# STUDIES ON AMPHIBIAN YOLK

## 1. The Ultrastructure of the Yolk Platelet

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ABSTRACT

The yolk platelets of mature eggs and young embryonic cells of all amphibian species studied (*Rana pipiens*, *Triturus pyrrhogaster*, *Diemictylus viridescens*, *Rana nigromaculata*, and *Bufo vulgaris*) have a superficial layer of fine particles or fibrils (*ca.* 50 A in diameter), a central main body with a crystalline lattice structure, and an enclosing membrane approximately 70 A in thickness. Electron micrographs of the main body reveal hexagonal net (spacing *ca.* 70 A), square net (spacing *ca.* 80 A), and parallel band (spacing from 35 to 100 A but most frequent at *ca.* 70 A) patterns. The crystalline structure is believed to be a simple hexagonal lattice made of closely packed cylindrical rods. Each rod is estimated to be about 80 A in diameter and 160 A in length.

### INTRODUCTION

Yolk platelets are the most prominent component in the cytoplasm of amphibian eggs and embryonic cells and have been generally designated as inert reservoirs of raw material to be utilized during subsequent embryonic and larval development. Recently, however, many investigations have indicated that yolk platelets have a unique structural organization with many interesting features, as well as a more complex relationship with their intracellular environment than had been supposed.

Lanzavecchia (22) and Ward (50), for instance, have suggested that the main body of yolk platelets may be laid down first within mitochondria or mitochondrion-like membranes of the early frog oocyte. In subsequent stages of oogenesis and early embryogenesis, however, this relationship is no longer apparent and the fully formed platelet is seen to be limited by a single membrane and to consist of an inner compact and crystalline main body and an outer non-crystalline superficial layer (16, 18, 23, 45, 50, 52). The superficial layer appears rather labile and frequently disappears from the surface of the main body in cell undergoing early differentiative processes (16, 23, 45). At even later stages when the dense main body itself begins to break down, membranous structures generally circumscribe the platelet (16, 21, 23) and frequently these membranes again appear distinctly mitochondrial in type (21, 23, 43). Concomitant with this breakdown, a quick increase in the mitochondrial population and elaboration of endoplasmic reticulum has also been noted (4, 16). Finally, many investigators have suggested that yolk platelets contain specific substances which may subsequently influence the development pathway of embryonic cells after they are released into the cytoplasm from their bound state (1, 7, 12, 14, 16, 31, 38, 46).

The precise role, if any, of yolk platelets in developmental processes is as yet ill defined, and the mechanisms of platelet formation and utilization remain a matter of speculation (7, 11, 16, 19, 22, 23, 31, 33, 50). Contradictory interpretations of yolk structure and chemistry in the current literature (9, 10, 16, 29, 30, 31, 34, 36, 39, 50)

show a need for a more precise understanding of these two aspects.

The fine structure of yolk platelets has been examined in such amphibian species as *Triturus pyrrhogaster* (16, 18, 45), *Diemictylus* (*Triturus*) *viridescens* (52), *Rana pipiens* (36, 50), *Rana esculenta* (21), and *Rhacophorus schlegelii* (45). These studies have indicated a periodic structure in the yolk platelets with a regular alternation of dark and light bands. The center-to-center spacing of the dark bands was about 70 A in each case. Prior to these observations, a birefringence had been noted for fresh and fixed yolk platelets of various amphibian eggs (10). During utilization, yolk platelets were found to split into parallel discs and rodlets (11). Such lamellar cleavage was observed also *in vitro* when platelets were exposed to weak acid, alkaline, or salt solutions (9, 10, 36). These observations might thus be indications of a basic structure for platelets analogous to that of other lamellar cytoplasmic systems (34, 36). On the basis of electron microscopic and electron diffraction patterns, the author has suggested rather that the periodic bands represent a fringe system corresponding to a three-dimensional network of units arranged within the planes of a crystalline lattice (16, 18). This paper is concerned with describing the yolk platelet ultrastructure in the mature oocyte and embryonic cell, and with defining the crystalline lattice found in the main body of the platelets.

## MATERIALS AND METHODS

Most of the observations were made on presumptive ectoderm cells of early embryos of *Rana pipiens* and *Triturus pyrrhogaster*, mature eggs of *R. pipiens*, and oocytes of *Diemictylus viridescens*. Oocytes of *R. pipiens* and *T. pyrrhogaster*, and embryos of *D. viridescens*, *R. nigromaculata*, and *Bufo vulgaris* were also studied.

Tissues were quickly transferred into a fixative of the following composition: 4 per cent $OsO_4$ (2.5 cc), M/3.5 sodium veronal (0.5 cc), M/3.5 sodium acetate (0.5 cc), M/10 HCl (1 cc), M/8 Holtfreter solution (0.5 cc), pH 7.2 to 7.4. After fixation for 30 minutes to 1 hour at 4°C, the samples were rinsed briefly in Holtfreter solution, passed through one change each of 50, 70, and 90 per cent and two changes of 100 per cent ethanol for 15 minutes each, and finally embedded in the Epon mixture described by Luft (24).

Blocks were sectioned (250 to 500 A) with a diamond knife on a Porter-Blum microtome. The sections were mounted on collodion-coated grids stabilized with a carbon film and subsequently stained with saturated lead acetate for 1 to 2 minutes under a flow of nitrogen (32) or with saturated uranyl acetate for 1 hour (51). For examination by the light microscope, thicker sections (0.5 to 2 $\mu$) from the same blocks were cut and stained with an aqueous solution of 0.1 per cent toluidine blue (pH 11) without removing the plastic.

Electron micrographs were taken with an RCA model EMU-3E microscope at 50 kv using a single condenser system and an objective aperture of 30 $\mu$. Most of the pictures were taken at an initial magnification of 32,000 times, as calibrated with a carbon

*Explanation of Figures*

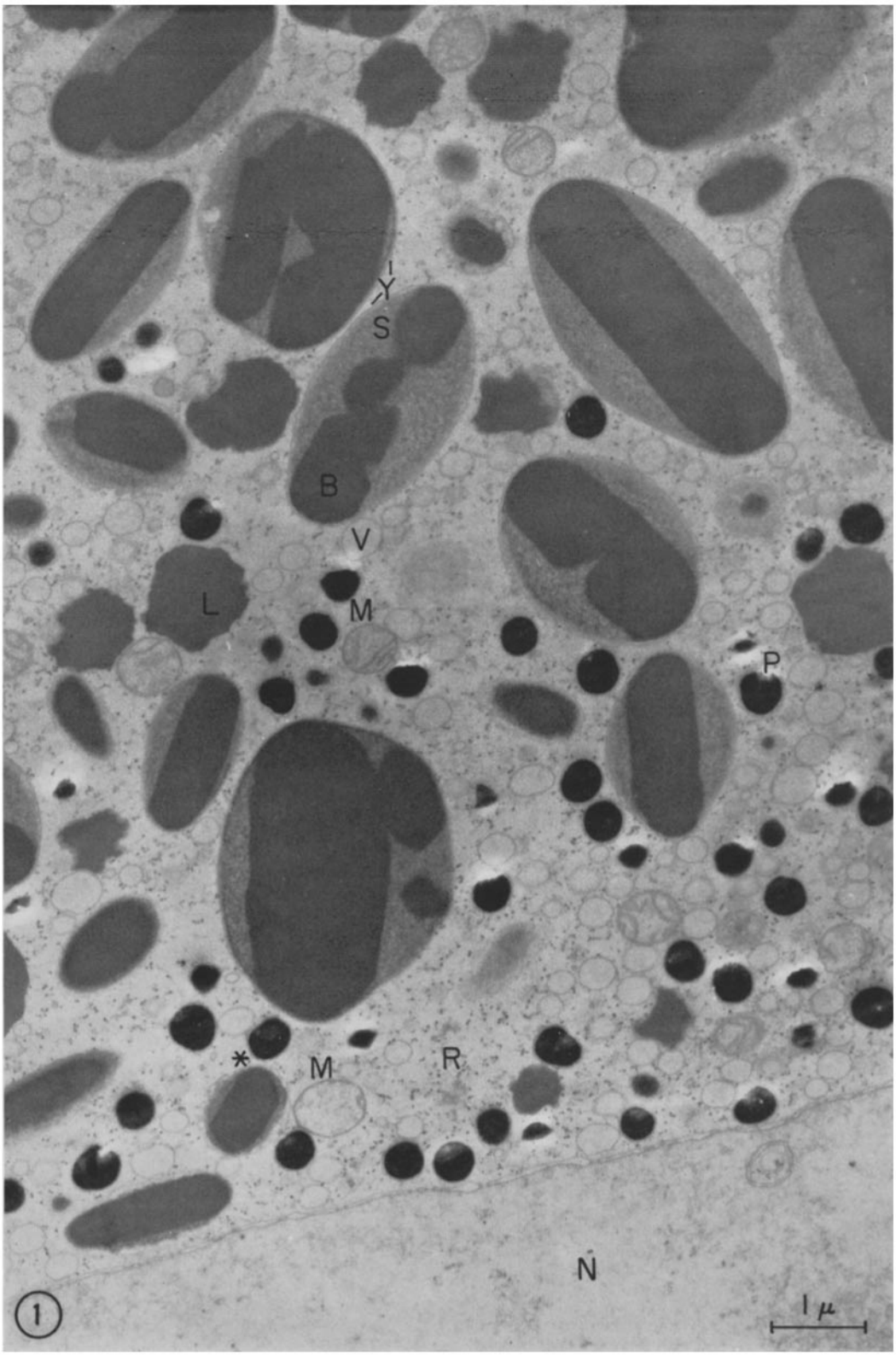
All figures except Fig. 13 are electron micrographs of osmium-fixed material embedded in Epon. Figs. 5, 6, 8, 10, and 11 are from sections stained with uranyl acetate; the rest are from sections stained with lead acetate.

*Key to Abbreviations*

*B*, main body of yolk platelet
*C*, cell membrane
*L*, lipoid body
*M*, mitochondrion
*N*, nucleus
*P*, pigment granule
*R*, cytoplasmic ribonucleoprotein particle
*S*, superficial layer of yolk platelet
*V*, cytoplasmic vesicle
*Y*, limiting membrane of yolk platelet

FIGURE 1

Part of a presumptive ectoderm cell in the blastula embryo of *Rana pipiens*. Each yolk platelet consists of an outer superficial layer (*S*) and a central main body (*B*), both enclosed within a single membrane (*Y*). The diameter of yolk platelets varies between 0.3 and 5 $\mu$, with an average of 2 $\mu$. Lipoid bodies (*L*), pigment granules (*P*), mitochondria (*M*), vesicles (*V*), and ribonucleoprotein particles (*R*) can be identified. The nucleus (*N*) of the cell is at the bottom of the picture. The yolk platelet indicated by an asterisk (*) is shown at a high magnification in Fig. 2. × 15,000.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grating replica (Ernest F. Fullam, 28,800 per inch) or latex spheres (Dow, 0.088 $\mu$). The microscope was tested and found free of astigmatism prior to examination of the platelets. The projector systems were normalized at each magnification step before photography. Kodak Contrast Projector Slide Plates, 3¼ by 4 inches, were used for the photographic plates. They were enlarged to 100,000 or 200,000 times, and all measurements were made on the enlarged images using a glass magnifier.

## OBSERVATIONS

### *General Morphology (Examination by Light Microscopy)*

In general, yolk platelets of mature amphibian eggs are scattered at random throughout the cytoplasm, but they are larger and appear more concentrated in the vegetal hemisphere. In *Rana* or *Bufo* the larger flattened bodies are distinctly rectangular, while those in *Triturus* and *Diemictylus* are more or less round. However, this difference is less obvious in the smaller platelets, whose outlines are more or less ovoid in both species. In most cases, the longitudinal axis of the body is slightly longer than the transverse axis, and the tertiary axis is from two to three times shorter than the longitudinal axis. The larger yolk platelets are clearly discernible with the light microscope and may range up to 50 $\mu$ in length. Smaller ones can be seen down to the limits of resolution with the light microscope.

### *Ultrastructure*

The basic structure of the yolk platelets is essentially the same in late oocytes, mature eggs, and early embryonic cells of each species studied. Under low magnification (Figs. 1 and 11), each yolk platelet appears to consist of an opaque homogenous body surrounded by or embedded within a less opaque superficial layer. Usually both of them are enclosed within a single membrane. The yolk platelet may thus be described as having three basic components: a main body, a superficial layer, and a limiting membrane.

#### The Main Body

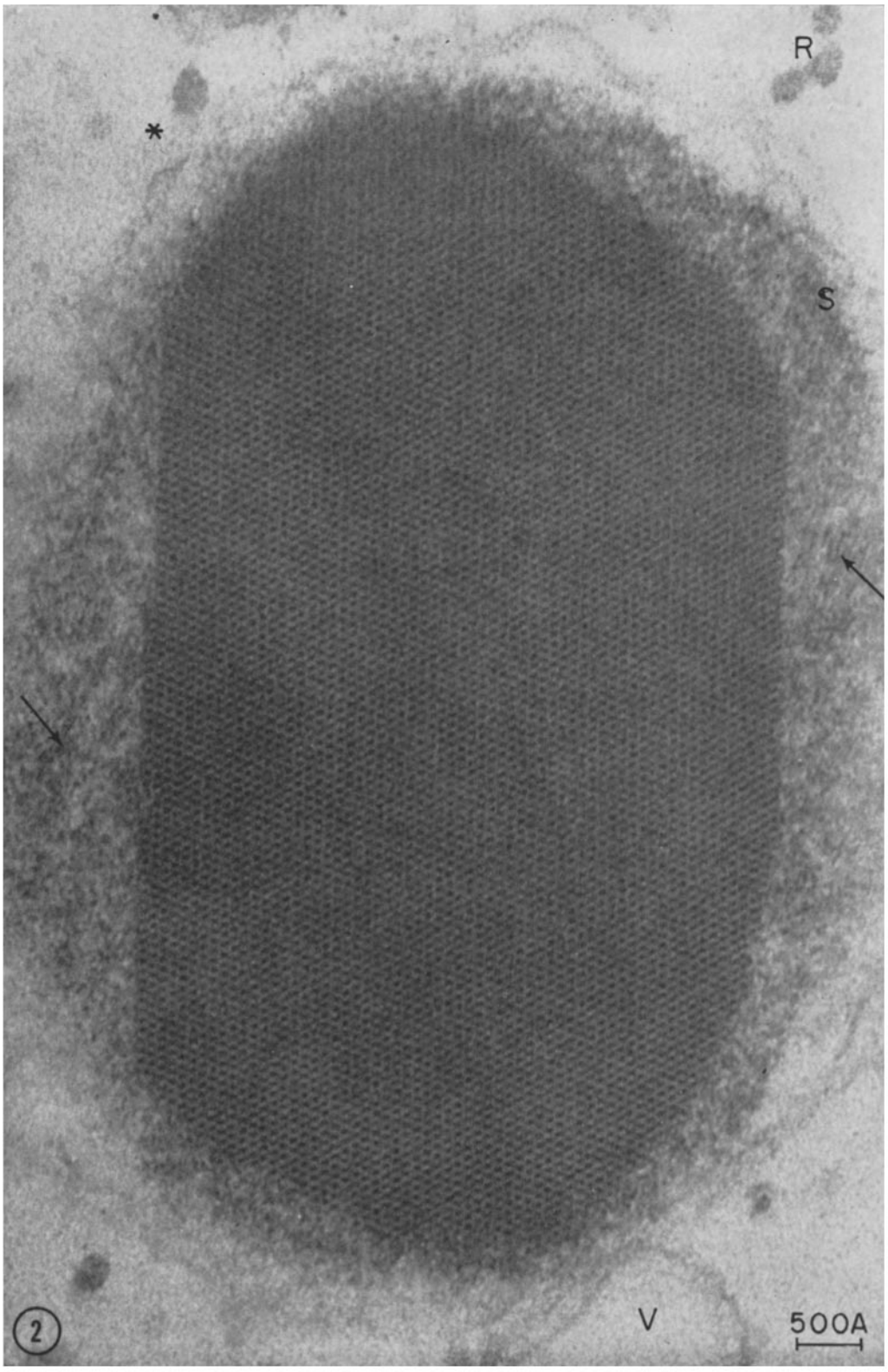
Profiles of the main body are usually oval or round, but occasionally they have flattened sides (Fig. 1). In *Rana* especially, the variety of the shapes among the smaller platelets is remarkable (Fig. 1), with triangular, rectangular (Fig. 9), rhomboidal, and hexagonal figures (Fig. 9) being observed. Although generally a single main body is present within a yolk platelet, in larger yolk platelets two or three separate main bodies may be embedded within a common superficial layer.

Under high magnification (Figs. 2 to 10 and 12), the main body has a highly ordered fine structure similar to a crystalline lattice. In some sections, a periodic pattern extends over the entire main body, which may be up to 3 $\mu$ in length. Two types of periodic pattern can be seen, tentatively described as "dot pattern" and "band pattern." The dot pattern consists of dark, roundish dots packed in an almost hexagonal array and separated from one another on all sides by a lighter interspace, as shown in Figs. 2 and 5. Most yolk platelets, however, have a band pattern, as shown in Figs. 3, 4, 6 to 10, and 12. The band pattern consists of straight, parallel, alternately dense and less dense bands which usually traverse most of the main body without any particular orientation to any of the axes of the platelet (Figs. 3 and 7). In some platelets a secondary system of similar but less distinct parallel bands intersects the primary band system at angles ranging from 20 to 90 degrees (Figs. 3, 4, 9, 10, and 12).

The center-to-center distance between dense lines or adjacent arrays of dots has been measured for yolk platelets from the eggs or embryos of *R. pipiens*, *T. pyrrhogaster*, and *D. viridenscens* (Fig. 13). Similar results were obtained with embryos of *R. nigromaculata* and *B. vulgaris*. Although a wide range of distances is found, the large majority fall between 65 and 85 A. The average spacing

FIGURE 2

High magnification of the small yolk platelet indicated by an asterisk (*) in Fig. 1. The entire main body displays a hexagonal array of dots approximately 45 A in diameter with an average center-to-center distance of approximately 80 A between dots. The superficial layer consists of fine particles or fibrils (arrows) (average diameter 50 A), which are much smaller than the cytoplasmic ribonucleoprotein particles (average diameter 200 A). × 200,000.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is 70.9 A in the oocyte of *R. pipiens* (Fig. 13 A), 71.3 A in the embryo of . *Rpipiens* (Fig. 13 B), 71.1 A in the embryo and oocyte of *T. pyrrhogaster* (Fig. 13 C), and 71.1 A in the oocyte and embryo of *D. viridescens* (Fig. 13 D). Thus in this respect no significant difference can be seen among the three different species or between the oocyte and the embryo. Also, there do not seem to be any differences in the spacing and types of periodic patterns among the platelets of various sizes or among platelets contained within oocytes or embryonic cells at different stages of development. In fact, all possible variations in the type of pattern and in the spacing of individual dots or bands were often seen in platelets within a single micrograph plate (see also Figs 4 and 8 in reference 49). Therefore, in the analysis which follows it is assumed that there are no consistent differences in the structure of yolk platelets between different species and different sizes. Of 362 close-to-focus pictures of yolk platelets, 290 displayed a periodic pattern. Among the latter, a hexagonal array of approximately isometric dots was seen in only 18 cases (Figs. 2 and 5), while band patterns were found in the remainder (Figs. 3, 4, 7 to 10, and 12). The center-to-center distances between the dots ranged from 70 to 90 A, with an average of 81 A. The spacing between rows of dots averaged 70 A. The exact diameter of the dots was difficult to determine because of lack of sharpness in the electron micrographic image, but is estimated to average 45 ± 5 A. Under high magnification the dots show no evidence of internal structure. When the plane of a section showing a clear hexagonal pattern of dots was slightly inclined with respect to the optical axis of the electron microscope, the dot pattern was transformed into a band pattern (Figs. 5 and 6). A similar phenomenon was observed when the focus at the section was shifted for $\Delta f = \pm 1\ \mu$. In some such sections a dot pattern at the periphery of the main body was observed to change into a band pattern in other regions (Fig. 8).

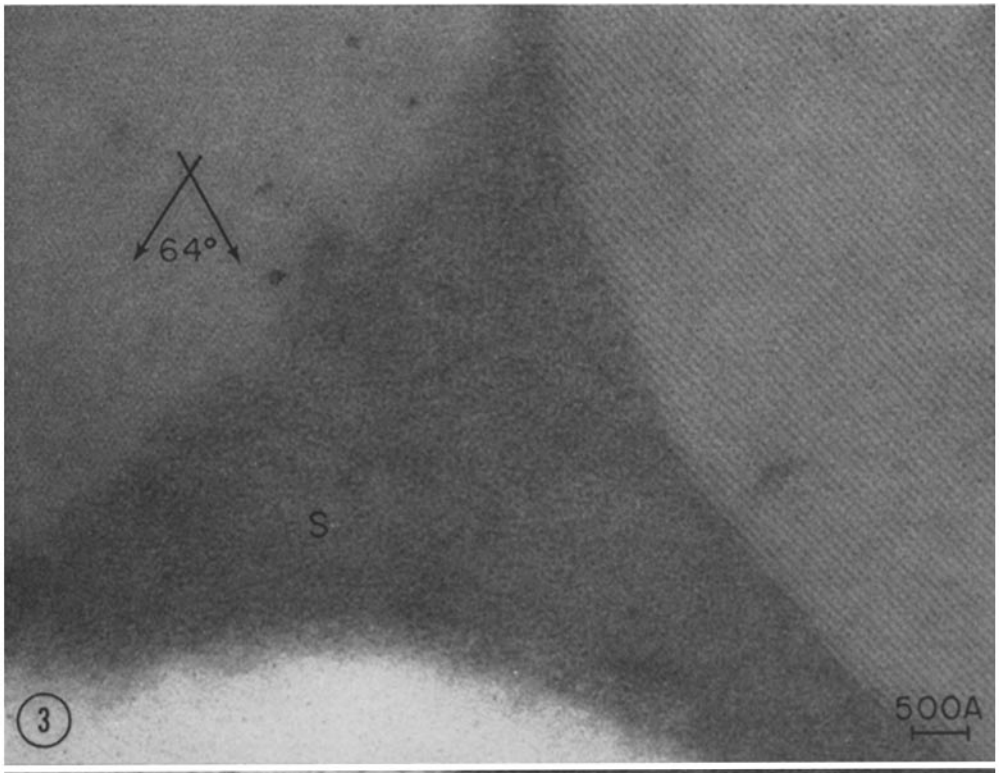
The spacing of the band pattern ranged from 35 to 100 A. As indicated previously, however, the majority of the values fell between 65 and 80 A and averaged 71 A. In 147 cases in which intersecting bands were observed, the acute angle of interception varied from 20 to 90 degrees with primary frequencies of 60 and 90 degrees. In 5 examples, the two intersecting bands were of almost identical density and spacing, thus displaying a square net (Fig. 4). In these cases the bands appeared as diffuse and broken lines. The spacing of both intersecting bands ranged from 70 to 85 A, with an average of 75 A. In 10 examples, three sets of intersecting bands having similar densities and spacing resembled hexagonal nets (Fig. 9), with an average spacing of 69A. In a number of cases, bands were limited to the periphery of the main body and blended into a homogeneous interior (Fig. 10). In 72 examples, the entire main body appeared homogeneous even though the section was cut thin and a through-focus series carefully performed. The exact width of the dark bands was difficult to determine because their outlines were not sharp, but usually their width was slightly greater than that of the light bands. Although the band patterns can be observed in unstained sections, the dark bands increased considerably in density (but not in width) when the section was stained with lead or uranium. However, the light bands (which have a low but definite density) do not show a pronounced increase in density after staining.

FIGURE 3

Part of a yolk platelet in a late oocyte of *Diemictylus viridescens*. Two separate main bodies with different periodic patterns are embedded in a superficial layer of high density particles. In the right body, the alternating dense and less dense parallel bands show a spacing of 86 A. In the left body, parallel alternating bands with a spacing of 53 A intersect one another at an angle of 64 degrees. × 140,000.

FIGURE 4

Part of a yolk platelet in a late oocyte of *Diemictylus viridescens*. The main body displays a rectangular lattice of parallel bands with a spacing of 73 and 80 A in both *a* and *b* directions. × 140,000.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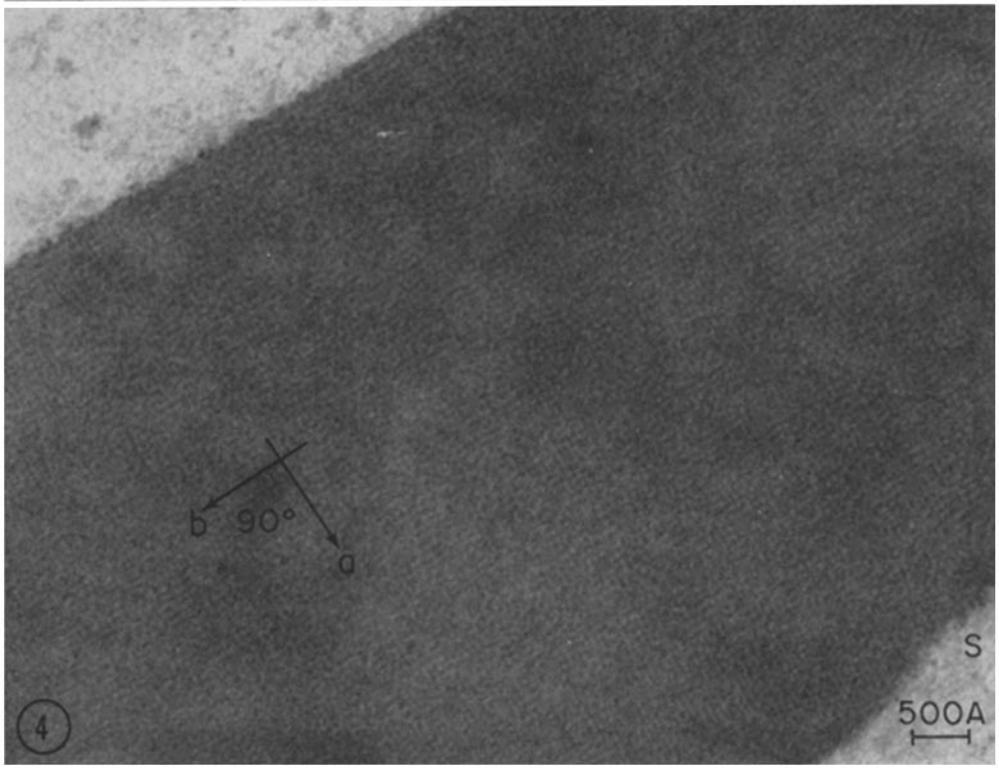
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### The Superficial Layer

The superficial layer immediately surrounds the main body of each platelet (Figs. 1 to 7 and 10 to 12). It has a medium density and a finely granular or fibrous appearance, and was observed in both small and large yolk platelets in all species studied. The particles or fibrils of the superficial layer (Fig. 2) have an average diameter of 50 A and are loosely scattered in some platelets (Fig. 12) and tightly packed in others (Fig. 3). Also, the size of the components appears much larger in methacrylate-embedded samples (16), perhaps because of aggregation. The precise form of particles is difficult to judge from the micrographs. Some of them appear to be fibrils up to 200 A long by 50 A wide (Fig. 2). In the superficial layer of a well preserved yolk platelet, two or three subdivisions may be distinguished: a relatively thin layer of dense, moderately packed materials (Fig. 11, at *a*); a thick layer of medium density (Fig. 11, at *b*); and a thin layer of a diffuse and less dense material close to the limiting membrane (Fig 12; best seen in Fig. 2, reference 49). Very often, the particles near the main body display a gradual transition from a disordered pattern to the regular periodic pattern of the main body (Fig. 7). Occasionally, a band pattern similar to that described for the main body was observed as a patch within the superficial layer (Fig. 12). This could, however, have been a piece of main body tangentially cut.

### The Limiting Membrane

The superficial layer and main body are usually surrounded by a single membrane about 60 to 80 A thick and of medium opacity (Figs. 1, 2, 5, 11, and 12; see also Fig. 2 in reference 49). Parts of the membrane display the triple-layered profiles of the unit membrane. The limiting membrane is separated from the superficial layer by a light zone or interspace, 70 to 200 A wide, in the best-preserved specimens. Occasionally, the membrane seemed to be fragmented and to form a series of small vesicles. When fixation was for longer than 1 hour, such fragmentation was extensive. Finally, the use of methacrylate as an embedding medium promoted the disappearance of the membrane, particularly when fixation was for longer than 1 hour.

## DISCUSSION

The data presented indicate that the main body, superficial layer, and limiting membrane represent the basic structural components of yolk platelets of all amphibian species. In this respect no difference is observed among yolk platelets of various sizes. Furthermore, periodic patterns of similar spacing and type were observed in all species and cell types examined. Therefore it is probable that most yolk platelets have the same fundamental structure, regardless of origin or size. This conclusion does not support the idea that a high degree of heterogeneity exists among amphibian platelets, as suggested by the chemical analyses of Panijel (31).

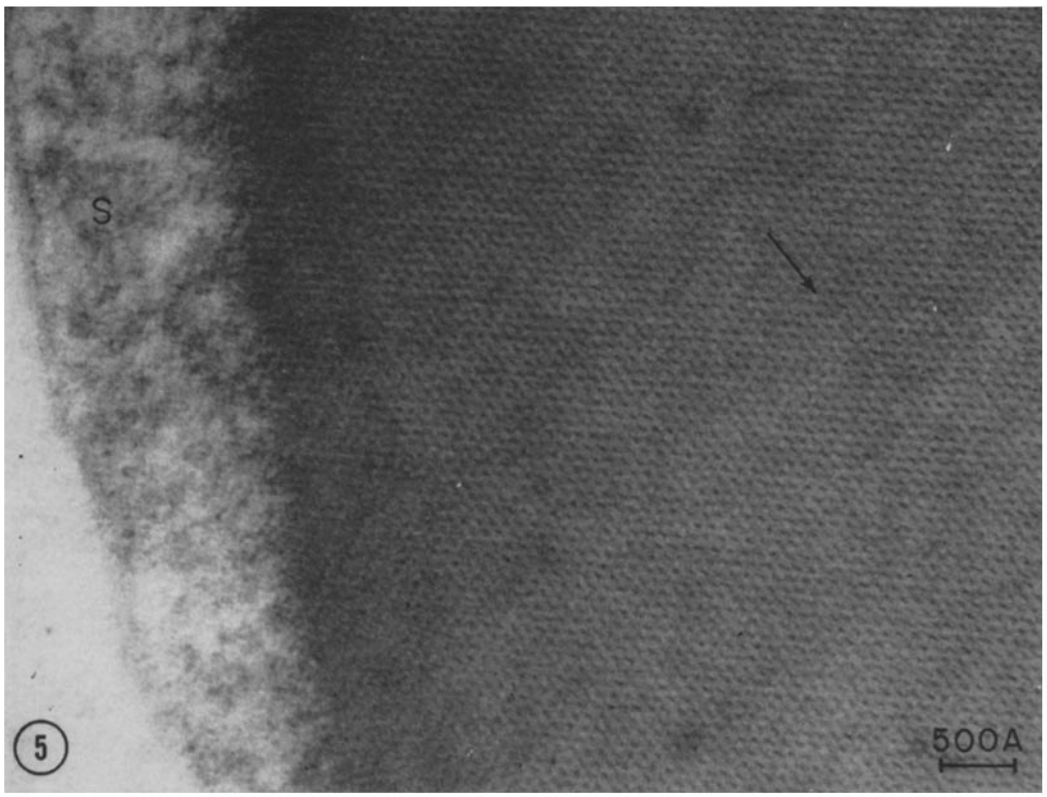
The primary concern of this study has involved the types and periodicity of patterns observed in the main body of the yolk platelets. The types of patterns observed seemed to depend entirely upon the angle at which each main body was sectioned, rather than upon artifacts due to magnification or differences among crystalline structures. In a number of recent papers (2, 3, 5, 6, 26, 27, 35, 42) it has been shown that intercellular crystals of protein have similar periodic patterns. In all these studies, dot, band, and transitional patterns also were obtained. Recently it has been shown that electron microscopic images of suitably oriented thin crystals of certain stable organic or metal organic compounds are

FIGURE 5

Part of a yolk platelet within a gastrula cell of *Triturus pyrrhogaster*. A hexagonal array of dots about 40 A in diameter is evident in the main body, with center-to-center distance averaging 80 A between the dots. The spacing between the lines of dots running in the direction of the arrow is 72 A. × 200,000.

FIGURE 6

The same region as in Fig. 5, photographed after the objective holder was slightly tilted in the direction indicated by arrows in Figs. 5 and 6. The hexagonal pattern of dots in Fig. 5 has changed into a band pattern, but the 72 A spacing remains the same. × 200,000.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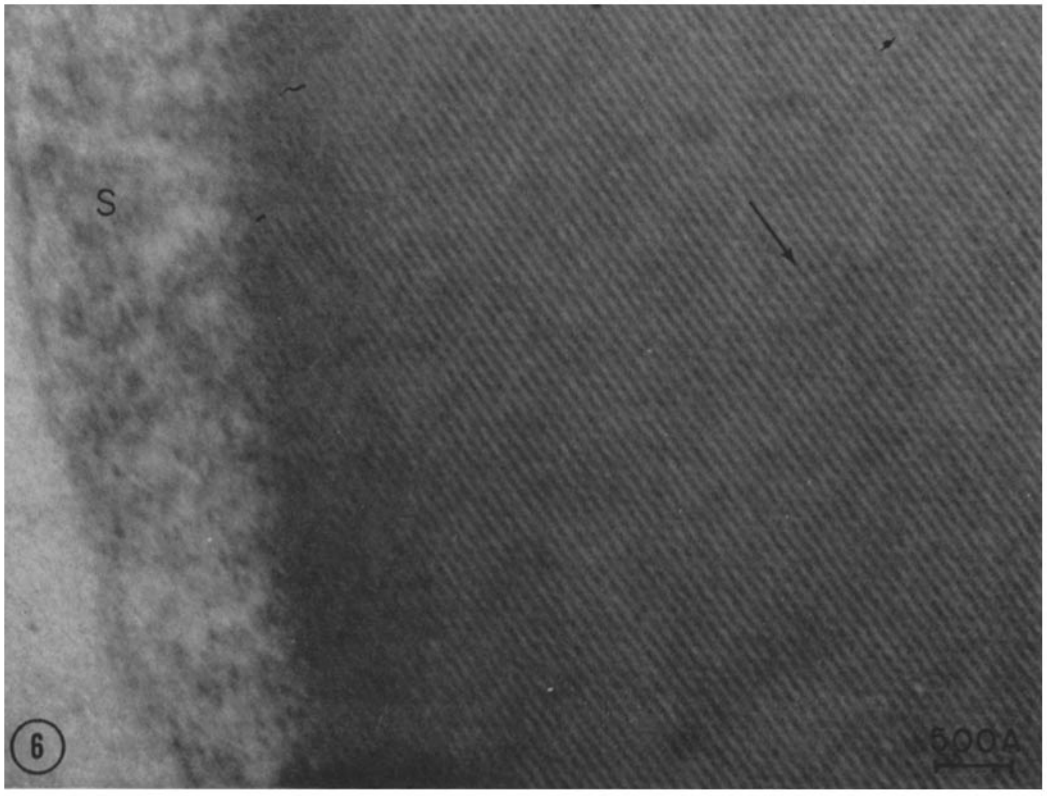
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striated (20, 25). The periodicity of these striations generally coincided with the spacing of the molecular planes within the crystals as determined by x-ray analysis. Menter (25) and Labaw (20) have interpreted such striated images as fringe systems arising from interference between the primary beam and beams diffracted from the molecular planes, and, as such, the fringe patterns do not directly represent the molecular array itself.

In previous papers, periodic patterns similar to those found in this study were observed in electron micrographs of yolk platelets (16, 18). These have been interpreted as fringe systems arising from net planes in a macromolecular crystal, since diffraction spots corresponding to a spacing of 72 A were observed in the back plane of the objective. In most cases the band pattern found in this study is essentially similar to a fringe pattern. On the assumption that the fringe patterns observed in microscopic sections correspond to three-dimensional net planes within a crystalline lattice, a possible lattice structure can be described. Consideration of and comparison with various crystalline models suggest that the fringe systems observed in amphibian yolk platelets may be expected from a face-centered cubic lattice or a simple hexagonal lattice, because with these structures a pattern of hexagonal nets and square nets is obtained from various section orientations. The determination of the crystal lattice structure from section micrographs, however, cannot be so certain as a determination based on a replica or pseudo replica as performed by Wyckoff (53).

According to Holtfreter (10), yolk platelets are negatively and uniaxially birefringent with respect to the long axis of the platelets. In weak acids, weak alkalis, and strong salt solutions, the platelets split into microscopic or ultramicroscopic laminae (9, 10, 36), so that the observed birefringence is increased. According to Picken (34), the individual laminae are positively birefringent with respect to their plane and isotropic when viewed along a plane normal to each lamina. The birefringence and ultramicroscopic lamination observed thus suggest a layered arrangement of molecules in the main body of yolk platelets. For this reason it is more logical to assume that the crystalline lattice in question is of the simple hexagonal type rather than of the face-centered cubic type, since the former has a layered structure whereas the latter is usually isotropic.

The micrographic images observed in this study cannot be completely explained as a fringe pattern, however, since the component protein macromolecules are probably sufficiently large and discrete to be resolved and visualized with the electron microscope. From a study of many micrographs, one may speculate that these

FIGURE 7

Part of a yolk platelet of the ectodermal cell of the *Rana pipiens* embryo (tailbud stage). The two main bodies show different band patterns. The spacing between bands is 68 A in the right body, and 75 A in the left body. The dense bands blend into the granular region. × 130,000.

FIGURE 8

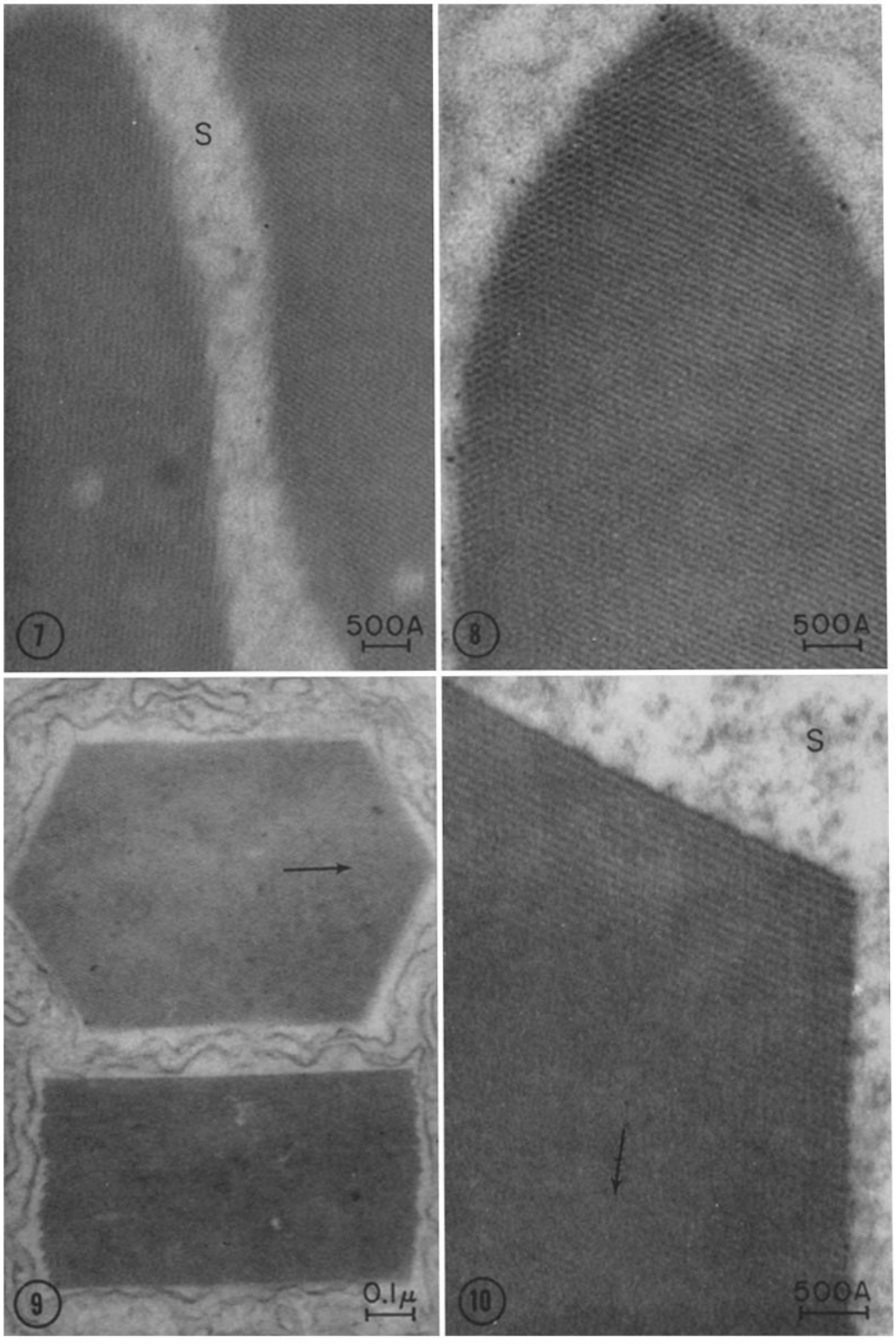
Part of the main body of a yolk platelet after the disappearance of its superficial layer in an ectodermal cell of *Triturus pyrrhogaster* (gastrular explant). The hexagonal dot pattern at the upper left periphery is transformed into a band pattern at the right. The average spacing between the bands is 70 A. A few very dense particles can sometimes be observed near the edge of the main body. × 180,000.

FIGURE 9

Two main bodies with hexagonal and rectangular shapes in an ectoderm cell at the tail bud stage of the *Rana pipiens* embryo. In the upper body, a hexagonal net (spacing 69 A) is evident (arrow). The lower body shows no distinct pattern. × 75,000.

FIGURE 10

Part of a main body showing a rhomboidal shape in a gastrula ectodermal cell of the *Triturus pyrrhogaster* embryo. The intersecting band pattern (spacing 70 A) loses its distinctness toward the interior of main body (arrow). × 200,000.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individual molecules are visualized in cross-section as dots in a hexagonal pattern. Thus a section angle which is not quite parallel to this plane would give an image of superimposed units forming parallel bands. One could also speculate that the crystal is made up of cylindrical rods or ellipsoids arranged parallel to their long axis. If such rods were sectioned perpendicular to their long axis, a dot pattern would result. However, a band pattern would be seen more frequently, because of a greater probability of oblique sectioning—and this is indeed the case. This assumption is strengthened by the observation that a slight tilt of the section or electron beam causes the dot pattern to transform into a band pattern. On the other hand, in some sections a dot pattern at one edge of a crystal changes into a band pattern in the interior and into a dot pattern again at the opposite edge. Such a pattern can also be seen in virus (28, 40, 53) and protein (5, 6, 35) crystals composed of individual spherical particles. It is thus suggested that the units observed in yolk platelets are spherical.

According to a recent biochemical analysis of amphibian yolk by Wallace (48), the crystalline main body consists of two moles of a highly phosphorylated phosphoprotein for every mole of a lipoprotein. Sedimentation, equilibrium, and viscosity measurements have revealed that the molecular weights of these two proteins are approximately 32,000 and 420,000, respectively. From the molecular weight and partial specific volume determinations of both proteins, spherical diameters have been estimated at 40 A (volume, $3.2 \times 10^4$ A$^3$) for the phosphoprotein and 101 A (volume, $5.4 \times 10^5$ A$^3$) for the lipoprotein. If the hexagonally arrayed dots observed with the electron microscope are similarly assumed to represent a spherical macromolecule, then the diameter of such dots ($45 \pm 5$A) falls within the range assumed for the diameter of the phosphoprotein molecule. As previously mentioned, uranium or lead staining of the sections was also found to enhance considerably the contrast of the observed dot or band patterns. Since uranium has been found to bind strongly to the phosphate groups of DNA (15, 41, 54), it may also be assumed that uranium (and probably lead) would become bound in a similar manner to the phosphate groups along the phosphoprotein molecules and effect the observed increase in contrast. Other types of protein generally take up considerably smaller quantities of uranium (53). Such considerations would thus seem to indicate further that the phosphoprotein is the component of the dots or bands.

Localization of the lipoprotein, however, is less certain. On the assumption that the protein macromolecules have spherical cross-sections and are closely packed in hexagonal unit cells, the center-to-center distance should then reflect the diameter of spherical units. This distance, 81 A at most, is considerably smaller than the diameter of spherical lipoprotein, 100 A, although the true dimensions could be reduced by shrinkage during the procedures of preparing samples for microscopy. However, if one assumes that two phosphoprotein and one lipoprotein molecules comprise a unit in the shape of a cylinder with hemispherical ends and are closely packed in a simple hexagonal lattice, the axial length and diameter of such a unit can be calculated as 160 A and 80 A, respectively (total volume, $6 \times 10^5$ A$^3$). The two phosphoprotein molecules in such a unit would then be about 80 A apart (center-to-center distance) from one another and also a similar distance apart from phosphoprotein molecules in neighboring units (see also Fig. 4 in reference 48).

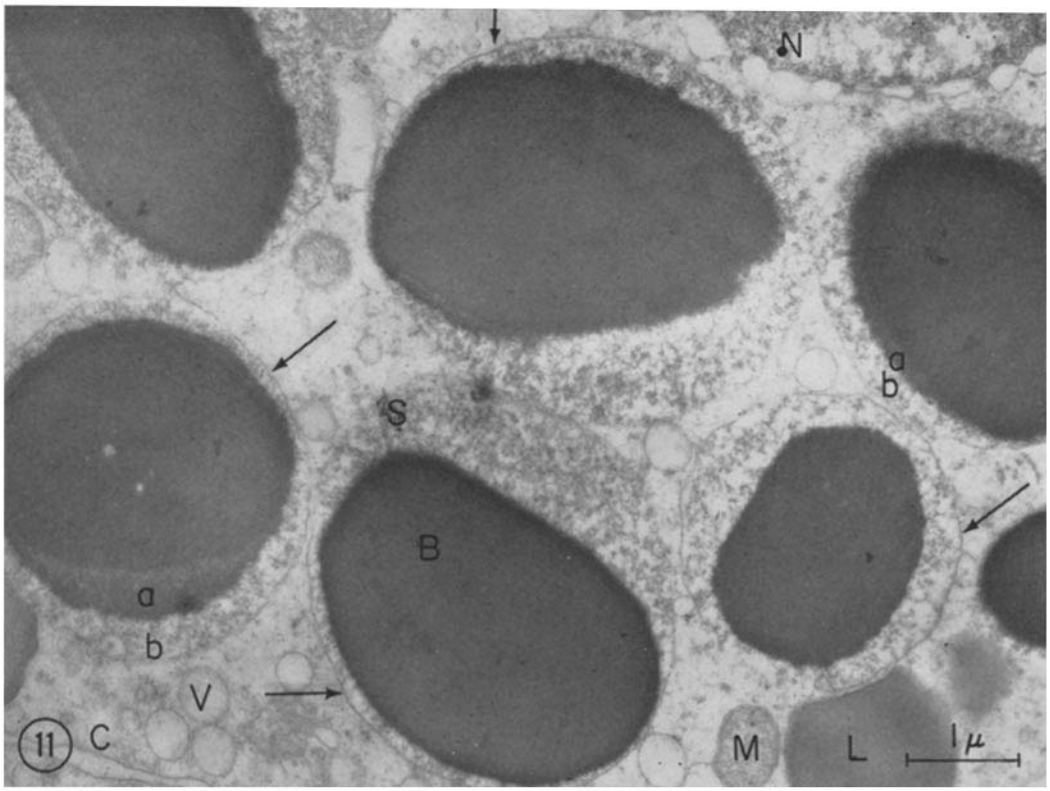
A recent x-ray diffraction analysis of amphibian

FIGURE 11

Part of an ectoderm cell in the *Triturus pyrrhogaster* embryo (gastrula). Each platelet is limited by a single membrane (arrows) and is composed of a compact main body (*B*) and superficial layer (*S*). Two subdivisions (*a*, *b*) can be distinguished in the superficial layer. × 15,000.

FIGURE 12

Two yolk platelets within a blastula cell of the *Rana pipiens* embryo. Each main body shows a parallel band pattern, the spacing between bands being 69 A in the right body and 77 A in the left. An isolated area with a periodic pattern similar to that of the main body is present in the superficial layer of the left platelet. × 80,000.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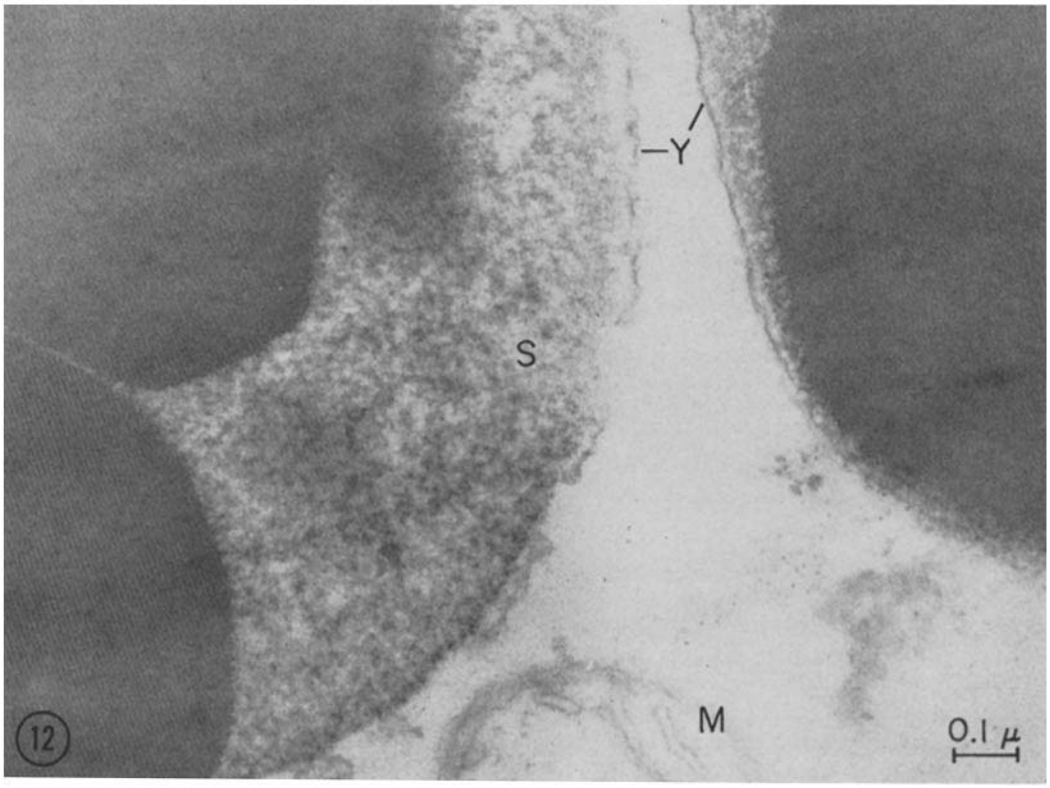
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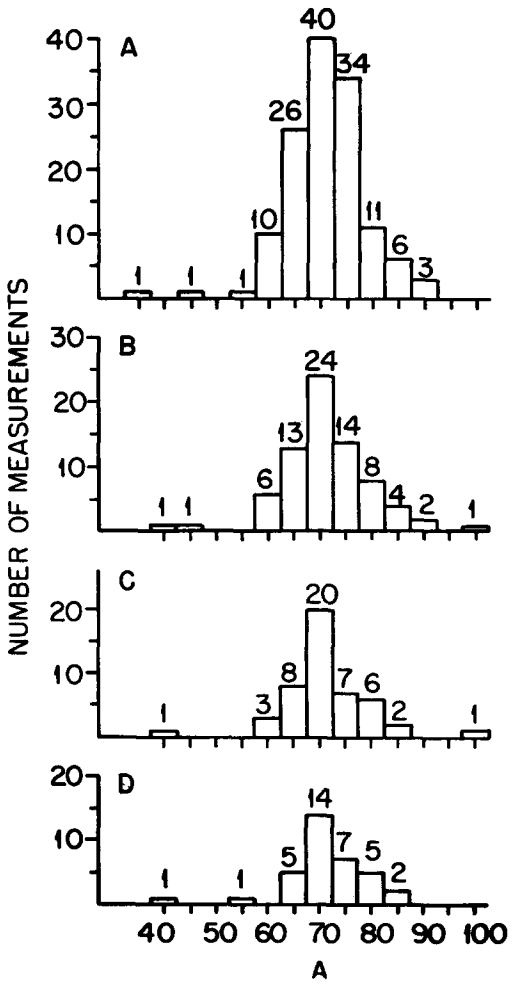


FIGURE 13

Frequency distribution of the spacing of periodic patterns in the main body of various amphibian yolk platelets. The average center-to-center distance from one dense line or one array of dots to the next was measured in each yolk platelet. A, oocyte and mature egg of *Rana pipiens* (average 70.9 A, 133 platelets). B, embryo of *Rana pipiens* (average 71.3 A, 74 platelets). C, embryo and oocyte of *Triturus pyrrhogaster* (average 71.1 A, 48 platelets). D, oocyte and embryo of *Diemictylus viridescens* (average 71.1 A, 35 platelets).

yolk by Honjin and Nakamura (13) has revealed that the repetitive unit for both fresh and formalin-fixed samples is 164 A, and that this value changes to 153 and 158 A, respectively, when the sample is dried. These x-ray diffraction values agree well with the values calculated from the combined electron microscopy and biochemical data, and thus may represent the length of a basic unit composed of two phosphoprotein and one lipoprotein molecules.

The above deliberations concerning the molecular architecture of the crystal have value only as a working hypothesis. Other physical and chemical studies are certainly needed to substantiate its relationship to the actual crystalline lattice.

In previous electron microscopic studies (16) of developing ectoderm cells, characteristic changes that yolk platelets undergo seem to be closely related to the progressive differentiation of the cells. One of the first changes to appear is a decrease in thickness and a subsequent disappearance of the superficial layer. The chemical and structural features of the superficial layer thus become of particular importance to the extent that they may be related to the first stages of embryonic differentiation. Lanzavecchia and Le Coultre (23) have indicated that the particles in the superficial layer are ribonucleoprotein granules, and suggest that the cytoplasm receives a supply of ribonucleoprotein when the platelets lose their superficial layer. This suggestion agrees with the frequent claim that the yolk fraction of the amphibian embryo contains an appreciable amount of RNA (8, 31, 37, 38, 46). In Epon-embedded cells, however, the diameter of particles or fibrils in the superficial layer (50 A) was appreciably smaller than that of the cytoplasmic particles of the ribosome type found in the same cells (100 to 250 A). Furthermore, recent cytochemical and chemical studies on yolk platelets (30, 44, 47) have provided no evidence for the presence of RNA in either the superficial layer or the main body. Rather, a localization of acid polysaccharide in the superficial layer is suggested (30).

Electron microscopy reveals certain similarities and differences between the components of the superficial layer and the main body. Although the resolution of electron micrographs did not usually permit a clear definition of components, it seems possible that similar protein components are present in both the main body and the superficial layer, since the boundary between these two areas frequently appeared to be transitional.

Finally, although Ringle and Gross (36) have thrown doubt on the existence of an outer membrane in the yolk platelets, the existence of such a structure can be clearly observed on the surface of the superficial layer in well preserved Epon-embedded cells. This membrane can be seen to

be a unit membrane on micrographs of the highest resolution. However, it is easily destroyed when other preparative procedures are employed, such as longer fixation with osmium tetroxide, longer dehydration in ethanol, or embedding with methacrylate.

### *Note*

Since this manuscript was written, Ward (50) has published an electron microscopic study on the origin of yolk platelets in the oocyte of *Rana pipiens*. His work indicates that the main body of the yolk platelet is composed of electron-opaque particles (35 to 40 A) arranged in a regular hexagonal array (spacing, 70 to 85 A). He also suggests the existence of the superficial layer consisting of a fibrous material. His observations are in essential agreement with the work reported here for a variety of species and developmental stages. Further comment on several conflicting points will be reserved for a future paper.

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