nuclear membrane, but it is not constant.

The first work on photography with ultra-violet light on living and fixed (unstained) cells was done by Köhler in 1904 with his original ultra-violet microscope. His photographs show very nicely the nuclei in living cartilage cells of Triton and the spireme threads and chromosomes of fixed epithelial cells of Salamandra. Very good ultraviolet photographs of grasshopper (*Melanoplus femur rubrum*) spermatocyte cells, showing spiremes and chromosomes in all stages of mitosis, were published by Lucas and Stark in 1931; they show no spindle fibers, though they state that these can be seen in unstained sections. Wyckoff and Ebeling (1933) show similar ultra-violet photographs of "grasshopper" spermatocytes. Caspersson (1936) shows similar photographs of such cells in other species of Orthoptera (*Chorthippus dorsatus* and *Gomphocerus maculatus*). These chromatic structures in Orthopteran sperm cells, however, show well in the living material with visible light (Chambers, 1914, in *Disosteira carolina;* Bělař, 1929, in *Chorthippus lineatus*). It is of interest that the giant chromosomes of the salivary gland cells of Drosophila show their banded structure well with ultra-violet light (Caspersson, 1936). In *Arbacia punctulata,* the chromatin network and chromosomes cannot be seen with visible light in the living egg, but show very nicely with ultra-violet light and appear exactly as they do in fixed and stained preparations. Judging from the absorption of ultra-violet light, compounds which absorb 2537 A° strongly are present in the Arbacia egg in the nucleolus, the chromatin network and the chromosomes, but not in the spindle fibers and asters, and not in the nuclear sap.

Cytoplasm of the Centrifuged Egg

When the *Arbacia punctulata* egg is centrifuged, the stratification is as follows: Oil at the centripetal pole, clear layer, a band of mitochondria, a large layer of yolk granules, and red pigment granules at the centrifugal pole; the nucleus lies in the clear layer under the oil cap (Harvey, 1932; 1940). In the living egg, the stratification is very striking (Photograph 10). The different materials stain selectively

with many vital dyes; e.g. the mitochondria stain purple with methyl green, the yolk and pigment stain blue with methylene blue (Harvey, 1941). When the centrifuged eggs are fixed, sectioned and stained with Heidenhain's haematoxylin, the layers are not so striking. After a Bouin fixation, the oil cap is not to be seen; it has apparently been dissolved by the fixative (Harvey, 1940, Photograph 124). However, with a formalin fixation the oil cap is preserved (Photographs 11, 12). The most striking difference between the living egg and the fixed and stained sections is in the clear layer. This is optically empty in the living egg, but is deeply stained in the haematoxylin preparations and is filled with very small granules (Photograph 12). In the formalin fixed sections, stained with haematoxylin, the different layers of mitochondria, yolk and pigment are scarcely distinguishable; they are apparent, however, in Bouin-fixed sections, especially when the haematoxylin preparations are counterstained (Harvey, 1940, Photograph 124).

It was found difficult to obtain good ultra-violet photographs of the living centrifuged egg, as it tended to burst with the pressure of the coverslip necessary to obtain the desired thinness for the light to penetrate. We therefore used unstained sectioned material; the Bouin fixative was not suitable for the ultra-violet, but the formalin fixation was quite satisfactory. The most absorbing area is the clear layer, which appears quite dark in the ultra-violet photographs, much darker than the nucleus (Photograph 13). A control, unstained, section photographed by visible light is shown in Photograph 11. The oil cap and the granular layers, mitochondria, yolk and pigment are somewhat but not markedly absorbing with ultra-violet light, and all about equally so. There is no special absorption by the mitochondria. The bulk of the protoplasmic nucleic acid compounds is therefore in the clear layer. This material is the ground substance or matrix of the uncentrifuged egg in which the granules lie. This is apparently also the important material for development, rather than the granules, since it has been shown that fractions of eggs, obtained by centrifugal force, may lack any one of the different types of granules and still develop (Harvey, 1932, 1936, 1940). It is of interest to find that it is the ground substance or matrix which contains the compounds which absorb in the nucleic acid region of the spectrum.

Summary

1. Photographs of living immature, mature and dividing eggs of *Arbacia punctulata,* taken with ultra-violet light of wave length 2537 A°, show an absorption, indicating presence of nucleic acid compounds, in the nucleolus, chromatin network and chromosomes. The photographs with ultra-violet light are similar to those of sections of fixed material stained with Heidenhain's haematoxylin taken with visible light. The chromatin network and chromosomes cannot be seen with visible light in the living egg.

2. In the centrifuged egg, with ultra-violet light, the clear layer shows greatest absorption, indicating the localization of nucleic acid compounds in this layer. With visible light, this layer appears dark and granular in sections stained with Heidenhain's haematoxylin, much as it does with ultra-violet light. It is optically empty in the living centrifuged egg with visible light. This layer represents the matrix or ground substance of the normal uncentrifuged egg. The matrix of the nucleus and the layers of granules (mitochondria, yolk, pigment and oil) are relatively non-absorbent with ultra-violet light.

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