Experimental studies on protein spindles

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INTRODUCTION

Amorphous and crystalloid protein bodies are to be found in the cytoplasm and the nucleus of different organs of numerous lower and higher plants. The shape and the structure of such protein bodies are very diverse.

The protein spindles, however, belong in respect to their structure, their origin, and their role inside the cell, to a distinctly separate group of protein bodies. Contrary to other bodies they are found in few plants only. According to G i c k l h o r n (1913) they were found in 6 families (about 30 species, mostly *Cactaceae*). Since then this number has not increased greatly.

The first papers on protein spindles (C h m i e l e w s k y 1887, Molisch 1885, Mikosh 1890) dealt mainly with the description of the spindles, the demonstration of their protein nature, and their role in the cell. Molisch (1885) thought that these spindles are reserve proteins, while Glicklhorn (1913) was convinced that they are a product of metabolism. Recently, due to the work of K ü s t e r (1948) and Weber and his collaborators (Weber 1951, 1952, Weber and Kenda 1952, Weber, Kenda and Thaler 1952, Weber and Kenda 1953, Rosenzopf 1951 and others) new light was thrown on protein spindles. It was confirmed that they contain RNA and are probably virus bodies which can be found only in infected plants; similarly inclusions are found in tobacco plants infected by tobacco mosaic virus (Bawden 1950, Rosenzopf 1951). It was pointed out that in structure and shape protein spindles resembled mitotic spindles, and that both these structures were similar to protein liquid crystals. They were also compared to tactoids (Bernal 1947, Östergren 1949, Freundlich 1927, Weber 1951). Shrader (1951) suggests that the elucidation of protein spindle structure may throw much light on the structure of the mitotic spindle.

In the present investigation the authoress was mostly interested in 3 problems:

- 1. types of protein spindles in Peireskiopsis,
- 2. influence of different chemical and physical factors on their shape and structure.
- 3. comparison of the influence of these factors on protein spindles with their action on mitotic spindle.

MATERIAL AND METHODS

As a material the upper epidermis of *Peireskiopsis spathulata* (O t t o) B r i t t. and R o s e from the Botanical Garden of the Jagellonian University was used. Observations were made during 8 monthes, from August till March. A phase contrast microscope, polarizing microscope and dark field illumination were used. Extreme temperatures were applied with the equipment described in a previous paper (B a j e r and M o l è - B a j e r 1953). The partly schematic drawings were done during observations by means of a drawing prism. The influence of each factor was examined several times on numerous, even up to several hundred, spindles and during the investigations about 4000 spindles were experimented with.

OBSERVATIONS

Protein spindles are numerous in the *Peireskiopsis* epidermis, though their number varies in leaves of the same age and coming from one plant. They usually are found in agglomerations at considerable intervals and mostly near the base of the main leaf nerve. In each agglomeration one or, rarely, two spindles may be found in almost each cell. Single spindles may be also found, dispersed throughout the leaf. Spindles are found both in chlorotic and healthy parts of the leaves. In stomata guard cells no spindles have been found which is in agreement with the observations of other writers (W e b e r 1951, W e b e r and K e n d a 1952). The dimensions of spindles are not dependent on cell dimensions i. e. in small cells large spindles have been observed, and vice versa, which is in disaccord with observations of W e b e r, K e n d a and T h a l e r (1952) on *Rhipsalis*.

In respect to shape and structure at least 5 types (a-e) of protein spindles may be distinguished in *Peireskiopsis*.

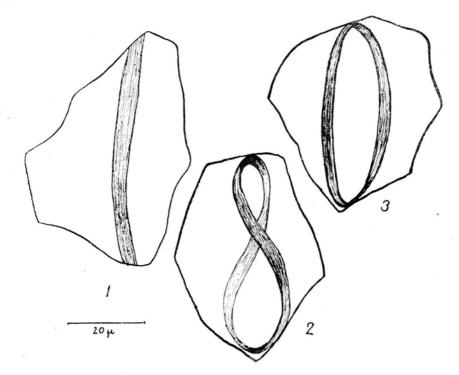
a. The spindles are typically cigar shaped (Tabl. I, fig. 1, 2, 3) and are 15—30 µ long and 4—8µ thick. They are uniform, fairly transparent, have no visible structure and are fairly stiff because they are not deformed during plasmolysis, though their pointed ends may be bent. During plasmolysis they do not pierce the cytoplasm, which was observed in the case of *Drosera* rhabdoids (B r a t, W e b e r and K e n d a 1951). In polarized light they are strongly double refractive in relation

to the long axis of the spindle. This was also noticed in other spindles of *Cactaceae* (K ü s t e r 1934). When the compensator plate is used interferention colours change from orange to violett. In dark field illumination spindles seem to be optically empty, though their outlines shine slightly. When light comes from one direction only and the asymuthe diaphgram is rotated the outlines do not shine evenly but sparkle. This indicates that the particles are longitudinal. Observations in dark field illumination and also those in other experimental conditions (see below) seem to indicate that the spindle is enveloped by a kind of membrane, and that the spindle structure in its outer parts is different from what it is in the center. About 90 per cent of examined spindles belong to this type and this paper deals mostly with them. They are to be found in the cytoplasm and sometimes on the boarder of a special kind of vacuoles. In this later case the spindle shape may be different (Table I, fig. 8).

- b. In the second type are classified all those bodies which, though they differ considerably in shape (exceptionally, even ball and ring shaped "spindles" are to be observed), have, similarly as in the first type, no visible structure and react in the same way to the action of different factors. Also spherical bodies irregular in shape, with the refraction index lower than other crystalloids, belong to this group. They are more plastic, easily change their shape when pressed, and resemble the amoeba-like inclusions found in cells of tobacco plants infected by tobacco mosaic virus B a w d e n 1950) as well as the inclusions of virus tumor in Rumex acetosa (L i t t a u and B l a c k 1952). In Peireskiopsis they have been found in different developmental stages (some smaller some larger, in the later case the refraction index is smaller an the Brownian movement more vigorous, while in the first, there is no trace of this movement). Examples are given in Table I, Figs. 4, 5, 6.
- c. These spindles have a characteristic fibrillar structure. In shape they resemble type a but seem to be braided of thin threads (Table I, Figs. 7, 9.
- d. These protein bodies are agglomerations of thin threads which were observed in cytoplasm and in vacuoles (Table I, Figs. 10, 11, 12, 13 and Table II, Figs. 14, 15).

They do not exceed 5 per cent of all observed spindles, and are slightly double refractive (positive in relation to the long axis). The threads may be arranged parallelly or be braided. Transition forms from bundles of threads to spindles with one and two poles were also found In this last case the protein spindles may resemble greatly fixed mitotic spindles.

e. In the last type are crystalloids similar to those described by K $\ddot{\text{u}}$ s ter (1934) in Epiphyllum. Their length equals at least that of the



Textfigs. 1—3, Fifth (e) type of crystalloids. Crystalloids with distinct fibrillar structure and different shapes (1 — band, 2 — double loop shape, \Im — ring).

cell and they may be looped or even double looped. Their structure is distinctly visible. They have been observed very seldom, not more than a few in a thousand. Examples are given in Textfigs. 1, 2, 3.

Throughout the experiments mutual numerical relation of bodies with the different spindle shape did not change. Observations of the types of spindles in *Peireskiopsis* show that in this species may be observed, almost all types of spindles described so far in various plants.

INFLUENCE OF PHYSICAL AND CHEMICAL FACTORS

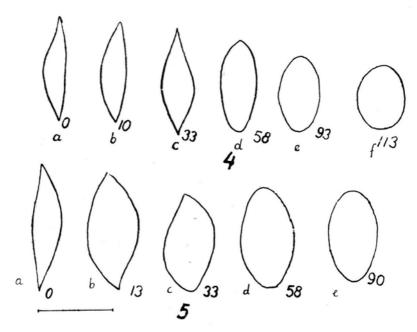
The resistance of various spindles to chemical and physical factors differs mainly in the time necessary for the reaction to take place and the degree of the observed changes. However, one factor causes similar changes in all cases.

1. Influence of extreme temperatures (-16 to 100°C)

a. High temperature at ure. Temperatures of $+48^{\circ}$ to $+100^{\circ}$ C lasting 5 mins to 2h in form of shocks have been applied. In consequence of high temperature action (52°C) the following changes have been observed in the cell: refraction indexes of nuclei, leucoplasts, and chloro-

plasts increase and these cell elements coagulate. Th viscosity of cytoplasm decreases, which is indicated by vigorous Brownian movements (this may cause changes in the position of protein spindles which makes observations more difficult). Also the growth of vacuoles have been observed.

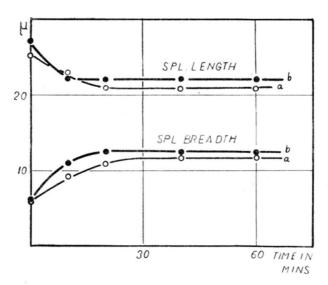
Medium liquid paraffine. After from several mins to 1h of high temperature action protein spindles swell and their refraction index decreases. The following two extreme kinds of changes have been observed: 1. The central part of the spindle swells, while the spindle poles remain sharply pointed, then slowly the shape of the spindle changes and after some time a more or less irregular ellipsoid is formed (Textfigs. 4 a-f, 5 a-e). The long axis of the spindle always decreases. 2. Even after

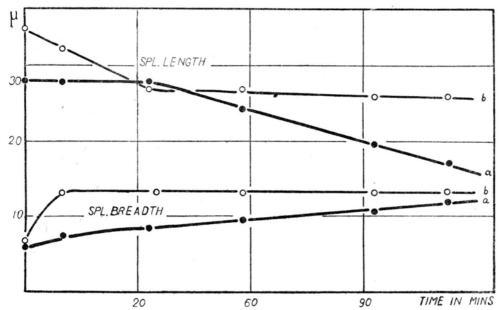


Textfigs. 4 a-f, 5 a-e. Influence of high temperature on shape of homogeneous protein spindles (medium liquid paraffine). Time in mins. is given. a — spindle in room temperature.

a considerable time (1h or more) of the high temperature action the shape of the spindle remains unchanged, and then suddenly within several seconds the spindle swells, and forms an ellipsoid or ball shaped body. Between these two types of reaction there is a continuous gradation of reaction types. The differences in reaction to high temperatures may be caused by differences in the submicroscopical structure of the spindle.

The different swelling reactions of protein spindles is best illustrated by graphs in Textfigs. 6, 7. Graph on Textfig. 6 a, represents the case of a simultaneous increase in length and descrease in breadth. In another spindle (Textfig. 6 b) the breadth increases after its length ceased to change. In Textfig. 7 a the central part of the spindle swells while its length does not change (the length decreases after 30 mins.).





Textfigs. 6 a-b, 7 a-b. Swelling of spindles in high temperature. Breadth and length of the spindles plotted against time. Curves for two spindles.

Spindles of the type e (large bodies with distinct structure) do not change in conditions described above.

If the temperature is not applied by shock but is raised slowly changes observed in the spindle are the same and to not appear until the temperature reaches 50°C. Changes caused by high temperature are irreversible even if the spindles ewell only slightly.

At 76°C and more the described changes take place instantaneously and spindles form vacuole like bodies.

A long lasting high temperature action and even a slight swelling of spindles do not destroy their double refraction, though ultimately after full swelling the birefringence desappears. Ball and ellipsoid shaped bodies are half transparent, with no visible structure, and their viscosity coefficient is low which is shown by their easy deformation.

It is necessary to stress that the winter material (January, February) was far more resistant to high temperature and even a number of spindles did not swell.

Medium: water. If as medium for observations tap or destilled water is used the observed changes in the spindles are similar though the percentage of changed spindles is lower. This is especially noticeable in winter.

b. Low temperature causes no visible changes in the shape and structure of protein spindles. This was observed when water or liquid paraffine was used as medium for observations.

2. Influence of K₄Fe(CN)₆, KNO₃, Ca(NO₃)₂ and hypertonic sacharose

Protein spindles of Peircskiopsis do not change when placed in water and even 48h after preparation no traces of changes in their shape and structure are visible which is in accordance with Mikosh's (1890) data in the case of Occidium. On the other hand they easily swell and dissolve in alkaline as well as several neutral salt solutions. Swelling is caused by K4Fe(CN)6 usually after several minutes to several hours and by KNO3 usually after 6h or more when hypertonic, hypotonic as well as isotonic solutions are applied. In this case the swelling is different from that caused by high temperatures as small vacuoles appear on the spindle boarders. Swollen spindles are ball or ellipsoid in shape and their refraction index diminishes; (Table III, Figs. 29-30 and 31-32). If high temperature acts on swollen spindles, the swelling process usually developes further. On the contrary, if the swollen spindles ar treated with Ca(NO₃)₂ or 25 per cent sacharose solution no influence of high temperature will be observed. This may indicate that dehydration prevents high temperature action.

3. Influence of ethanol and JKJ

The influence of 20—95 per cent ethanol (Table II, Figs. 25—26) and JKJ (Table II, Figs. 27—28) on the shape and structure of spindles is similar in both cases. Spindles change their structure to a more regular one, become thinner, and the structure of braided fibrills with a higher birefringence appears in them. Thus they may resemble spindles of the typ b described above. However, not in all the spindles such structure appears, and only sometimes changes in shape may be observed.

These observations do not agree with Molish'es (1885) and Küster's (1934) data. The first writer reports that protein spindles dissolve in alcoholic solutions, and the latter one that in JKJ they swell and dissolve. If the action of alcohol on spindles of *Peireskiepsis* is short in duration, the spindles, if treated with KNO₃, swell and reverse to their original shape. This, however, is not the case if the action of alcohol is long lasting. Spindles treated with JKJ or alcohol and then placed in liquid paraffine do not change in consequence of high temperature action.

These experiments show that the swelling and dissolution of protein spindles are closly connected with hydration processes, while deswelling is connected with dehydration.

4. Influence of chloral hydrate.

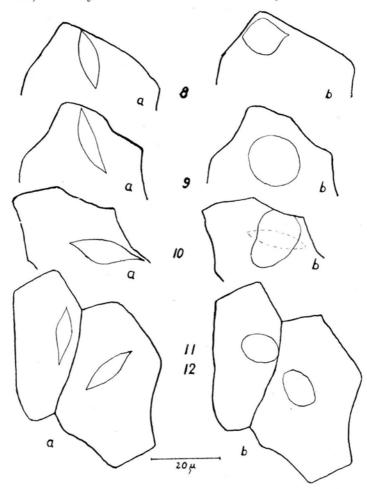
2 per cent water solution of chloral hydrate was used. Its action is quick (which probably is partly the result of the small dimensions of the particles) and after several mins. the spindle breadth decreases and its length increases. Simultaneously the ends of the spindles sharpen and a structure of braided fibrills resembling that of the type b spindles appears (Table II, Figs. 20—21, and 22). Here the effect of chloral hydrate is quite different from what K ü s t e r (1934) observed in *Epiphyllum* spindles where swelling, liquifaction, decrease of spindle length, and a change in the direction of birefringence took place.

High temperature (liquid paraffine used as a medium) applied to protein spindles of *Peireskiopsis* after acting on them with chloral hydrate causes no swelling nor rounding but may either slightly lengthen and sharpen the spindle poles or may produce no visible effect.

5. Influence of colchicine

5 per cent colchicine solution in 20 per cent ethanol was used. The influence of colchicine is slow and changes caused by it in the spindle structure are very similar in all cases. After 12h 95 per cent of all observed spindles are destroyed and in their place granulated grey bodies

appear. These bodies are usually irregular in shape and there need not be necessarily a direct relation between the size of the spindles and their product (Table II, Figs. 16—17 and 18—19; Textfigs. 8 a-b, 9 a-b, 10 a-b, 11 a-b, 12 a-b). Usually these bodies are not exactly in the same place as



Textfigs. 8—12 a-b. Influence of colchicine on shape and structure of protein spindles, a — before, b — after colchicine action.

the spindles from which they originated, and often near such a body an empty outline of the spindle is visible (Textfig. 10b). This may indicate that as a result of colchicine action the content of the spindle has flowed out from the "spindle membrane" into the cytoplasm. The influence of colchicine on protein spindles of *Peireskiopsis* is irreversible and 24—48h after colchicine has been washed away with water no changes are noticeable.

6. Influence of quinine chloride

Influence of quinine chloride on striated muscles is well known (weak concentrations stimulate, and strong ones prevent their contraction). According to Frey-Wyssling (1948) factors stimulating or preventing contraction of muscle proteins ought to act similarly on other fibrillar proteins. In view of this statement the action of quinine chloride on protein spindles is most interesting. Quinine acts very quickly and causes visible effects within several mins. The content of the spindle desappears and only an empty outline of the spindle is visible (Table II, Figs. 23—24). This process is preceded in numerous cases by a slight swelling and is irreversible.

7. Influence of pH

It is well known that suitable conditions inside virus infected cells are necessary for the inclusions to originate. One of these factors is the value of pH. X bodies in Aucuba are resitant to acids up to pH = 2, while in solutions of tobacco mosaic virus the virus is not soluble when pH is 3—4 and precipitates in the shape of microscopical needles. It was proved that value of pH in which these needles are soluble is the same in vitro, as inside the cells.

The spindles in Peireskiopsis are very sensible to pH value of surrounding medium and they are stabile, only when pH is 5-8. Type e is most resistant to acids and bases. Other types are easily dissolved in bases and acids, the easier the stronger the concentration of the medium. Table III, Figs. 33-36 and 37-40 illustrate how spindles dissolve. The dissolution of spindles occurs differently in acid than in basic solutions. In acid medium swelling procedes the dissolution and in this the process resembles high temperature action. After spindles dissolve there are in the cytoplasm in the places previously occupied by the spindles agglomerations of small granules. These desappear after some time probably as a consequence of cytoplasm movements. In basic mediums spindle dissolution is usually preceded not by their swelling but by a vigorous particular pulsations (each pulsation, i. e. a contraction and an expansion of the spindle, lasts about 0,5 sec). Such pulsations last for several seconds, up to 2 minutes, simultaneously the spindle changes its shape irregularly, and finally dissolves.

It is necessary to stress that pH values given above refer to the medium only, and pH values inside the cell have not been examined.

Experiments on a joint action of both acidity of medium and high temperature have been carried out. In neutral solutions, e. g. water, some spindles do not swell in high temperature. When pH is below 4 or above 8 soon after a high temperature is applied the spindles swell quickly, change their shape becoming spherical and simultaneously their refrac-

tion index diminishes. It thus seems probable that the differences in behaviour of spindles under the influence of high temperatures might be due to differences in pH values inside the cells.

DISCUSSION

Observations on the influence of different factors on the shape and structure of protein spindles in *Peireskiopsis* indicate that in consequence of their action 2 kinds of changes may take place.

I. Swelling and rounding of the spindle and even complete dissolution with simultaneous decrease of the refraction indexes. These changes are caused by: high temperature, $\mathrm{KNO_3}$ and $\mathrm{K_4Fe}(\mathrm{CN})_6$ solutions, and different acidity values of medium (pH). It is necessary to point out that each of these factors may cause strong dehydration of proteins. This group of factors causes the liquifaction and destruction of protein spindles, which seems to be indicated by the desappearance of birefringence.

2. Thinning and pointing of spindle ends and a simultaneous appearance of a structure (threads of fibrills braided together). Such changes are caused mainly

by agents dehydrating the proteins (chloral hydrate, JKJ, alcohol).

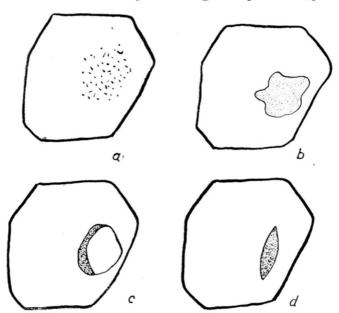
Quinine chloride and colchicine produce results which do not fit in these two groups.

1. Origin of protein spindles

The hypotheses on the origin of protein spindles are numerous, though the number of observations is insufficient. Only Sheffield (1931) using ciné-micrography followed through the development of virus inclusions in Aucuba of tomato mosaic. She observed that after infection the movement of cytoplasm within the cell is quicker and that small granules join together as a result of cytoplasm movement to form X bodies. There are such observations on the origin of protein spindles in Cactaceae. Weber, Kenda and Thaler (1952) observed agglomerations of tiny crystals in Epiphyllum and Rhipsalis cells. According to these writers the crystals may be arranged parallely and form double refractive bundles. It is not known whether this arrangement is caused by cytoplasm movements or factors which lead to the formation of tactoids similarly as in virus solutions. Such an explanation reminds greatly a hypothesis made long ago by Chmielewsky (1887). Chmielewsky thinks that small single fibrills placed at some distance one from the ohter originate from the cytoplasm. Intususception causes them to lengthen. Bunches of fibrills may assume different shapes. Also according to Molisch (1885) the spindle originate from threads arranged parallely as a result of an increase in their number and volume. Mikosh (1890) thinks that spindles are formed from granules displaced in the cytoplasm. Similar conclusions were drawn by She ffield (1931). All these authors are convinced that it is in the cytoplasm that spindles originate.

In Peireskiopsis all fully developed protein spindles are formed in the cytoplasm (i. e. homogeneous bodies, spindles, rings. etc.). However homogeneous bodies, the shape of which it is difficult to define, have been observed on boarders of vacuoles, and single threads arranged more or less parallely have been seen inside the vacuoles. In these cases spindles seem to originate not in the cytoplasm, but inside the vacuoles. These observations may be in agreement with the reports of other writers, as it seems probable that the granules found in cytoplasm are the origin of those spherical X bodies which have a low viscosity and refraction indexes. This may be the first stage of inclusion formation, similar to that observed in Solanum (Sheffield 1931). As a result of demixing, i. e. kind of coacervation, two phases might be distinguished: cigar shaped agglomerations of longitudinal particles (tactoid with small amount of liquid) and the liquid phase which later is resorbed by the cytoplasm. This may be the second stage of protein crystal formation from amorphous bodies (cf. Sheffield 1931). In Peireskiopsis all the stages, i. e. granules, differently formed amorphous bodies, and almost formed spindles, have been observed. The formation of protein spindles is illustrated in Textfig. 13 a, b, c, d.

One more, though very rare, process of spindle formation has also been observed in *Peireskiopsis*. In regular spherical "protein vacuo-



Textfig. 13 a—d. Schema of origin of homogeneous protein spindles. a — agglomerations of granules in cytoplasm, b — amoeba like X body, c — demixing in X body, in consequence protein body is found in cytoplasm.-d

les" in which the structure is hardly discernible, two poles of the future spindle appear. The vacuole lengthen, becomes thinner, and change into spindles. It is probable that the resorption of liquid is simultaneous to the process of crystalisation.

The swelling as well as the vacuole formation, which may be caused in *Peireskiopsis* spindles by numerous factors (cf. text), seem to support the above suggestions of *Peireskiopsis* protein spindles origin. This is in accord with what Weber (1953) reported on the origin of spindles in *Peireskiopsis pititache*.

In *Peireskiopsis*, bunches of protein fibrills are found in vacuoles and in the cytoplasm. In this latter case fibrills are usually closer to each other and form separate groups Table I, Figs. 10, 11, 12, 13, Table II, Figs. 15, 16, show the probable way in which such crystalloid with loose structure originate. If this supposition is correct, the protein fibrills arranged more or less parallely crystalise within the "protein vacuole" (Textfigs. 10, 11,). While the number of these fibrills increases they intercross and braid together (Table I, Fig. 12, Table II, Fig. 14) and in late stages spindle shaped bodies are formed. Here also, as in the case described above, the vacuole liquid is resorbed by the cytoplasm.

The relation between the loose bundles of fibrills and the homogeneous fibrills is not fully understood. Some of the authors quoted maintain that bundles of fibrills transform into homogeneous spindles as a result of their increase in number. The action of chloral hydrate and alcohol, resulting in the appearance of structure, seem to indicate that there is a close connection between homogeneous and b type spindles, which are composed of braided fibrills.

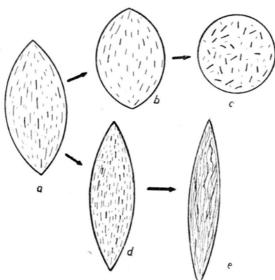
Probably dehydration processes inside the cell cause a change of a part of homogeneous spindles into b type spindles. On the other hand it does not seem probable that a close connection between homogeneous spindles and balls composed of fibrills (type d) exists. The relation of these two types of *Peireskiopsis* spindles to chemical and physical agents is very different. This fact and the great variety of forms with fibrillar structure indicate, in my opinion, that the character of this two types of crystalloids is different. It does not seem probable hat such differentiated bodies may be a simple transformation from homogeneous spindles. Very probably the origin in *Peireskiopsis* of these two type of crystalloids is independent of each other.

2. Protein spindle structure

According to a concordant opinion of many writers, the protein spindles are composed of a virus substance and the substance derived from cells of the infected plant is the product of disturbed metabolism in the infected cell. Opinions on the internal structure of the spindle differ. Most authors suggests that the spindle is composed of fibrills and interfibrillar substance (stroma or matrix according to Weber 1952) which in homogeneous spindles has the same refraction index as the fibrills. According to Weber (1952) the spindle is a positive tactoid inside which is a stroma composed of negative small tactoids.

It is probable that in *Peireskiopsis* in the different types of spindles the structure is not identical in details. Homogeneous spindles are probably typical tactoids (i. e. longitudinal particles are parallely arranged to the long axis of the spindle and loosly connected together). This opinion is supported by such facts as: 1. birefringence in relation to the long axis, which is characteristic for fibrillar proteins, 2. the easy swelling which causes the birefringence to desappear and 3. the increase of birefringence and the return of the spindle to its original shape under the influence of dehydrating agents applied in the first stages of swelling while birefringence though low still exists.

It is well known that dehydration of proteins usually increases in higher temperatures (Lecomtedu Noüy 1945). Probably this also happens in protein spindle. Water entering intermicellar spaces causes the acting forces between the micelles to diminish, and the surface tension forces the shape of the tactoid to change. This process is reversible in its early stages. When the swelling is advanced the regular arrangement of micelles is destroyed, which is indicated by vigorous pulsa-



Textfig. 14. Schema of changes in the spindle caused by dehydrating and hydrating agents. a — spindle in normal conditions, b—c — changes in the spindle caused by hydrating processes. d—e — changes in the spindle caused by dehydrating processes.

tions and movements inside the spindle before it changes its shape. Similar facts were also reported by K ü s t e r (1934) and explained by him as the consequence of a change in position of particles.

Dehydrating agents (chloral hydrate, alcohol) cause the appearance of structure as water is removed from the intermicellar spaces and particles are connected more closely and probably denaturated. Such structure cannot be loosened by high temperature. These changes are illustrated by schema Texfig. 14.

So far it has not been explained whether the whole spindle has an uniform structure or whether it is enveloped by a special membrane. This problem has been hardly dealt with. It is only M i k o s h (1890) who suggests that the surface of the spindle is densier and more resistant to different factors. This is fully confirmed by the present observations. The empty outline of the protein spindle which is visible after treating with colchicine or quinine and the observations in a dark field indicate that the outward surface of the spindle has a different structure, or that the spindle is enveloped by a membrane.

The other types of the spindles with visible structure do not resemble tactoids. They differ from type a in their reaction to hydrating and other (i. e. colchicine) agents. It seems probable that in these spindles the particles are strongly connected together and that the structure of these spindles is much more complicated.

- 3. Comparison of high temperature and colchicine action on protein and mitotic spindle
- a. Temperature. The influence of high and low temperatures on shape and structure of protein and mitotic spindles differs. In Hymenophyllum (Bajer and Molè-Bajer 1953) in metakinesis, metaphase and anaphase, under the influence of high temperature, half spindles of the mitotic spindle lengthen, sharpen, and stiffen, also inside the spindle a fibrillar structure joining chromosomes to spindle poles appears in some cases. In protein spindles high temperature causes swelling and liquifaction while in mitotic spindles stiffening and probably dehydration. Reaction to low temperatures also differs. The mitotic spindle is rounded and most probably partly destroyed while no visible changes in the protein spindle are to be found. The influence of low temperature on mitotic spindles was studied by Ehrenberg (1946) who found that a fall in temperature increases the curvature of the mitotic spindle and suggests that it is caused by contraction and stretching out of protein molecules. These facts indicate the different character and structure of mitotic and protein spindles. Protein spindles (type a) may

be considered as tactoids, while the structure of mitotic spindles is much more complicated (cf. S w a n n 1951 a,b, W a d a 1950).

b. Colchicine of colchicine on mitosis was very extensivly studied (for references see Schrader 1944, Hughes 1952) on fixed material and also in vivo (Wada 1944, Gaulden and Carlson 1952, Inoué 1952). The results obtained by different authors are more or less the same. Colchicine causes a desorganisation of the formed mitotic spindle, prevents it to be formed anew, and so makes the cell division impossible. Gaulden and Carlson (1952) were the first to observe what happens with the substance which remains from the destroyed spindle, and found that it may be either dispersed between chromosomes or form in the cell an irregularly shaped body (named by Gaulden and Carlson "hyaline globule"). easily deformed by mechanical factors. Hyaline globules are composed of half transparent substance with a high viscosity coefficient and are not enveloped by a membrane. Their position in the cell is not the same as that of the mitotic spindles, which probably is due to a decrease of cytoplasm viscosity. These facts indicate that the action of colchicine on mitotic and protein spindles is similar. In both these cases colchicine destroys the spindle structure and causes the appearance of bodies with irregularly arranged particles.

SUMMARY

- 1. 5 types of protein spindles have been found in the epidermis of *Peireskiopsis* leaves. This paper deals mainly with homogeneous protein bodies typically spindle shaped. Also amoeba like protein bodies, similar to X bodies found in virus infected plants have been observed.
- 2. Crystallisation of homogeneous spindles from amoeba like bodies have been followed through and it has been observed that as result of particular demixing vacuoles and protein bodies are formed. Protein bodies become spindles, and vacuoles are resorbed by cytoplasm.
- 3. Influence of numerous agents on shape and structure of protein spindles has been examined and 3 groups of factors distinguished:
 - a, Factors causing swelling and rounding of the spindle and, later, their dissolution while simultaneously birefringence desappears and viscosity decreases (high temperature, KNO₂, K₄Fe(CN)₆, high and low pH values).
 - b) Factors causing sharpening and thinning of the spindles with simultaneous appearance of fibrillar structure (chloral hydrate, alcohol, JKJ).
 - c. Factors not belonging to these two groups. They are colchicine which causes spindle desorganisation and quinine chloride which causes its desappearance.
- 4. On the basis of the experiments conclusions concerning homogeneous spindle structure and its changes produced by different factors are drawn.
- 5. Comparison of action of extreme temperature and colchicine on shape and structure of mitotic and protein spindles is given.

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EXPLANATION OF THE PLATES

Plate I

- Figs. 1—3. First type (a) of crystalloids; homogeneous spindles. 1. spindle in phase microscope. 2 the same spindle in ordinary microscope.
- Figs. 4—6. Second type (b) of crystalloids. 4 ball shaped crystalloid. 5 spindle with long thin pole (Spindle mit einer Peitsche). 6 ring.
- Fig. 8. Crystallisation and formation of the spindle on the boarder of the vacuole. Figs. 7, 9. Third type type (c) of spindles. Note coarse structure of interweaved thick threads.
- Figs. 10-13. Fourth type (d) of the spindles, bundles of fibrills. 10-12 in vacoule fibrills placed parallely. 14 fibrills are more numerous and are not parallely arranged but interweave each other. 13-15 bodies more compact and placed in cytoplasm.

Plate II

- Figs. 14—15. Explanation given in Plate I, Figs. 10—13.
- Figs. 16—17. Influence of colchicine on protein spindles. 16 spindle before colchicine action. 17 the same spindle after 12 h of colchicine action.
- Figs. 18—19. Spindle before (18) and 12 h after colchicine action (19).
- Figs. 20—21. Influence of chloral hydrate on protein spindle. Spindle before chloral hydrate action (20) and 20 mins. after chloral hydrate action (21), 22 another spindle changed in result of chloral hydrate action.
- Figs. 23—24. Influence of quinine chloride on protein spindles. Spindle before (23) quinine action and the same spindle changed by quinine action (24). The contence of the spindle has desappeared and its empty outline remains.
- Figs. 25—26. Influence of ethanol on protein spindle. Spindle before (25) and the same spindle after ethanol action (26).
- Figs. 27—28. Influence of JKJ on protein spindle. 27 spindle before, 28 after JKJ action.

Plate III

- Figs. 29—32. Swelling of the spindles in result of KNO_3 action. 29—31 spindles before KNO_3 action, 31—32 the same spindles after KNO_3 action.
- Figs. 33—40. Dissolution of spindles in acid medium (pH 3). 33—36 and 37—40 the same cells. Times in mins. are given.
- Fig. 41. Spindle swelled in result of KNO3 action.



Plate I

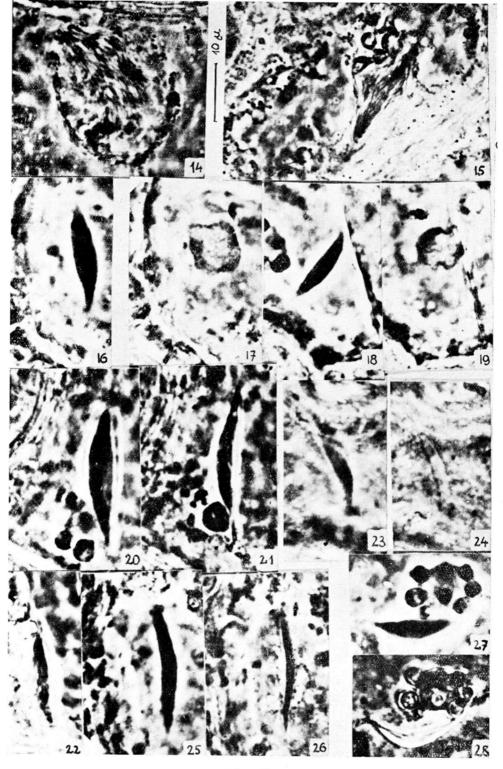


Plate II

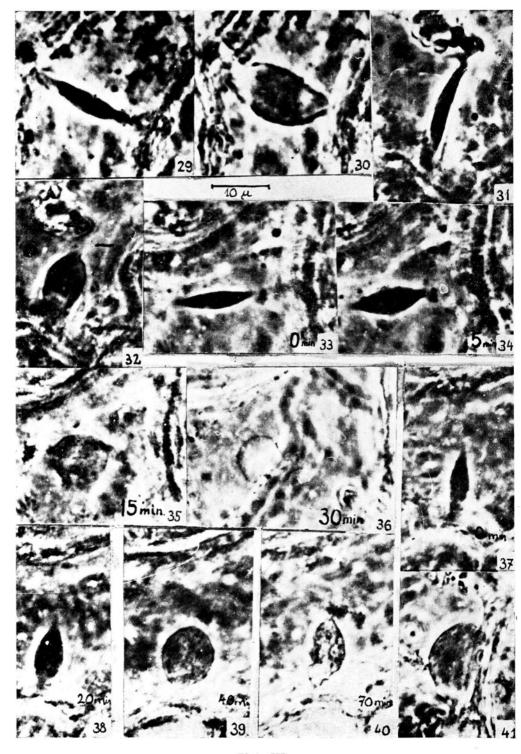


Plate III