(13) As the word "anti-biotics' symbolically exemplifies, modern medicine principally bases itself on a vast understanding of how to cleverly administer death just in the right place (apart from a few inevitable wrong places, called 'side-effects').

(14) Some excercises to develop a thinking of this kind are described in more detail in : Rudolf Steiner, Knowledge of the Higher Worlds and its Attainment, 1904.

Is the Copper Chloride Crystallization Method a Test for Detecting Formative Forces in Living Materials?

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Abstract

This work was undertaken to investigate whether or not crystallizations from aqueous Copper chloride solutions to which synthetic polymeric substances such as polyvinyl-pyrrolidone (PVP) has been added have similar effects on the crystal morphology of $CuCl_2$ as additions of plant juices, blood, or extracts from animal organs. PVP of various degrees of polymerization (K-values) have been applied. It was found that PVP K90 (which possesses the largest average molecular weight of all PVP species tested) yields $CuCl_2$ crystallization patterns which have all essential features of those which are obtained when adding substances to $CuCl_2$ solutions which are derived from living materials.

In further experiments, molecular weight measurements involving gel permeation chromatography have been conducted on several fresh as well as on aged vegetable juices. It was found that fresh plant juices have the highest weight average molecular weight and the largest polydispersity. These values decrease rapidly in the first three to four days of aging. A link between the results of these two types of experiments is made in the paper.

Introduction

It has been reported in the literature (1-4) that minute additions of plant juices, blood, or liquid extractions from animal organs to a 5% $CuCl_2$ aqueous solution would change, during the evaporation of the solution, the arrangement of the individual needles of the crystallized copper chloride. These additions yield a well coordinated, unified $CuCl_2$ pattern, whereas a control experiment of the same $CuCl_2$ solution without any additions of biological substances results in irregularly scattered (random) aggregates of needles, which have a large number of crystallization centers (Compare Figure 1 with Figure 2a). These unified patterns in which the needles are arranged in "dendritic bundles" (4) are observed to be structured right up to the boundaries of the test trays and have very often near the center a characteristic twirl around the nucleation site (Figures 2a and 4a). If, however, the plant juice (for example mashed leaves mixed with distilled water) has been aged for several days, many of the characteristic features of the $CuCl_2$ crystallization patterns that are typical for fresh plant juice have waned, that is, the uniform structure of the copper chloride needles fall apart into non-typical aggregates of needles (Figure 2b). The difference between $CuCl_2$ crystallization patterns of fresh and aged plant juices has lead to the speculation (4) that the copper chloride crystallization method is an extremely sensitive tool which reveals those forces which shape leaves or biological substances in nature. These processes are believed to deteriorate through aging or possibly other treatments. The statement above is said to be substantiated by the observation that inorganic, that is, "lifeless" substances, when added to a

$CuCl_2$ solution, do not yield the above mentioned unified crystallization patterns.

The most thorough and systematic study on this subject was published by M. Engquist (4) who presented in more than 200 photographs an impressive spectrum of $CuCl_2$ crystallizations of aqueous extractions of roots, leaves, flowers, seeds, flour and other biological substances in various stages of freshness or other conditions. Differences in the crystallization patterns are clearly visible.

The speculation that the $CuCl_2$ method is a sensitive test for the forces which give plants and other living materials their shape was essentially endorsed by the well known theoretical physicist Walter Heitler (4). He argues in a preface to Engquist's book (4) that the form of a plain crystal is mainly determined by physical laws which cause predictable and simple shapes. In contrast to this, the form of a leaf is neither random nor can it be derived by the laws of physics because the term "form" in the above mentioned definition is not contained in the terminology of physics. Physical laws act only from one point into the surrounding neighborhood and can therefore, according to Heitler, not create a well- coordinated, unified form. The forces which bring about the physical form of a plant are therefore called by Engquist, and many others (1-4), "formative forces" or "life forces" and are said to cause the above mentioned unified patterns in $CuCl_2$ crystallizations of living materials.

We have confirmed the observations made by Engquist and others in a limited number of plant juice experiments by varying several crucial experimental parameters. The main concern of this paper, however, deals with the question of whether

or not the well coordinated, unified copper chloride patterns could be also explained in a different way. To shed light on this we conducted two sets of experiments. First, we added to the $CuCl_2$ solutions a few milligrams of a synthetic organic polymer (polyvinylpyrrolidone, or PVP) which is available in a wide range of chain lengths, i.e. molecular weights, and which is derived from basic petrochemicals. Second, we measured the molecular weight distribution of fresh and aged plant juices by applying gel permeation chromatography. An interesting correlation between the results of these two types of experiments was found.

Experimental Procedure

The $CuCl_2$ crystallization method has been repeatedly described in the literature (1-4). In our case, 5 ml of a 5% aqueous solution of $CuCl_2 \cdot 2H_2O$ (a greenish-blue salt) is left for several hours at 35° C and about 40% relative humidity in a tray consisting of a carefully cleaned 10 x 10 cm^2 mirror glass plate on which a clean glass ring of 9 cm diameter is glued from the outside, using paraffin. Because of adhesion effects on the interior tray walls, the thin (1-2 mm) layer of liquid is usually concave so that the crystallization commences at the center of the plate and proceeds slowly to the perimeter. (In a few cases, probably because of some residual paraffin at the ring/plate interface, the crystallization also occurs simultaneously at several points on the perimeter.) After the primary dendritic growth, secondary crystallization usually takes place which produces, among other things, a finer pattern in the boundary region or in the remaining open spaces. The copper chloride crystallization

pictures shown in this paper were obtained by inserting the crystallization trays, like a photographic negative, into a dark room enlarger. The white areas in the photographic prints thus obtained are an image of the $CuCl_2$ needles.

The water-rinsed plant parts were crushed by mortar and pestle, or in some cases in a food processor. Subsequently, 10 parts of distilled water per 1 part of juice was added. After about 15 minutes the solution was filtered through a disposable milipore filter with the application of a moderate vacuum. (Since the pore width of the filters was 0.45 mm, large molecules and colloid size components could have been held back.) The plant juice thus obtained was either immediately added to a 5% $CuCl_2$ solution (about 2.2 mg per 5 ml tray) or stored in a sterilized, covered beaker for several days, in preparation for future experiments.

Polyvinylpyrrolidone (PVP) is a synthetic petrochemical which is synthesized from acetylene and formaldehyde. It is readily soluble in water and consists, as other polymers, of molecules with no uniform molecular weight but with a characteristic width of the molecular weight distribution (polydispersity). The properties of the pharmaceutical grade PVP used for our study are listed in Table 1. For the experiments reported below, about 2.2 mg PVP were added per 5 ml copper chloride tray.

Table 1

Physical Properties of Polyvinylpyrrolidone used in the present experiments (5)

Average degree of polymerization (K-value)	Weight average molecular weight $\bar{M}_w$ (6)
K 15	7,000
K 30	43,000
K 90	1,249,000

Gel permeation chromatography (GPC) is a technique which optically identifies molecules of varying molecular sizes. The diluted plant juice is run through a column whose packing consists of gels which have different pore sizes. The smaller the molecules of the test solution the greater is the number of pores available for permeation and hence, the longer is their residence time in the column. GPC gives a molecular weight distribution (rather than a single molecular weight)(6). The instrument used for this study was a Perkin Elmer Series 3B with a TSBKW 6000 PWXL column which has been previously calibrated with polyethylenoxide. The carrier solvent through the column ("eluting solvent") was a 50% methanol solution which diluted the test liquid about 20 (and in some cases 40) times.

Results and Discussion

Figures 3a-c depict copper chloride crystallization images which were obtained by adding three types of PVP having different degrees of polymerization (K-values) to a 5% $CuCl_2$ solution before drying.

When adding PVP K15 a large number of primary nucleation sites (approximately 20-30) can be observed (Figure 3a). This number is, however, considerably smaller than that seen for $CuCl_2$ crystallizations without additions (more than 100 in Figure 1). The needles are relatively long, unobstructed, and occasionally end in fine-structured, secondary crystallizations. It appears that the added polymer had some inhibiting effects on the nucleation of the $CuCl_2$ solution.

The crystallization image depicted in Figure 3b was obtained by adding the more highly polymerized PVP K30 to $CuCl_2$. The number of primary nucleation sites is reduced compared to Figure 3a (about 8-12 nucleation sites). We observe long, unobstructed crystals as well as places where short needles emanate, starlike, from a given center. The zone on the periphery is finely-structured and completely filled. The general appearance is somewhat denser than in Figure 3a.

Finally, PVP K90 causes the number of nucleation sites during the $CuCl_2$ crystallization to be reduced to essentially only one (Figure 3c). The characteristic twirl-like appearance, as known from crystallizations of fresh plant juices (Figure 2a) is clearly visible (See also Figures 4a and 4b). The secondary crystallization along the spear-like needles and those at the end point of these shoots make the overall picture appear the densest of the three images depicted here. Figure 3c has the appearance of a unified and well-coordinated pattern.

The results presented here suggest that the characteristic unified crystallization picture, as shown in Figure 2, is not limited to plant juices but can also be observed by adding synthetic polymers to the $CuCl_2$ solution. It is noted that

the resemblance of the crystal morphology of the $CuCl_2$ patterns with additions of PVP becomes closer to the patterns with plant juice additives when the molecular weight (or the viscosity) of the synthetic polymer is higher. It appears that additions of high polymeric substances, (that is, substances of high viscosity) delay the crystallization process so that eventually only one crystallization center remains which is located near the point where the thickness of the $CuCl_2$ solution was smallest. It is therefore suggested that the gradual reduction of a unified pattern during aging of plants or plant juices (as for example shown in Figure 2) is the result of a gradual reduction of the chain lengths of the organic polymers which are found in plant extracts. In order to test this hypothesis, the following experiments were conducted.

Three different types of vegetable juices, obtained from carrots, lettuce, and cauliflower were subjected to molecular weight measurements immediately after juicing, and again after certain time intervals up to 20 days. The results are shown in Figure 5. It is observed that a sharp decrease in the weight average molecular weight ($\bar{M}_w$) (6) occurs during the first 3 to 4 days of aging, after which $\bar{M}_w$ decreases more slowly. It is interesting to note that lettuce juice apparently degrades the fastest, whereas cauliflower had the smallest reduction in $\bar{M}_w$ over the time period chosen.

The distribution of the molecular weights of the polymers, that is, the polydispersity (6) was also determined. It is observed that the difference between large and small polymeric chains is most pronounced immediately after juicing and rapidly becomes smaller after aging (Figure 6). This again is interpreted to mean

that the long polymeric chains in plant juices break down into smaller pieces due to aging. The polydispersity difference is seen to be largest for lettuce juice.

It is known that plant extracts contain natural polymeric materials such as starch, sugar, or enzymes. These long chain molecules are likely to be broken into smaller molecules by degradation processes such as oxidation or enzyme reactions. The observations presented in Figures 5 and 6 are therefore believed to be in accord with the current understanding.

Conclusions

The experiments presented here clearly demonstrate that certain synthetic polymers which are not derived from living materials cause unified copper chloride crystallization patterns, which possess all essential features of those known from similar experiments involving plant juices, blood, or organ extracts. The characteristic features of these pictures, such as the number of nucleation sites, a distinguished twirl around a given nucleation site, (Figure 4) the frequency of secondary nucleations, and a well-coordinated appearance of the entire crystal morphology are linked to the average molecular weight of the polymers involved. The larger the average molecular weight (i.e. the viscosity) of the polymers, the more unified a copper chloride crystallization seems to be. It is concluded that polymeric materials have an inhibiting effect on the crystallization process which causes nucleation and growth to commence from one site only.

The contention that the copper chloride crystallization technique provides a decisive test for the existence and the deterioration of

formative forces in living material, as proposed in the literature, is not supported by the present experiments.

Acknowledgements

I am indebted to Mr. Larry Seiks for performing the $CuCl_2$ crystallizations, and to Ms. Mingrong Ma and Mr. Satoshi Sakurai for the molecular weight measurements. Mrs. Magda Engquist has kindly permitted the reproduction of Figures 2 and 4a.

Figure Captions

(SEE CENTERFOLD)

Figure 1 $CuCl_2$ crystallization without additions (5% solution).

Figure 2 $CuCl_2$ crystallization of 5% solution to which (a) fresh, diluted carrot juice was added; (b) same as in (a) above, but the juice was aged for 15 days after pressing (4).

Figure 3 $CuCl_2$ crystallizations of 5% solution to which 2.2 mg PVP was added per 5 ml copper chloride tray. (a) K15; (b) K30; (c) K90. (See Table 1.)

Figure 4 (a) $CuCl_2$ crystallization of pine seed extract (4).
(b) Center area of Figure 3c.

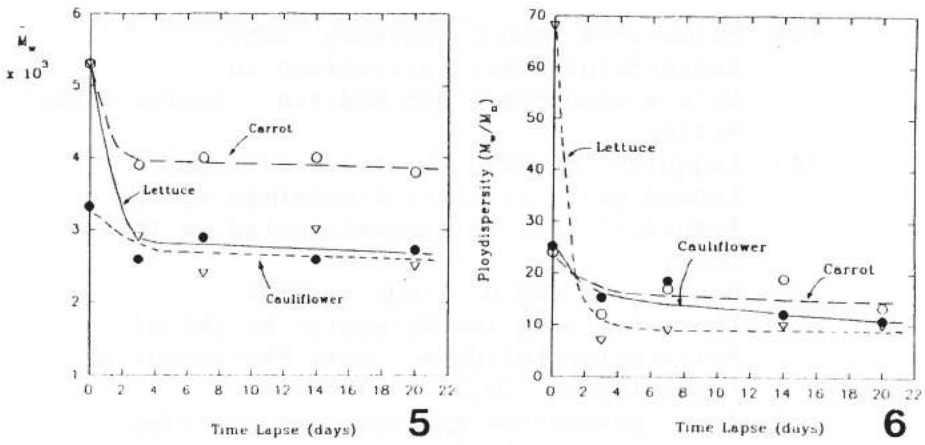

Figure 5 Weight average molecular weight (6) of three types of diluted vegetable juices (carrot, lettuce, cauliflower) as a function of aging time as obtained by gel permeation chromatography.

Figure 6 Polydipersity (6) of three types of dilute vegetable juices as a function of aging time as obtained by gel permeation chromatography.

References and Footnotes

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(5) Bühler, V. and U. Klodwig. 1984. Characterizing the Molecular Weight of Polyvinylpyrrolidone. Acta Pharmaceutica Technologica, 30,317.
(6) A gel permeation chromatogram provides essentially a bell-shaped curve in which the detector response is plotted versus the residence time of the test liquid in the column. The chromatogram is sliced into a large number of vertical segments (time slicing). The weight average molecular weight is defined as:

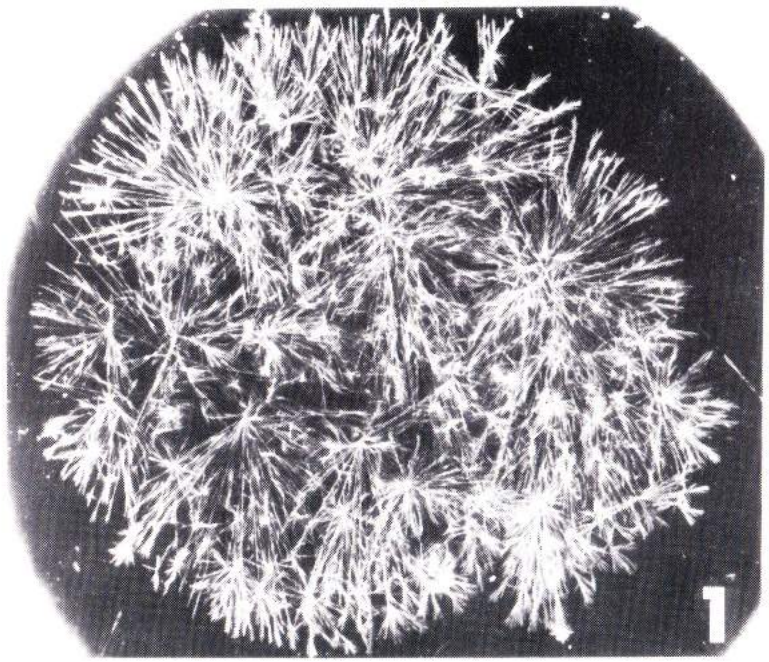
$$\overline{M_w} = \frac{\sum_{i=1}^{n} A_i M_i}{\sum_{i=1}^{n} A_i}$$

where n is the number of slices taken over the relevant data, A_i is the corrected area of the i^{th} slice, and M_i is the molecular weight at the midpoint of the i^{th} slice.

The number average molecular weight is defined as:

$$\overline{M_n} = \frac{\sum_{i=1}^{n} A_i}{\sum_{i=1}^{n} \frac{A_i}{M_i}}$$

The polydipersity is defined as the ratio between $\bar{M}_w$ and $\bar{M}_n$.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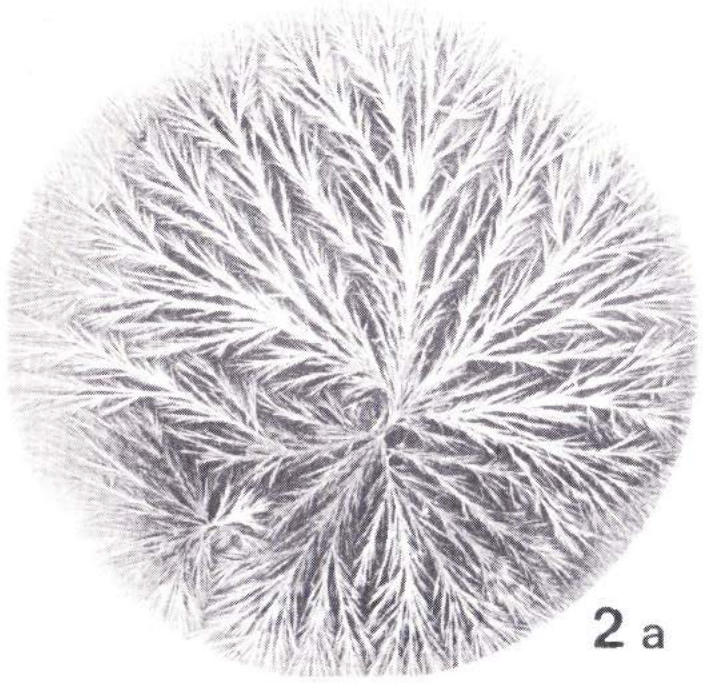
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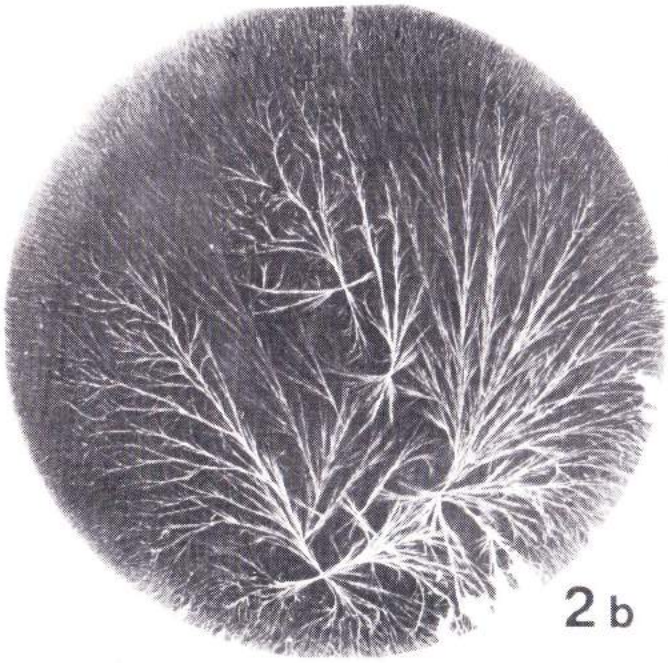

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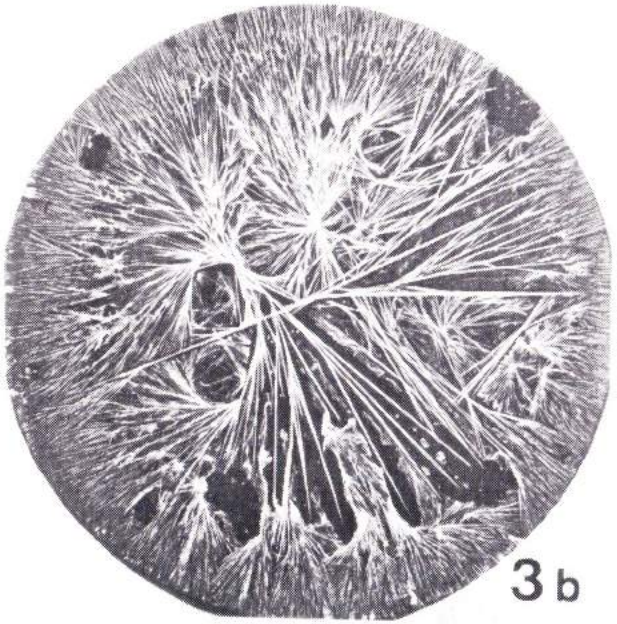

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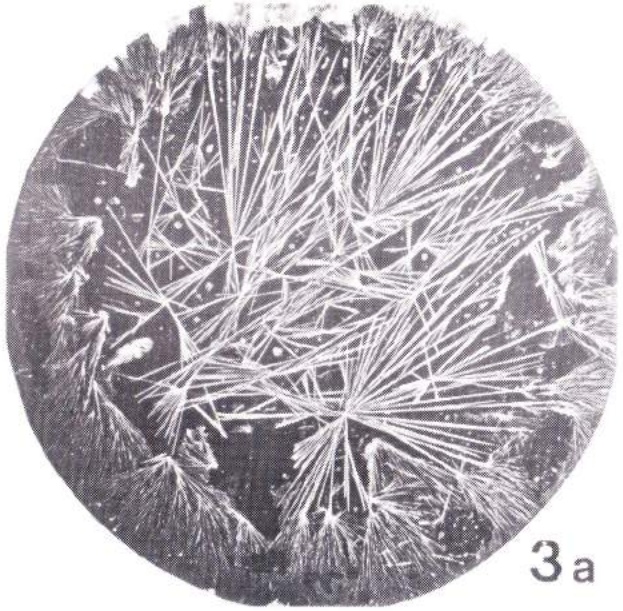

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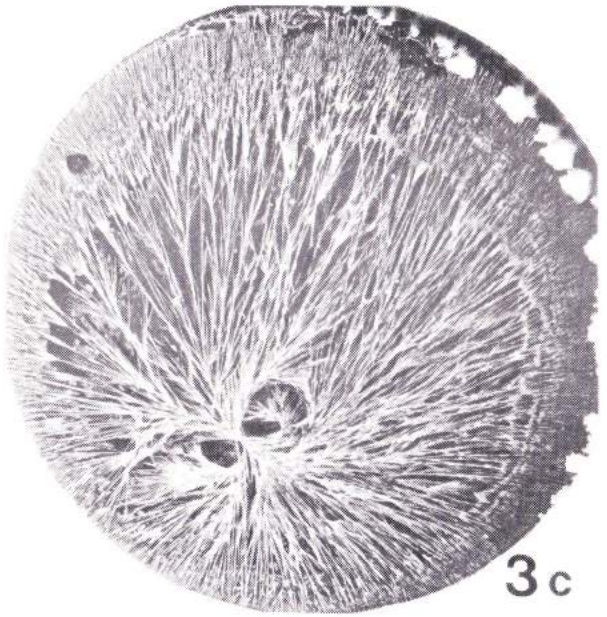

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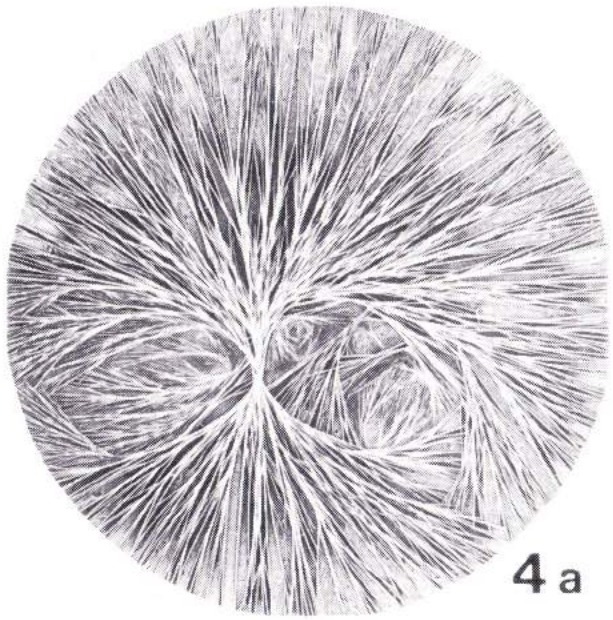

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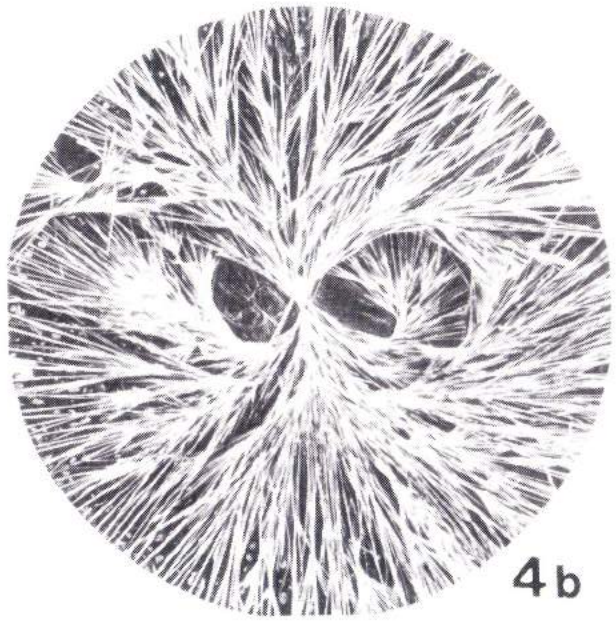

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