3. EXPERIMENTAL STUDIES ON BOTRYOCOCCUS COLONIES FROM HUNGARIAN UPPER TERTIARY OIL SHALE

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Abstract

In this contribution the results of the following new investigations are presented: 1. Symmetry operations on the partially degraded and fragmented colony of fossil *Botryococcus braunii*. Quasi-crystalloid biopolymer skeleton and quasi-equivalent highly organized units are observed to be present within the same fragment. Four pentagon biopolymer units were selected from the bordering zone of the two kinds of symmetries and their structure was studied by rotation method. These observations have revealed different relationships between quasi-crystalloid and quasi-equivalent biopolymer system. 2. Colonies were partially dissolved with diluted diethylamine and merkaptoethanol for 30, 60, 90, 120 and 150 days. Then the fragmented organic remnants were investigated with TEM method. This experiment dissolved in the first place the molecular system sensu strictu. Different results were observed when the colonies were partially degraded with concentrated and dilute organic solvents (diethylamine, merkaptoethanol).

Key words: Botryococcus, fossil, Upper Tertiary, Hungary, TEM, biopolymer structure.

Introduction

The alga *Botryococcus braunii* KÜTZ., generally occurring in fresh waters has been determined to possess a high hydrocarbon production potential. It has attracted the attention of various investigators with regard to the different aspects of studies (GUY-OHLSON, 1992, VÉR, 1994, BATTEN and GRENFELL 1996). TEM structure of fossil *Botryococcus* colonies isolated from Hungarian oil shale were published by KEDVES (1983). The oil reservoir function of the holes, present in the mucilage was also emphasized in this paper. Later, biopolymer structure and symmetry operations results were published (KEDVES 1986a,b, 1987, 1988, KEDVES, ROJIK and VÉR, 1991). The peculiarity of the regular basic pentagons was discussed (KEDVES, TÓTH and FARKAS, 1993). The radial rotation method was used for the first time on the fossil *Botryococcus* colonies (KEDVES, TÓTH and VÉR, 1993, 1995). The highly organized biopolymer units may be modelled with fullerenes. Quasi-periodic and quasi-equivalent biopolymer structures are present in the wall of the colonies of *Botryococcus braunii* KÜTZ. These two kinds of structures are completely different.

The aim of our new investigations are as follows:

1. To decipher the relationships between quasi-periodic and quasi-equivalent biopolymer structures.

2. To determine the effect of the long-duration partial dissolution with diluted diethylamine and merkaptoethanol on the material under study. These chemicals were used succesfully to degrade the recent pollen grains.

Materials and Methods

The *Botryococcus* colonies were isolated from the oil shale with HNO_3 aq. dil. The isolated colonies were washed carefully in distilled water, and were investigated with the LM and the TEM method. To discover the biopolymer structure of the fossil *Botryococcus* colonies we have used several chemicals. In this paper we are dealing with methods which were used for the present studies only.

1. Partial degradation and fragmentation of the *Botryococcus* colonies

Experiment No: 924. – 1 ml merkaptoethanol cc. was added to 10 mg of dried *Botryococcus* colonies. Material was then kept for 24^{hrs} at 30 °C temperature. After washing, the residues were fragmented with a magnetic stirrer for 30 minutes. The fragments were investigated on a TESLA BS-500 TEM (resolution 6 Å) at the J. A. University, Faculty of Science, Electron Microscope Laboratory.

The modified Markham rotation method was first published in 1989 (KEDVES) and several further methodological papers were published later.

2. Partial degradation with diluted organic solvents

5 ml distilled water and 0.2 ml organic solvent were added to 5 mg dried *Botryococcus* colonies. The dissolution was made in a thermostat at 30 °C.

No of experiment	Solvents		Length of time in days
	diethylamine	merkaptoethanol	
1895	+		30
1896		+	30
1897	+		60
1898		+	60
1899	+		90
1900		+	90
1901	+		120
1902		+	120
1903	+		150
1904		+	150

The washed and fragmented material was investigated with an OPTON EM-902 (resolution 2-3 Å), and on a TESLA BS-540 (resolution 5 Å).

Results

The ultrastructure of the fossil *Botryococcus braunii* KÜTZ. colonies from the Hungarian oil shale (Pula) was described in the paper of the senior author in 1983. On Plate 3.1. we publish again the TEM structure of the outer part of the non-experimental colony. The figure illustrates the mucilage with spongy ultrastructure, and four cupules. The wall of the cupula is more or less compact and possesses small holes.

Partially degraded and fragmented colonies are composed of large globular units. This peculiar biopolymer structure was first published by KEDVES, ROJIK and VÉR in 1991. Plate 3.2., figs. 1,2, represents the TEM picture of more or less complete bucky-ball-like units composed of further smaller elements, and the desintegration of the larger units (Plate 3.2., fig. 2). We observed on ultrathin sections cupula composed of large globular biopolymer units.

Plate 3.3. represents the partially degraded and fragmented part of the *Botryococcus* colony. The upper part of the fragment is composed of large buckyball-like globular units. The lower part is more desintegrated quasi-crystalloid skeleton. Regular basic pentagonal biopolymer units (No 1–3) were investigated by KEDVES, TÓTH and FARKAS (1993). Biopolymers IV and V were first used for radial rotation, as an introduced new method (KEDVES, TÓTH and VÉR, 1995).

The results of the recently rotated biopolymer units, which are at the bordering of the two kinds of symmetry structures of biopolymer units may be summarized as follows.

BIOPOLYMER VI

(Plate 3.3., plate 3.4., figs. 1–6, plate 3.5., figs. 1,2)

Primary rotations

C.P.5.A.5.5. (Plate 3.4., fig. 1)

Around the basic regular pentagon altogether four circles of points of symmetry appeared. Two are composed of light and another two of dark, positive units. The first "light pentagon" is a little darker than the basic biopolymer.

C.P.5.A.5.10. (Plate 3.4., fig. 2)

There are five surrounding circles of the basic, central dark circle. The points of symmetry of the outermost dark circle are interesting and between the ten dark units there are further units surrounding a peculiar rotation area.

Secondary rotations

C.S.$X_{+2/2}$.5.5. – C.P.5.A.5.5. (Plate 3.4., fig. 3)

An irregular light pentagonal area appeared, each apex of this is connected to a more or less "U shape" units. These are surrounded by a pentagonal outer rotation area.

C.S.$X_{+4/1}$.5.5. – C.P.5.A.5.5. (Plate 3.4., fig. 5)

Interesting is the dark regular circular area around the fivefold rotation centrum. This, is followed by an irregular light area, and the outermost is a more or less pentagonal rotation area.

C.S.$X_{+4/4}$.5.5. – C.P.5.A.5.5. (Plate 3.5., fig. 1)

Dark pentagonal field is surrounded by ten dark points of symmetry surrounded by another less dark pentagon. This is followed by light and dark zones, the outermost area is not so regular, pentagonal star form.

C.S.$X_{+2/2}$.5.5. – C.P.5.A.5.10 (Plate 3.4., fig. 4)

The rotation centrum is surrounded by a dark star forming area. This is followed by five light areas which enclose dark points of symmetries. Near these areas there are secondary dark fields which are connected to the central dark star forming area.

C.S.$X_{-3/3}$.5.5. – C.P.5.A.5.10. (Plate 3.4., fig. 6)

Around the rotation centrum it is a not so dark pentagon. The apices of this pentagon are connected to further radially oriented large regular fields. No rotation area appeared, but at the outermost part a dark regular pentagonal basic unit appeared. This regular unit may be embedded into the so-called fullerene-like biopolymer structure.

C.S.$X_{-5/7}$.5.5. – C.P.5.A.5.10. (Plate 3.5., fig. 2)

After this rotation a regular pentagonal unit appeared. Each edge of this unit appears to be radially elongated which is enclosed within a pentagonal area. At the sides there are further five dark biopolymer units. This characteristic area is followed by not so well characteristic light and dark pentagonal areas. The outermost rotation area is not well delineated.

BIOPOLYMER VII
(Plate 3.3., plate 3.5., figs. 3–6, plate 3.6., figs. 1–4)

Primary rotations

C.P.5.A.5.5. (Plate 3.5., fig. 3)

The disposition of the surrounding points of symmetries is not so regular. Five light points are on the sides of the central dark pentagon. Following the basic unit radially oriented dark and light points appeared. The outermost five dark points are very characteristic. The outermost rotation area is not well delineated.

C.P.5.A.5.10. (Plate 3.5., fig. 4)

The circles of the dark and light points of symmetries are not so characteristic. But the outermost rotation area is well delimited.

Secondary rotations

C.S.$X_{+4/1}$.5.5. – C.P.5.A.5.5. (Plate 3.5., fig. 5)

Around the rotation area a dark pentagonal field appeared. Light and dark points of symmetries are around this field surrounding a pentagon. The outermost dark points of symmetries of the pentagon are similar to the outermost circle of the primary rotation.

C.S.$X_{+4/2}$.5.5. – C.P.5.A.5.5. (Plate 3.6., fig. 1)

The rotation centrum is surrounded by a light pentagonal field. This is the difference from the previous rotation. The outermost dark points of symmetry surrounding a pentagon are essentially identical to the previous secondary rotation.

C.S.$X_{+4/3}$.5.5. – C.P.5.A.5.5. (Plate 3.6., fig. 3)

In contrast to the previous one the results of this rotation are quite different. A dark star is in a light circle surrounded by a light pentagon. This is a negative rotation area the limiting zone being irregular and zigzag.

C.S.$X_{+5/1}$.5.5. – C.P.5.A.5.10. (Plate 3.5., fig. 6)

Around the rotation centrum a not so well-defined dark pentagon may be recognized. This is surrounded by light and dark points forming circles. The outermost large rotation area is more or less circular. The dark circle is surrounded by five light areas of "L" or "T" shape.

C.S.$X_{+5/2}$.5.5. – C.P.5.A.5.10. (Plate 3.6., fig. 2)

The rotation centrum is surrounded by five light points of symmetry forming a regular pentagon. This is followed by five dark points of symmetry. The outermost area is not so characteristic but it is a perceptible pentagon.

C.S.$X_{+5/3}$.5.5. – C.P.5.A.5.10. (Plate 3.6., fig. 4)

The rotation centrum is surrounded by a dark pentagonal field. At the edges there are five light points of symmetry. Between the light points there are five half moon shaped fields. The outermost rotation area is a pentagon with dark points of symmetry at their edges.

BIOPOLYMER VIII

(Plate 3.3., plate 3.6., fig. 5, plate 3.7., figs. 1–6)

Primary rotations

C.P.5.A.5.5. (Plate 3.6., fig. 5)

This rotation picture represents well the symmetry situation of the bordering of the quasi-periodic and quasi-equivalent biopolymer structures. Around the centrum of the rotation there is a light circular field. Following this a light coloured star shaped area is observed which is constituted by five radially oriented triangles. Each apice of this star shaped area is connected to large dark biopolymer units. At the inner part of it is a light pentagon. In the middle of the sides of this light pentagon there exist further radially oriented protuberances which are essentially the apices of the light central star.

C.P.5.A.5.10. (Plate 3.6., fig. 6)

In the centrum exists a light circular field, surrounded by ten dark points of symmetry. This is followed by another lighter zone with ten radially oriented projections. A similar, but dark circle corresponds to the large dark units of the previous rotation picture. Finally a large outermost rotation zone, with ten lobes may be observed.

Secondary rotations

C.S.X.$_{+3/1}$.5.5. – C.P.5.A.5.5. (Plate 3.7., fig. 1)

This rotation did not reveal a so characteristic pentagon. Four curved large areas forming large unit and a not so characteristic fourth arms. This rotation may be characterized by the presence of irregular units.

C.S.X.$_{+3/2}$.5.5. – C.P.5.A.5.5. (Plate 3.7., fig. 3)

Altogether four pentagonal fields appeared as a result of this rotation. Two dark fields at the centre are surrounded by ten radially oriented dark fields. This is followed by a pentagon in the outermost region.

C.S.X.$_{+3/3}$.5.5. – C.P.5.A.5.5. (Plate 3.7., fig. 5)

Extremely obscure picture appeared after this kind of rotation. Around the centrum of the rotation a dark pentagon is surrounded by a lighter pentagonal field. There are large radially oriented star-shaped dark fields in the outermost region.

C.S.$X_{+3/2}$.5.5. – C.P.5.A.5.10. (Plate 3.7., fig. 2)

Around the rotation centrum a regular pentagon appeared which is connected with pentagons further, but these connections are by one of the edges and not by the sides. Probably, this is also one of the ways for the connections of the two kinds of biopolymer systems.

C.S.$X_{+3/5}$.5.5. – C.P.5.A.5.10. (Plate 3.7., fig. 4)

The rotation centrum is surrounded with one light pentagonal field. This is followed by a narrow light and dark pentagonal fields. The outermost dark field is composed of ten not so characteristic dark units.

C.S.$X_{+3/9}$.5.5. – C.P.5.A.5.10. (Plate 3.7., fig. 6)

This rotation revealed a light regular basic unit. Each sides are connected to further units which may represent a negative quasi-crystalloid system embedded in a periodic biopolymer structure.

BIOPOLYMER IX.
(Plate 3.3., plate 3.8., figs. 1–6, plate 3.9., figs. 1–3)

Remark. – The biopolymer units which were used for symmetry operations are a little away from the bordering of the quasi-crystalloid and quasi-periodic biopolymer structures.

C.P.5.A.5.5. (Plate 3.8., fig. 1)

The rotation centrum is surrounded by a light star-shaped field. Around this several light and dark points of symmetry appeared. At least six circles of alternating light and dark points may be observed. The outermost five dark points form a regular pentagon.

C.P.5.A.5.10. (Plate 3.8., fig. 2)

The large central light field is surrounded by ten dark points of symmetry. Near to this circle ten light points of symmetry appeared. This is followed by further ones. But the arrangement of the points of symmetry which are far from the centrum becomes by and by irregular.

Secondary rotations

C.S.$X_{5/1}$.5.5. – C.P.5.A.5.5. (Plate 3.8., fig. 3)

The most interesting result in this rotation is the appearance of a light pentagon surrounded by ten light points of symmetry. Several light and dark points of symmetry are around this field. The outermost rotation area is not so well-defined, but it forms a field of pentagon.

C.S.$X_{2/2}$.5.5. – C.P.5.A.5.5. (Plate 3.8., fig. 5, plate 3.9., fig. 3)

This negative network is also very important. The central pentagon is surrounded by tetragons, connected by sides, but the outermost rotation area is a pentagonal field. Within the quasi-periodic symmetry there are quasi-equivalent periodic units also.

C.S.$X_{5/3}$.5.5. – C.P.5.A.5.5. (Plate 3.9., fig. 1)

Negative regular pentagon appeared around the rotation centrum. This is followed by a dark not so regular pentagon. An irregular star-shaped light field represents the outermost rotation area.

C.S.$X_{2/1}$.5.5. – C.P.5.A.5.10. (Plate 3.8., fig. 4)

A dark regular pentagon appeared around the rotation centrum. This is followed by ten dark points of symmetry, which form another pentagon. Around this pentagon there is a light area and after this the disposition of the points of symmetry becomes irregular.

C.S.$X_{2/2}$.5.5. – C.P.5.A.5.10. (Plate 3.8., fig. 6)

A dark pentagon appeared in the centrum. The light star-like area is not so regular. The outermost rotation area is ten edged with irregular borderings.

C.S.$X_{2/3}$.5.5. – C.P.5.A.5.10. (Plate 3.9., fig. 2)

A dark area appeared after rotation composed probably of the points of symmetry. This is encircled by a light more or less pentagon composed of ten unequal points of symmetry. The outermost rotation area is irregular.

The results of the long-duration partial dissolution of the material with diluted diethylamine and merkaptoethanol may be summarized as follows: Experiments No: 1/7 – 1895 (Plate 3.10., fig. 1) and 1/7 – 1896 (Plate 3.10., fig. 2). It is clearly shown in the pictures, that from these experiments the biopolymer structures were not observed. But after 60 days of dissolution (Plate 3.10., figs. 3,4) with both solvents electron dense globular particles exhibiting different kinds of disposition were observed. Regular pentagons, linear chains and further regular and irregular patterns were observed. After 90 days of dissolution (Plate 3.10., figs. 5,6) the results are similar and/or identical to the previous one. Dissolution of material with diethylamine (Plate 3.10., fig. 7) for 120 days revealed a peculiar pattern of biopolymers. Dark globular units and peculiar alveolar system within the dimension of the biopolymers were observed. Further smaller units were also present within the molecular dimension sensu strictu. By the dissolution for 120 days with merkaptoethanol (Plate 3.10., figs. 8,9, plate 3.11., fig. 1) the ultrastructure of the pedunculus was also discovered. The originally channelled part of the wall of the pedunculus is in the state of desintegration on molecular level. Different kinds of biopolymer patterns were observed in this part also. Finally very complex molecular and biopolymer system was discovered after 150 days of dissolution (Plate 3.11., figs. 2–5).

Discussion and Conclusions

Based on symmetry operations during the present investigations we can establish the following.

1. The new results support the peculiarities of the quasi-crystalloid skeleton of the biopolymer system of the *Botryococcus braunii* colonies isolated from oil shale.
2. With regard to the relationships between the quasi-crystalloid and quasi-equivalent biopolymer structures the following results are worth mentioning:

2.1. The appearance of the larger units after fivefold rotation (Plate 3.4., fig. 4,6, plate 3.6., fig. 5, plate 3.7., figs. 1–6).

2.2. Presence of regular pentagonal biopolymer unit connected by one apex to the central pentagon (Plate 3.7., fig. 2).

2.3. Tetragonal unit being connected to the central regular pentagon by sides (Plate 3.8., fig. 5, plate 3.9., fig. 3).

It has been noticed that the use of concentrated organic solvents is better for the study of different kinds of biopolymer structures of the fossil *Botryococcus* colonies. It seems that for the fossil material in which the aromatization process is of higher degree the diluted solvents are not so effective but these can be used for the study of the recent less aromatized biopolymer structures.

Finally taking into consideration the previously obtained results on the biopolymer structures of the *Botryococcus braunii* colonies isolated from oil shale it is evident that further experiments are necessary to better understand the structure and organization of the molecular and biopolymer system of this complicated algal organism.

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Plate 3.1.

Detail of the ultrastructure of the non-experimental colony of *Botryococcus braunii* KÜTZ. isolated from Hungarian (Pula) Upper Tertiary oil shale. 15.000x. This negative (no: 3101) was used first in the paper of KEDVES 1983, Revue de Micropaléontologie 26, plate 1, fig. 7.

Plate 3.2.

Biopolymer structure of partially degraded and fragmented *Botryococcus braunii* KÜTZ. colonies.

1. More or less complete large buckyball-like globular biopolymer units. Experiment No: 925, negative no: 596, 500.000x.
2. Desintegrated large buckyball unit. The electron density of the smaller globular units are different. Linear and network-like arrangement was observed at these smaller units. Experiment No: 946, negative no: 999, 500.000x.

Plate 3.3.

TEM picture of partially degraded and fragmented *Botryococcus braunii* KÜTZ. colony. Lower part of the fragment is clearly a quasi-crystalloid biopolymer skeleton, composed of regular pentagons whereas the upper part is composed of large globular units, which may be modelled with fullerenes. The previously published biopolymer units are also enumerated (I–III, published in KEDVES, TÓTH and FARKAS, 1993, IV, V, published in KEDVES, TÓTH and VÉR, 1995). The recently investigated biopolymer units (VI–IX) are also indicated together with the globular units of the basic regular pentagon. Experiment No: 924, negative no: 586, 250.000.

Plate 3.4.

Biopolymer VI

1. Five-fold primary rotation picture. 500.000x.
2. Ten-fold primary rotation picture. 500.000x.

3–6. Secondary rotation pictures. 500.000x.

Plate 3.5.

Biopolymer VI

1,2. Secondary rotation pictures. 500.000x.

Biopolymer VII

3. Five-fold primary rotation picture. 500.000x.
4. Ten-fold primary rotation picture. 500.000x.

5,6. Secondary rotation pictures. 500.000x.

Plate 3.6.

Biopolymer VII

1–4. Secondary rotation pictures. 500.000x.

Biopolymer VIII

5. Five-fold primary rotation picture. 500.000x.
6. Ten-fold primary rotation picture. 500.000x.

Plate 3.7.

Biopolymer VIII

1–6. Secondary rotation pictures. 500.000x.

Plate 3.8.

Biopolymer IX

1. Five-fold primary rotation picture. 500.000x.
2. Ten-fold primary rotation picture. 500.000x.

3–6. Secondary rotation pictures. 500.000x.

Plate 3.9.

Biopolymer IX

1,2. Secondary rotation pictures. 500.000x.
3. Secondary rotation picture. 1,500.000x.

Plate 3.10.

Ultrastructure of partially dissolved *Botryococcus braunii* KÜTZ. colonies.

1. Experiment No: 1895, negative no: 3443, 250.000x.
2. Experiment No: 1896, negative no: 3454, 100.000x.
3. Experiment No: 1897, negative no: 3702, 25.000x.
4. Experiment No: 1898, negative no: 3707, 25.000x.
5. Experiment No: 1899, negative no: 4220, 250.000x.
6. Experiment No: 1900, negative no: 4224, 100.000x.
7. Experiment No: 1901, negative no: 4232, 1,000.000x.
8. Experiment No: 1902, negative no: 4234, 100.000x.
9. Experiment No: 1902, negative no: 4235, 250.000x.

Plate 3.11.

Ultrastructure of partially dissolved *Botryococcus braunii* KÜTZ. colonies.

1. Experiment No: 1902, negative no: 4236, 500.000x.
2. Experiment No: 1903, negative no: 4296, 250.000x.
3. Experiment No: 1903, negative no: 4297, 500.000x.
4. Experiment No: 1904, negative no: 4300, 100.000x.
5. Experiment No: 1904, negative no: 4301, 250.000x.

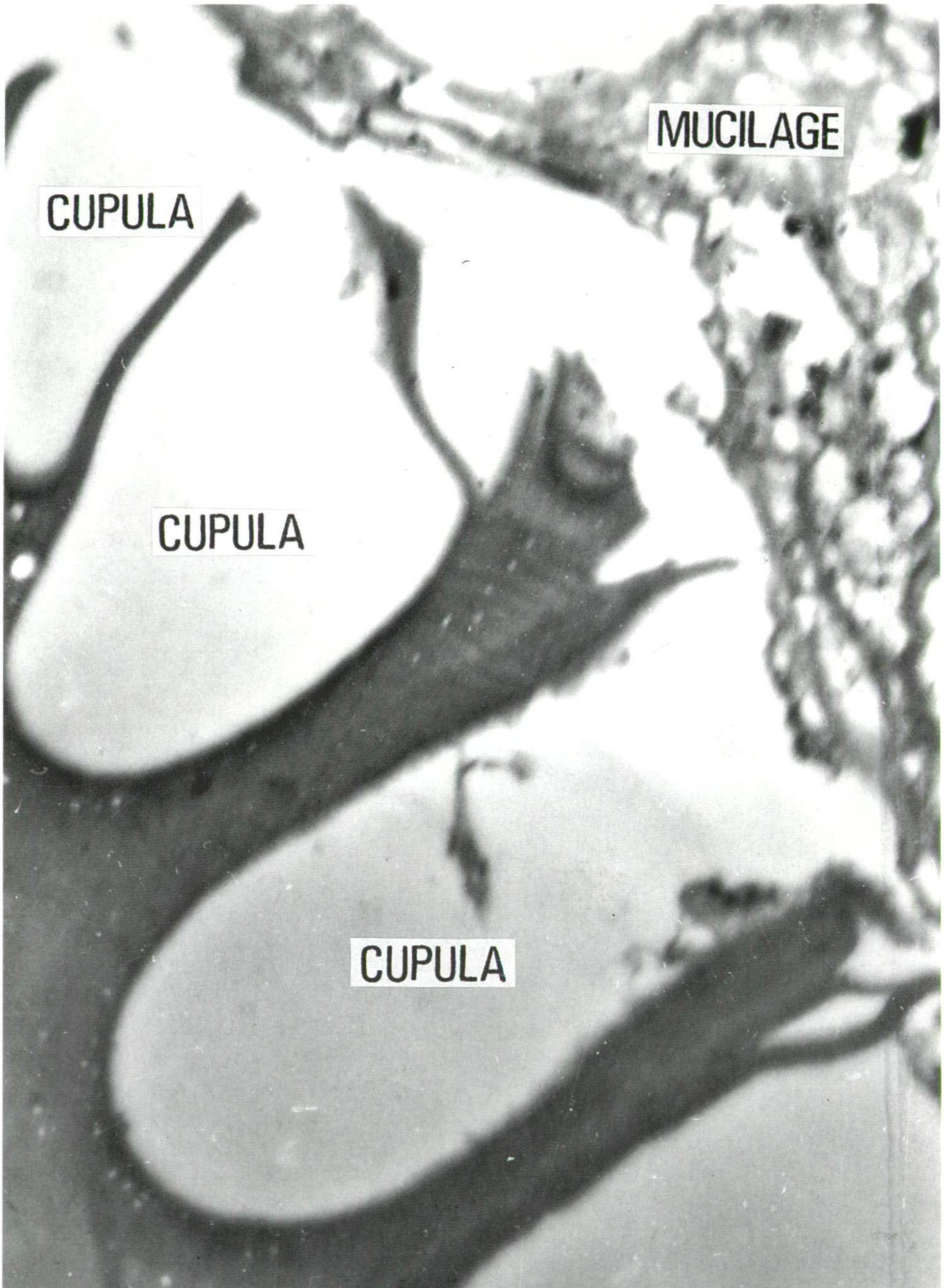


Plate 3.1.

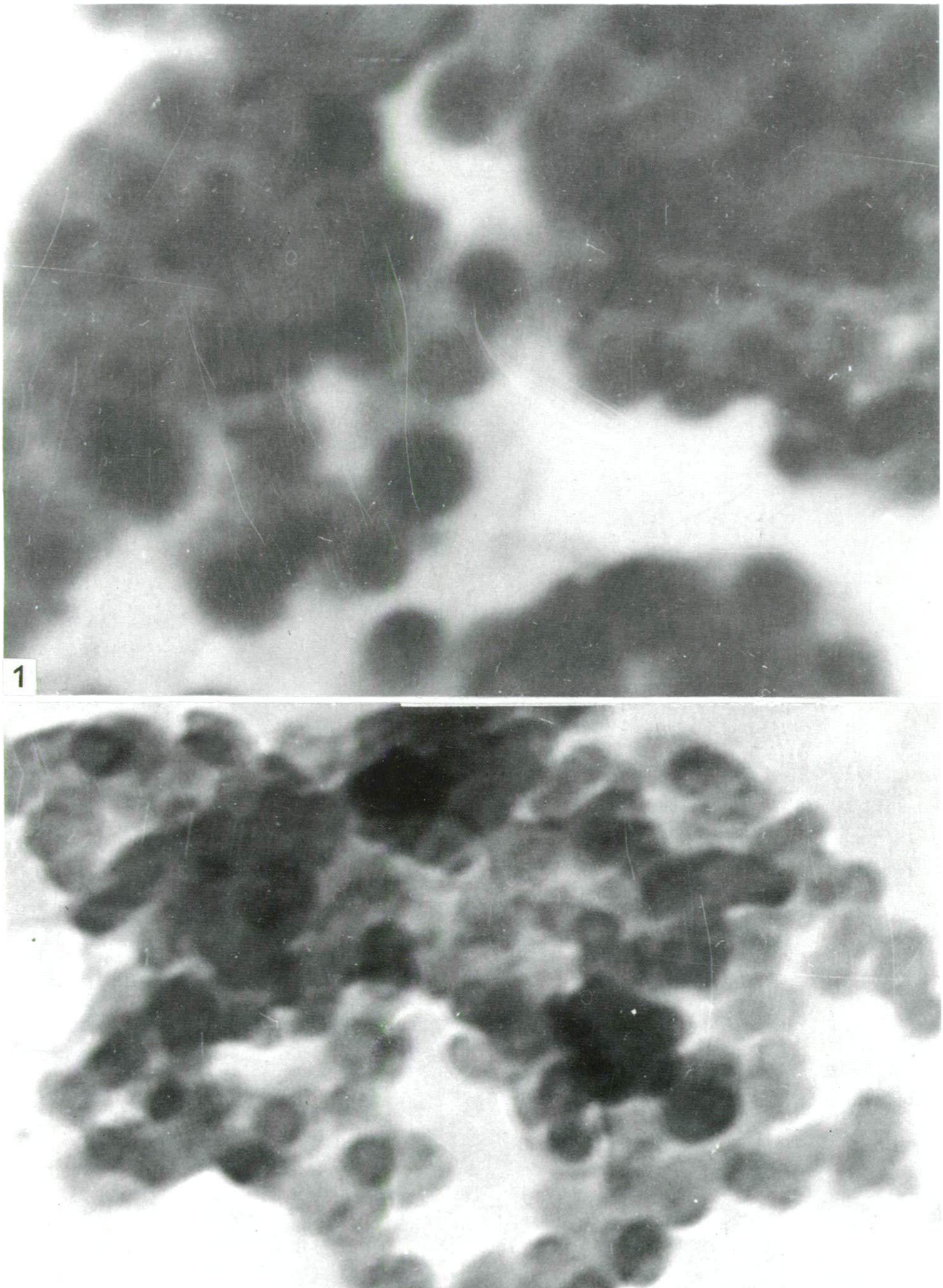


Plate 3.2.

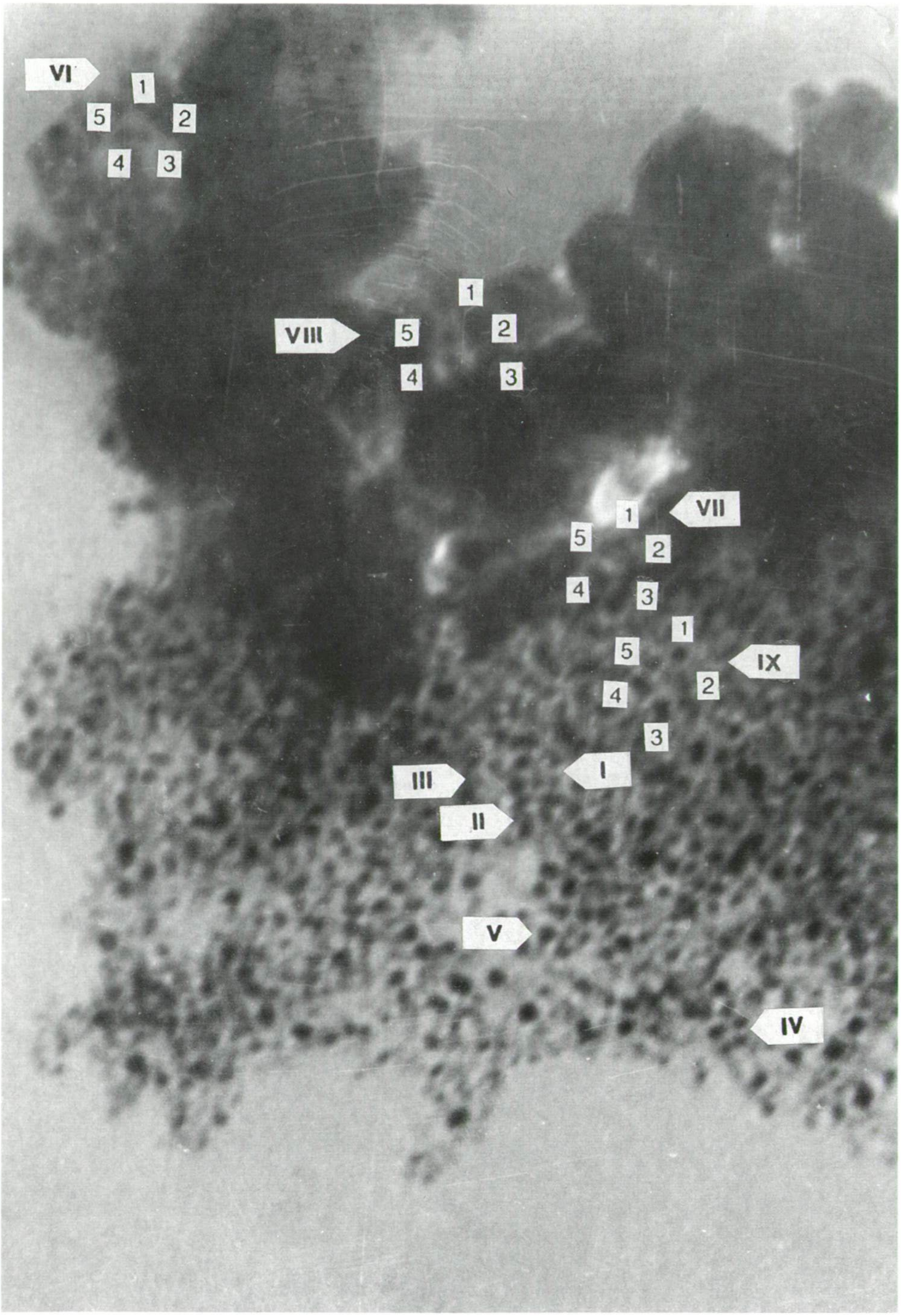
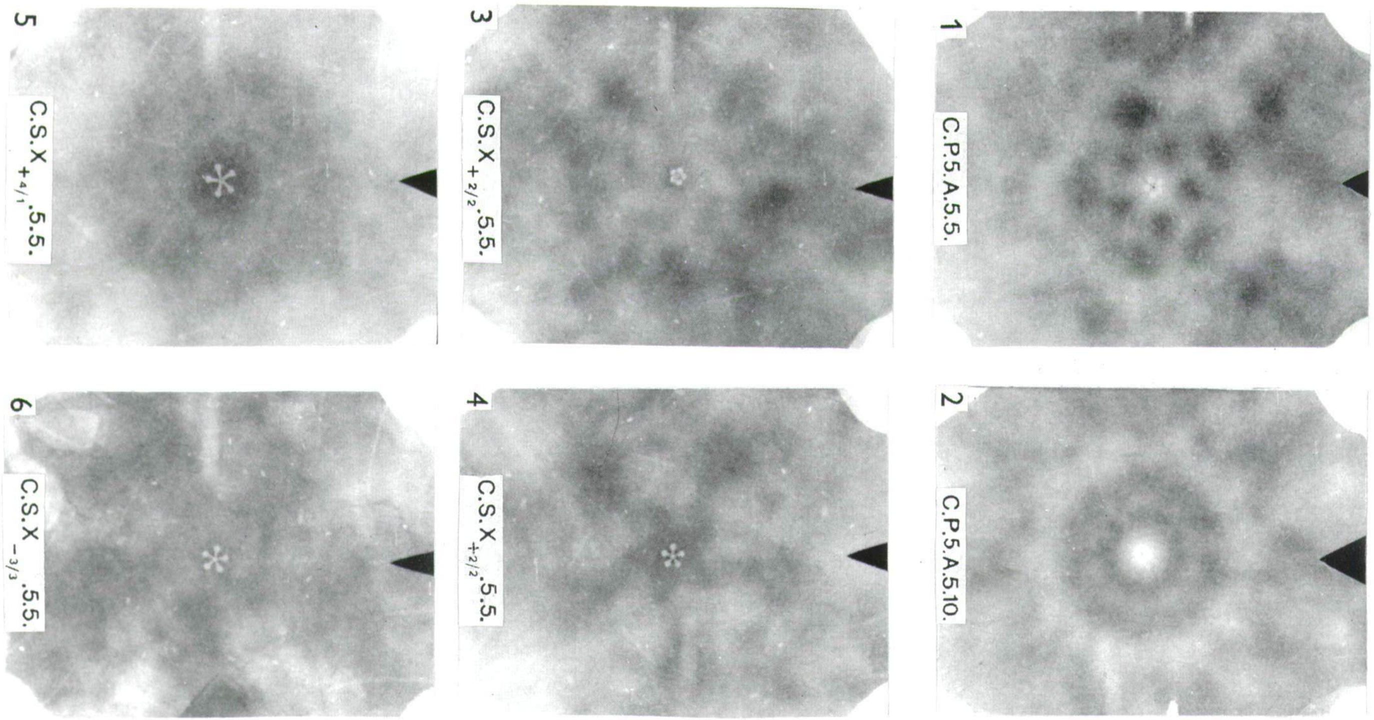


Plate 3.3.

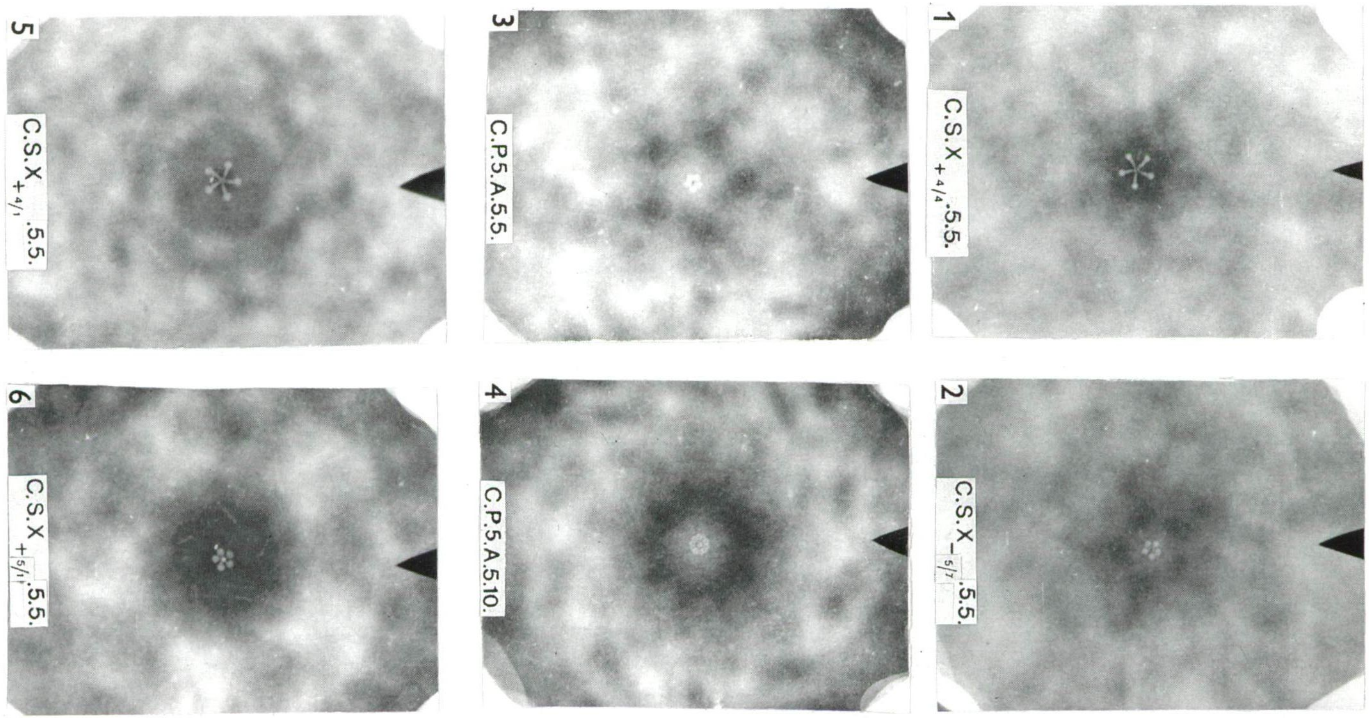
B I O P O L Y M E R VI



SECONDARY ROTATIONS PRIMARY ROTATIONS

Plate 3.4.

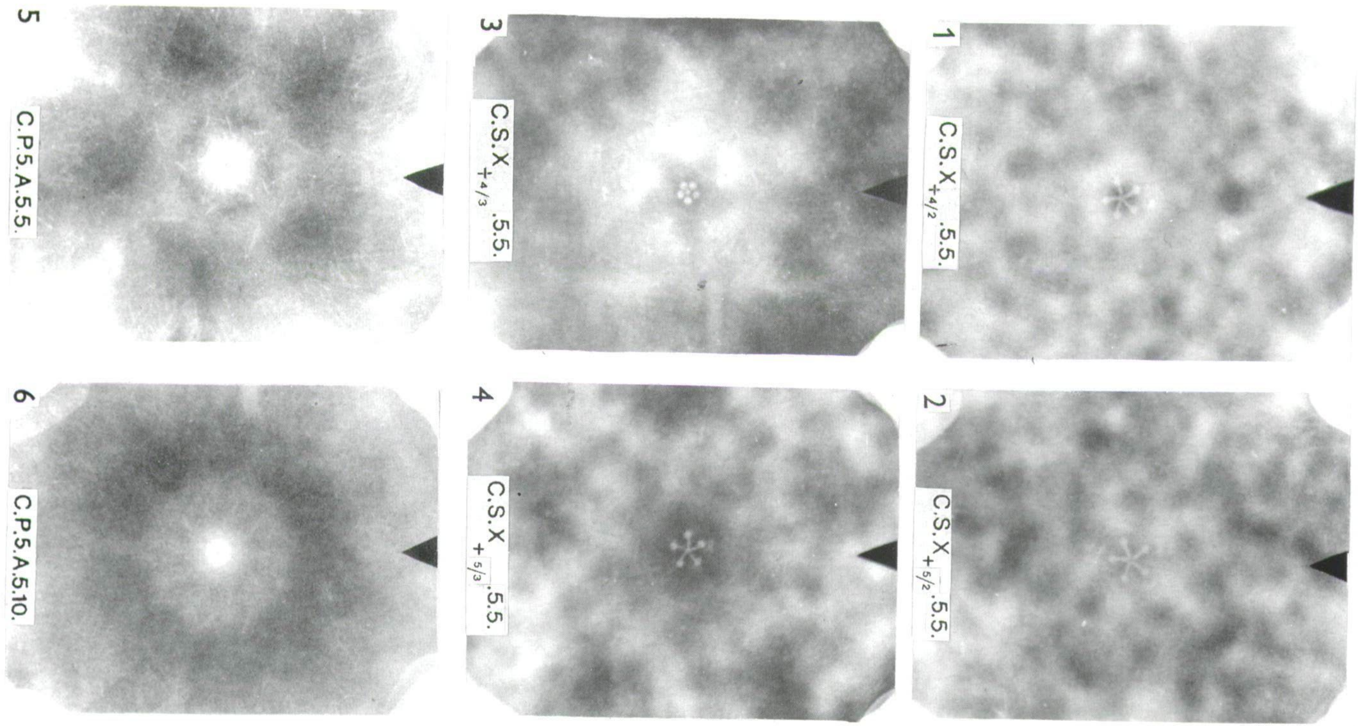
B I O P O L Y M E R VII BIOPOLYMER VI



SECONDARY ROTATIONS PRIMARY ROTATIONS SECONDARY ROTATIONS

Plate 3.5.

BIOPOLYMER VIII BIOPOLYMER VII



PRIMARY ROTATIONS SECONDARY ROTATIONS

Plate 3.6.

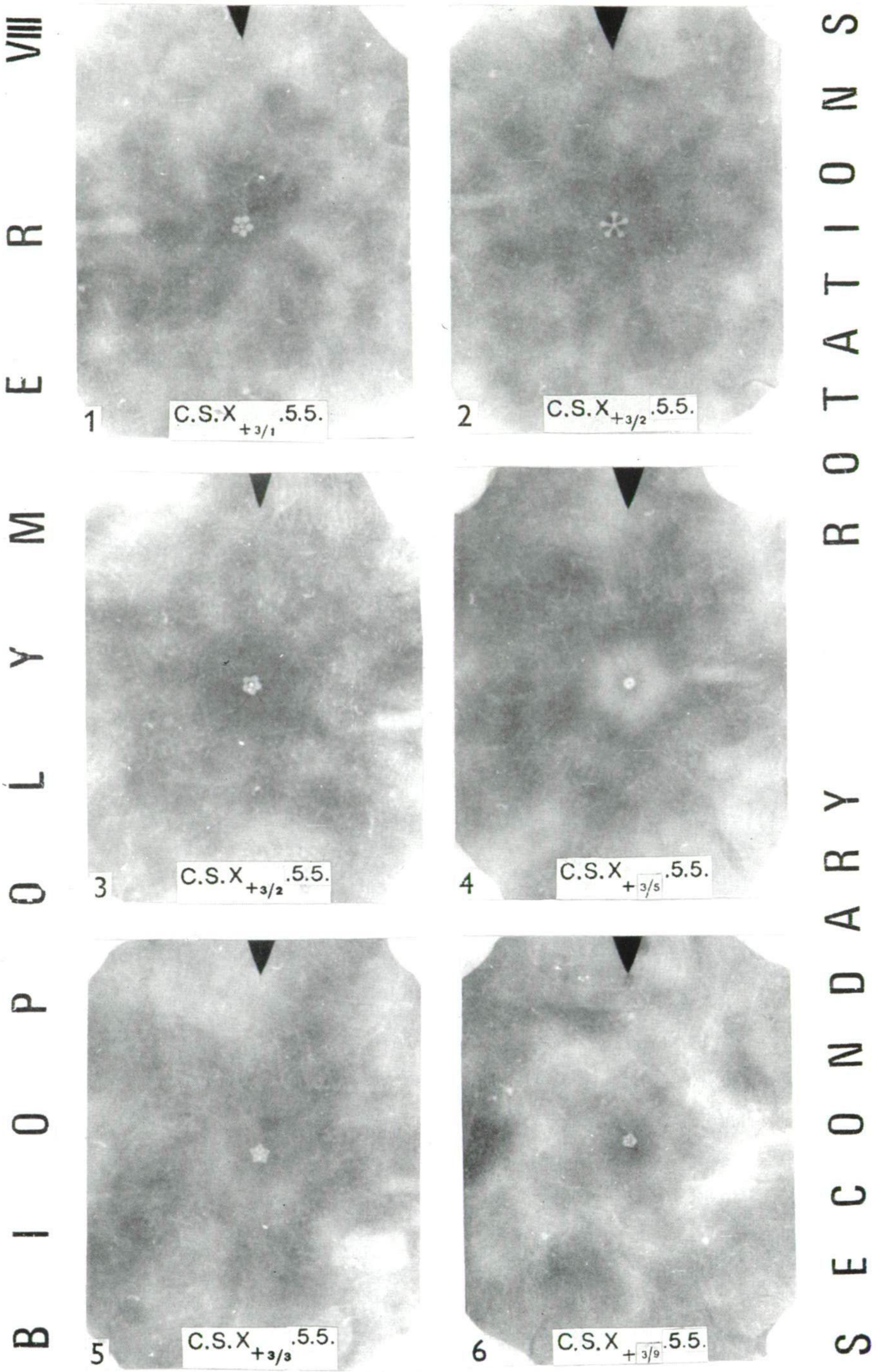
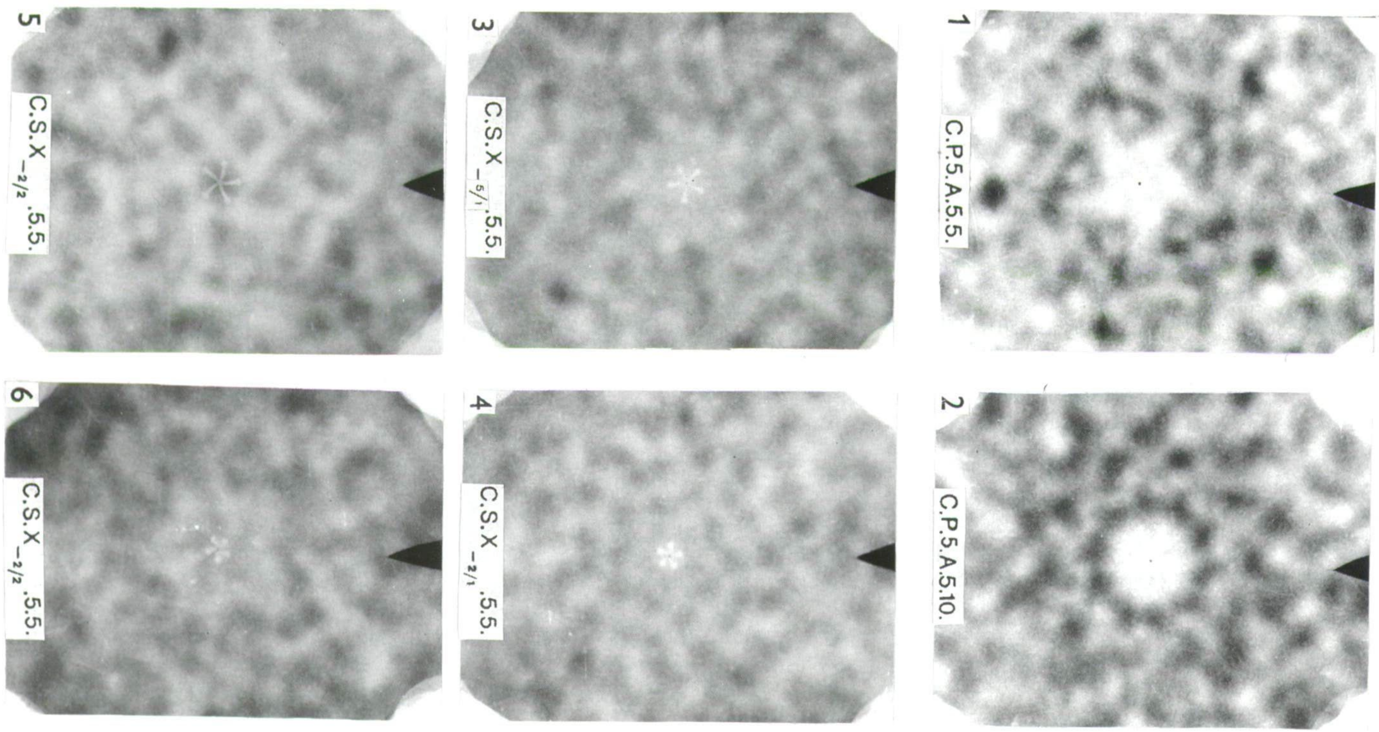

BIOPOLYMER VIII
SECONDARY ROTATIONS
1
C.S.X +3/1 .5.5.
2
C.S.X +3/2 .5.5.
3
C.S.X +3/2 .5.5.
4
C.S.X +3/5 .5.5.
5
C.S.X +3/3 .5.5.
6
C.S.X +3/9 .5.5.

Plate 3.7.

B I O P O L Y M E R IX



SECONDARY ROTATIONS PRIMARY ROTATIONS

Plate 3.8.

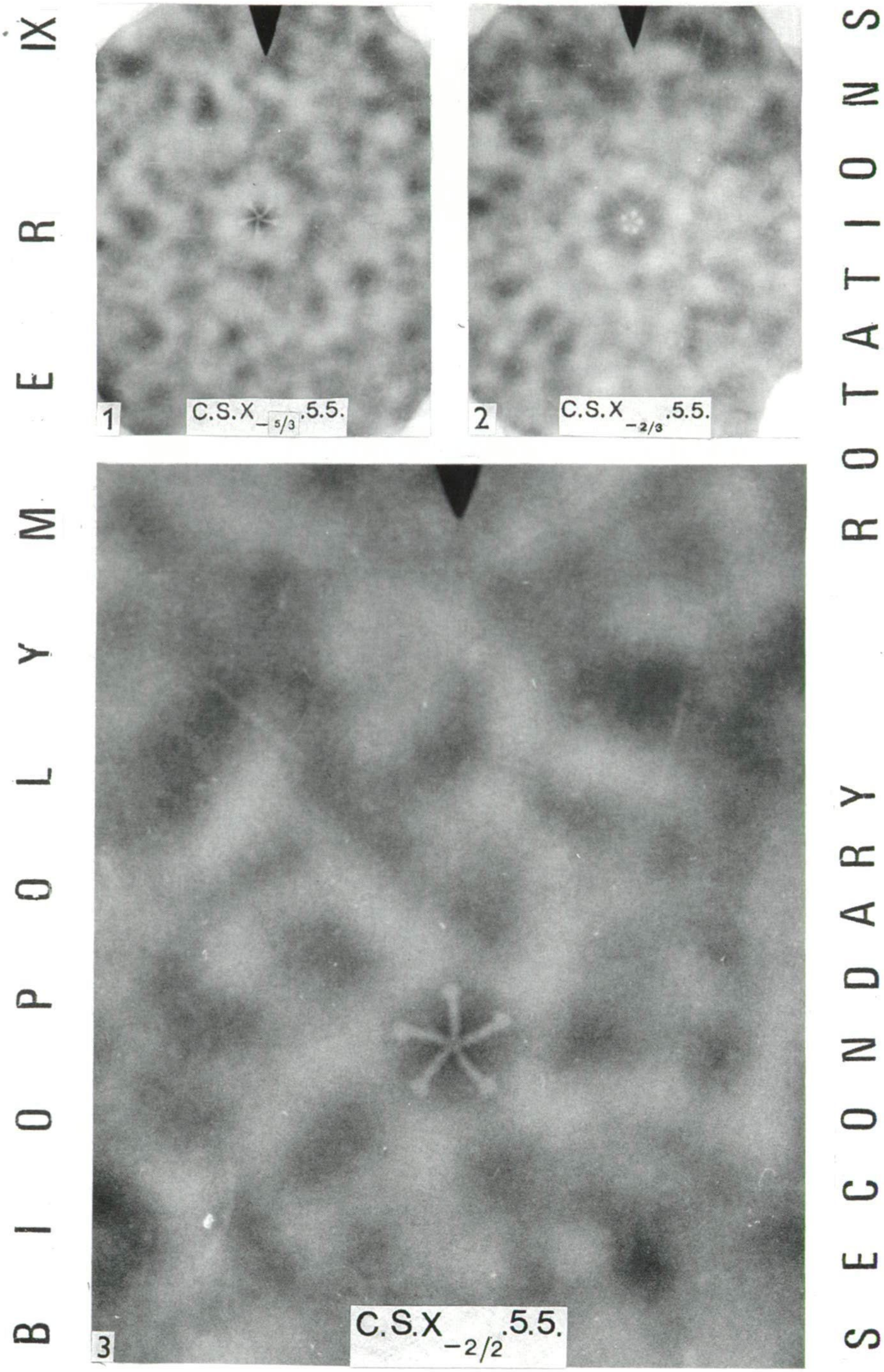

BIOPOLYMER IX
SECONDARY ROTATIONS
1
C.S.X −5/3 .5.5.
2
C.S.X −2/3 .5.5.
3
C.S.X −2/2 .5.5.

Plate 3.9.

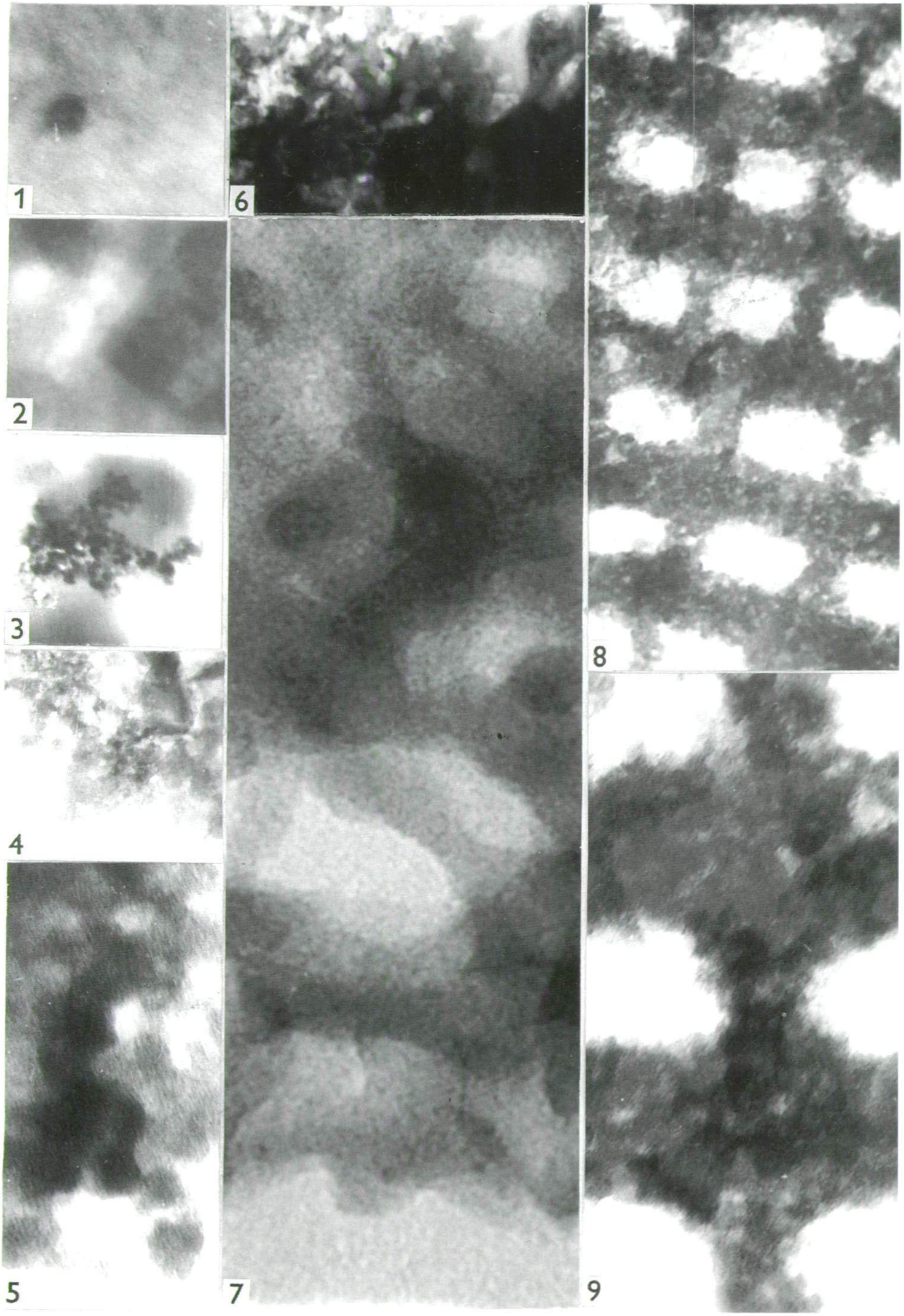


Plate 3.10.

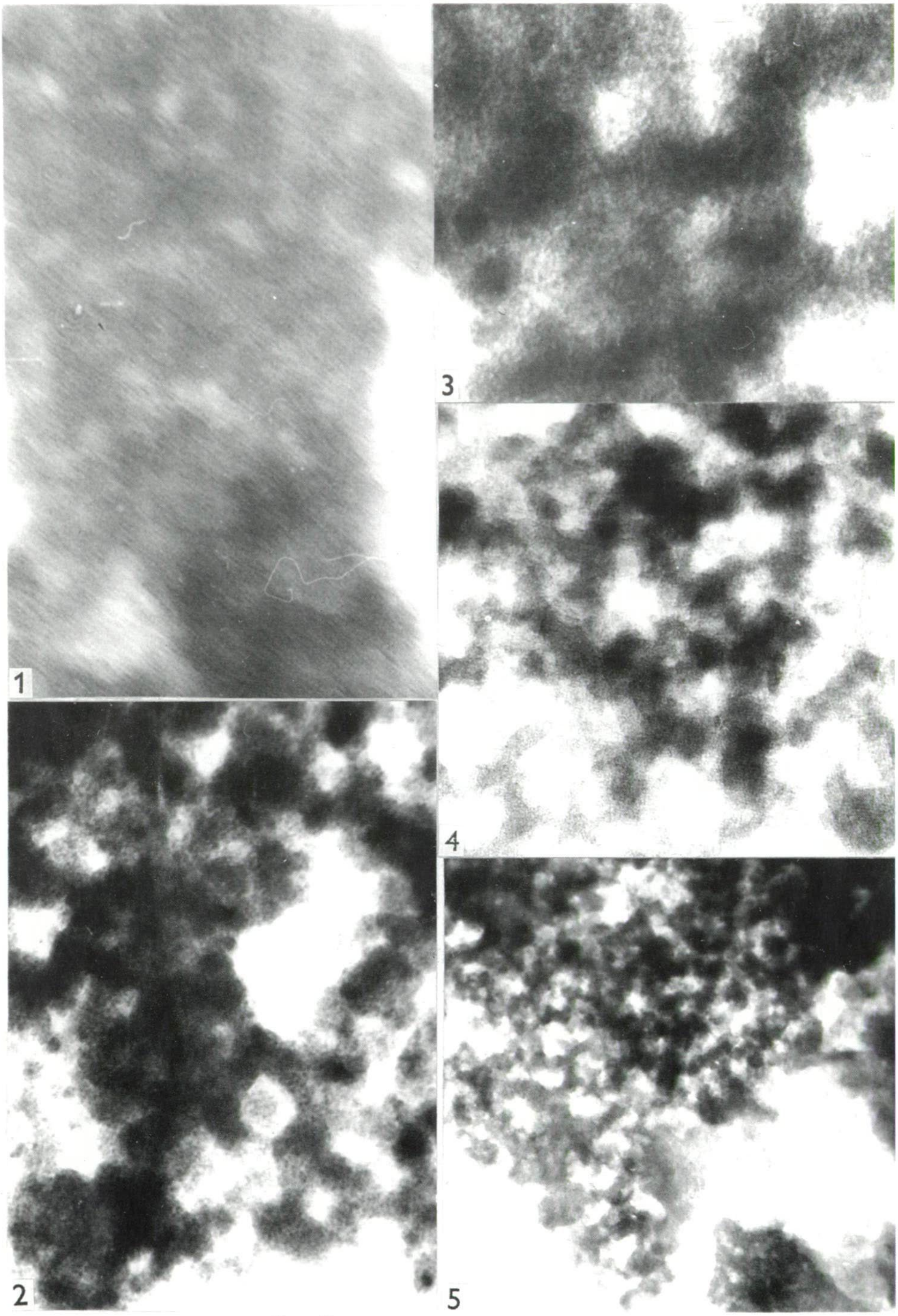


Plate 3.11.