Chemistry. — *Tissues of prismatic celloidin cells containing biocolloids IX. Experimental factors favourable for the integrity of the cell walls. Correlation of this integrity with the morphology of the films, cellgroups and cells.* By H. G. BUNGENBERG DE JONG and R. C. BAKHUIZEN VAN DEN BRINK.

(Communicated at the meeting of April 26, 1947.)

1. *Introduction and Methods.*

In the very first communications [1]) of this series the celloidin films containing groups of prismatic tissue cells were obtained by dripping some drops of the emulsion (water or a solution of a hydrophilic colloid in water, emulsified in a solution of celloidin in a mixture of amylalcohol and ether) on the surface of some water in a Petri dish.

The properties of the films obtained in this way depend, however, on a number of casualnesses and in order to obtain reproducible results we thought it necessary to standardize the method and to know which factors have a great, which a slight influence on the properties of the film. For our purposes it is particularly important that the cellwalls show no lesions. By lesions we mean here not only microscopically visible lesions of the cell walls [2]), but also those lesions, which cannot be detected under the microscope. The latter can be detected by the loss of cells in the cell groups to show accumulation of a basic dye, if gum arabic sol is used when preparing the original emulsion.

If not stated otherwise the following procedure was followed: 4 cc of the celloidin containing emulsifying liquid and 1 cc of a 3 % gum arabic solution in water was put in a measuring-flask of 10 cc with ground glass stopper. The measuring flash was then attached by means of a rubber string to a wooden disk, which was driven round its axis by a synchrone motor at a rate of 22 revolutions per minute. After 5 minutes the (coarse) emulsion is ready for spreading on the surface water/air. To investigate the influence of variables connected with the technique of spreading, four circular dishes (zinc) of different diameters are arranged around a stative, fitted with an arm which by rotating can be brought above the centre of each dish and which carries at its end the spreading pipette (a graduated micro pipette of 1 cc). The distance of the pipette point from the water surface in the dishes can further be varied at will.

The pipette is connected by a rubber tube via a three way cock with a reservoir of reduced air pressure (water manometer) or with the air.

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[1]) H. G. BUNGENBERG DE JONG, B. KOK and D. R. KREGER, Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, **43**, 512 (1940).

[2]) H. G. BUNGENBERG DE JONG and R. C. BAKHUIZEN VAN DEN BRINK, Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, **50**, 436 (1947).

Thus the pipette can be first filled with emulsion to the height desired, then the stopcock is closed and the pipette turned above the dish desired and afterwards its contents are run out by turning the stopcock, so that the pipette is connected with the air. The film obtained on the water surface is then first examined as to its macroscopical features (diameter, type of folds between the cell groups).

Part of the film is lifted from the watersurface by means of a microslide, and the latter is then laid down as a cover on the cuvette described previously [3]) (so that the original air side of the film is now in contact with the destilled water in the cuvette). Now the microscopical details of the cell groups and cells are observed, and afterwards the accumulation of toluidine blue [4]) in the cells is followed from a very dilute solution which streams continually through the cuvette.

In the two next sections we will try to give first a total impression of the results obtained and in the last section 4 a systematic survey of the influence of each of the variables investigated will follow.

2. *Morphology of films, cellgroups and cells and the correlation of the morphology with quality of the films.*

Generally speaking there exists a distinct correlation between the morphological features of a film and its quality judged by the percentage of cells in the cellgroups which have the power of accumulating Toluidine blue (that is have neither visible nor invisible lesions of the cell wall, by which the gum arabic has been washed out or diffused out the cell).

We usually have a film of good quality when after spreading a film results with a great diameter, and which immidiately after spreading extends to its maximal extent and shrinks only in a relatively small degree.

Further the quality is usually good, if after the above short shrinking a regular pattern of bundles of folds has developed which connect the individual cellgroups. Another criterion for good quality is often, that the cells within each cell group are distributed regularly, (in the middle of the cell group the larger cells and towards the periphery the smaller ones) while the cell groups themselves show a sharp boundary.

Unfavourable for good quality are films, which shrink to a greater amount and for a longer period. The bundles of folds connecting the cell groups are lacking then or do not run from cellgroup to cellgroup; the cells within the cell groups have no sharp boundaries (i.e. many solitary cells lay close to the irregular boundary of the cellgroup).

With reasonably good films there are 5 to 8 bundles of folds between the cell groups around each cellgroup (see fig. 1 A). We have the impression that if the diameter of the film is enlarged by some factor to such an extent that this number is only two or three, the quality of the

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[3]) See note [1]).

[4]) H. G. BUNGENBERG DE JONG and B. KOK, Proc. Kon. Ned. Akad. v. Wetensch., Amsterdam, **43**, 728 (1940).

film (higher percentage of accumulating cells in the cell groups) is still better (see fig. 1 B).

This, however, is not always the case, as we will see when discussing variable A 6 in section 4.

As the morphological details of the fold systems and of the cellgroups are correlated with the quality of the film, and also with the diameter of the film (other factors being held constant), a factor which enlarges the diameter of the film is in general also a factor which improves its quality.

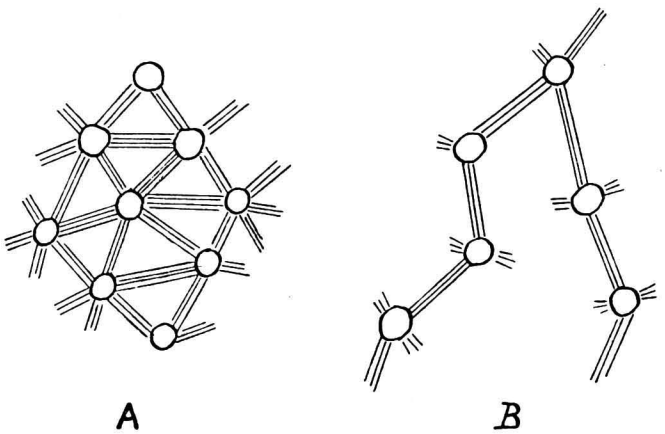


Fig. 1.

With a given emulsion and a given technique of spreading the diameter of the resulting film on a fresh water surface (tapwater), depends primarily on the area of the water surface. A greater diameter of the dish containing the water, gives films with greater diameters (the border of these films is often irregular), but the film never covers the whole water surface.

Under most favourable circumstances the diameter of the film (after the short shrinking immediately after spreading) is circa 60 % of the diameter of the dish (and often it is less).

This is caused by an invisible and more rapidly spreading very thin film, which after reaching the border of the water surface, solidifies and thus the spreading of the thicker film mechanically hinders (compare fig. 2). At the border of the thicker film the transition to the thinner film often betrays itself by groups of folds (fig. 2, *a*).

After the shrinking of the thick film, this thinner film (fig. 2, *b*) has usually detached itself from the border (fig. 2, *d*) of teh dish (though it remains attached to the thicker film). This appears from the fact, that one can stir with a glass rod near the border of the tray (at *c* fig. 2) without the thicker film moving. However, when one stirs closer to the latter (at b fig. 2) the whole film is moved.

The very thin film between border of the dish and the thick film is not visible as a rule. If one removes the thick film form the dish, part of the thin film remains on the water surface.

This explains, that if we now spread a fresh film, its diameter will be smaller than the first time, the available free water surface being smaller. In order to obtain films with reproducible properties it is therefore necessary to renew the water surface each time before spreading a following film.

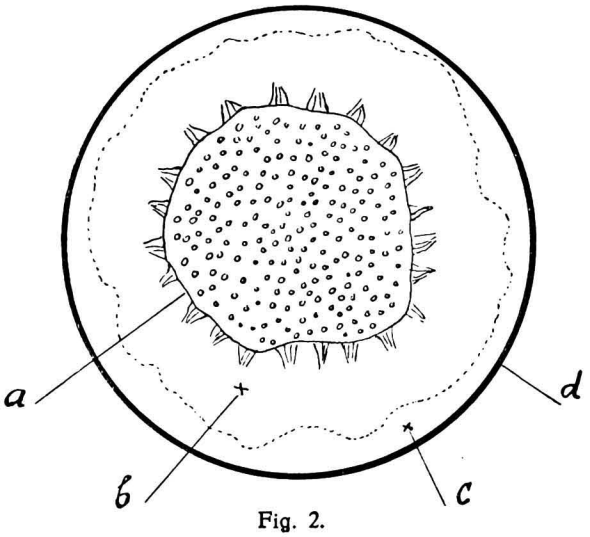


Fig. 2.

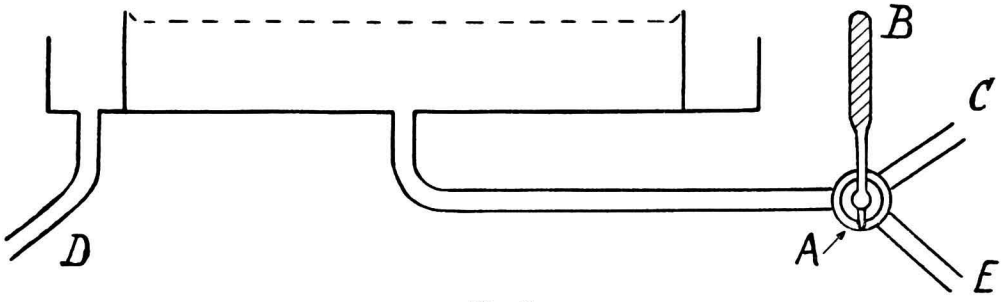


Fig. 3.

Fig. 3 gives a section of the dishes used. A is a brass three way stopcock (with handle B), with the aid of which tapwater via C can be let into the dish, D is the outletpipe of a circular gutter, surrounding the dish proper. If the water has sufficiently been refreshed, the dish is connected with E, to let out a certain quantity of water till the meniscus of the water surface stands as in fig. 3. This is necessary, for otherwise the film after spreading will not remain in the dish, but will slip over the brim in the gutter.

3. *Systems of folds.*

We saw in 2. that films of moderately good quality are often characterised by the presence of systems of folds connecting the individual circular cellgroups. If not an emulsion but the emulsifying liquid (e.g. celloidin

dissolved in a mixture of organic liquids) itself is spread, a film is also formed, but now the characteristic starlike folds systems are no longer present.

The film obtained (apart from a detail discussed further below) may be totally free from foldsystems (2 % nitrocellulose "C" in ether-benzol-amylalcohol = 2 : 1 : 1) or fields of parallel folds (the same sample in ether-amylalcohol 1 : 1) may originate as shown in fig. 4 B. If lycopodium powder of potato starch grains are suspended in the emulsifying liquid, we find after spreading the grains accumulated in clusters and the latter surrounded by the typical starlike bundles of folds. This clearly shows that these starlike systems of folds originate in general around heterogenities in the emulsifying liquid. In our case the heterogenities consist of drops of the emulsified gum arabic sol, which have also drifted together.

The latter drops are surrounded by a celloidin film, which at this stage must still have a semifluid character, enabling these films to coalesce to separate cellwalls, thus transforming the original cluster of drops into a characteristic tissue of prismatic cells.

The system of folds around the groups originate from the shrinking of the film, which shrinking is hindered by the groups of grains or emulsion drops. It can thus be foreseen that a number of velocities of detail processes must have appropriate values to obtain the typical morphological structure of a film of good quality.

a. The film after spreading may not solidify too fast, so as to enable the separate emulsion drops not only to drift together to groups, but also to arrange themselves within a cluster according to their sizes (larger ones in the centre, the smaller ones more peripherically).

b. The shrinking of the film must take place while the membranes around the emulsion drops are still semilfluid, so that they are not damaged by mechanical tensions.

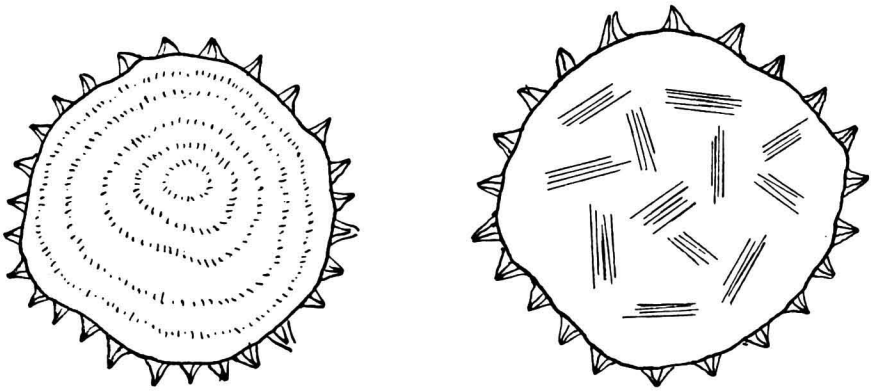
c. Still in this stage these membranes must not be too fluid, but soon solidify, otherwise coalensence of drops might occur and the transformation of the cluster of drops into the typical tissue of prismatical cells might be endangered.

As to factor a, the following observation on the spreading of the emulsifying liquid is of importance. The emulsifying liquid used showed such viscosity that it fell from the pipette in a series of rapidly succeeding drops. The film originated showed a series of concentric rings of small folds, each consisting of very short radial folds (see fig. 4 A).

The following table gives the diameters d of the fold rings [5]), of the above mentioned emulsifying liquid (2 % C in ether-amylalcohol-benzene = 2 : 1 : 1) spread on a dish of 30 cm diameter, the values $\sqrt{n}$ (in which n is the series of the whole numbers beginning with $n = 1$) and the quotients $d/\sqrt{n}$.

[5]) See note [6]).

Six drops in all were delivered from the pipette and 5 concentric fold rings originated in the film. If the margin of the film is also reckoned as



A B

Fig. 4.

d (cm)	$\sqrt{n}$	$\frac{d}{\sqrt{n}}$
5.1	$\sqrt{1}=1$	5.1
7.4	$\sqrt{2}=1.414$	5.2
9.2	$\sqrt{3}=1.732$	5.3
10.4	$\sqrt{4}=2$	5.2
11.1	$\sqrt{5}=2.236$	5.0
12.4 (margin of the film)	$\sqrt{6}=2.45$	5.1

fold ring, the surface of the central fold ring and the surfaces between every two succeeding fold rings correspond with 1 drop of the emulsifying liquid.

Supposing the film has a uniform thickness, the above surfaces should be equal in area. If this is really so, the diameters of the concentric rings must consequently be proportional to $\sqrt{n}$. The table shows that this proportionality is reasonably fulfilled, so that we may conclude that the film has a uniform thickness.

But this means also, that during the short time that the six drops were delivered, the emulsifying liquid has retained its liquid character, so that any heterogenuous particles (such as e.g. drops of emulsified gum arabic-sol) still have an opportunity to drift together to groups, before the gelating of the film would render this impossible

4. *Survey of the variables investigated.*

The variables investigated can be divided into two groups: A and B. Group A contains variables considered as regards the composition and

preparation of the emulsion, group B variables as regards the technique of spreading itself.

In the present survey the results are given when of a whole set of variables each of these are changed at a time, while the others remain constant.

As a rule we start in doing so from the following values for a set of variables, which together give a reasonable result. These are: emulsifying liquid consisting of a relatively low concentrated (1—2 %) solution of nitrocellulose in 2 vol. ether + 1 vol. amylalcohol + 1 vol. benzene. The emulsion is made from 1 cc 3 % gum arabic solution in water + 4 cc emulsifying liquid as described above under 1.

Distance from the pipette point to the water surface = 3 cm.

Quantity of emulsion delivered from the pipette = 0.33 cc.

Spreading dish of 30 cm diameter filled with fresh tapwater, depth of the water is this dish = 6 cm. Roomtemperature = 18° C.

A 1. *Kind of nitrocellulose.*

Some technical nitrocelluloses were investigated, inter alia "C" (used in the cellulose lacquer industry) and "Celloidin" (Schering-Kahlbaum)

Impression: the variables have qualitatively the same influence, though there are differences quantitatively. With "C" a 1 % solution in the emulsifying liquid is not yet too low, with "Celloidin" the concentration must be higher (2—2.5 %). High viscosity of the emulsion is not desirable, as in this case the drops delivered from the pipette succeed one another too slowly.

A 2. *Composition of the solvent for the nitrocellulose.*

Both with "C" and "Celloidin" we found that the solvent used in our first communications (1 vol. ether + 1 vol. amylalcohol) is not so favourable for good quality.

Though macroscopical and microscopical morphology seem to predict a reasonably good quality, the actual behaviour as regards accumulation of toluidinblue reveals that a great many cells in the cell groups often have lesions. A high percentage of accumulating cells is not easily reached here. A great improvement is obtained if the solvent contains benzene as well. As far as we could trace, the most favourable composition for the solvent is obtained when we have the following volume ratio ether : amylalcohol : benzene = 2 : 1 : 1. With the nitrocellulose sample "C" we investigated the influence of a variation of this ratio. The results can be discussed best with the aid of a so called ternary diagram. See fig. 5. Corner E stands for 100 % ether, Corner A for 100 % amylalcohol, corner B for 100 % benzene. The solvent originally used (1 ether : 1 amylalcohol) is represented in it by point 1 half way side A E of the triangle and the solvent approximately optimal for quality (2 ether : 1 amylalcohol : 1 benzene) by point 2. As emulsifying liquids we used 1 % solutions of "C"

in mixtures of the three liquids. "C" was soluble in 100 % E, not soluble in 100 % A nor in 100 % B. Solubility was restricted in the area of the

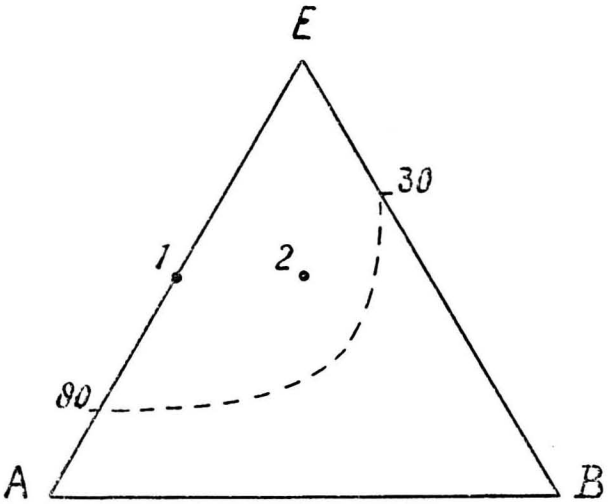


Fig. 5.

triangle, comprised between the side E—A up to ± 80 % amylalcohol, the side E—B up to ± 30 % benzene and the curve in the triangle connecting these latter points.

With 100 % E as solvent a film is really formed the first moment, it is true, but it is soon torn to rags, which scatter all over the water surface.

We see the same with a solvent on the E—B side of the triangle. The cells in the film rags have accumulated into groups after all, but the latter are also partially torn off the film.

The cells are more or less spherical and accumulate toluidinblue slowly. The cells themselves do not appear to show lesions, but because of the fact that the film carrying the cellgroups shows these cleaving phenomena, it is completely unsuitable for any use.

If we investigate the solvents situated on side E—A of the triangle, we meet with sufficient amylalcohol in the mixture just the reverse situation. The film bearing the cell groups, does no longer explode spontaneously, but the percentage of cells accumulating toluidinblue, is greatly reduced. So now we have lesions of the cell membranes and no lesions in the film. In many cell groups a great percentage of the central cells do not accumulate. Sometimes another type of defect cell groups is obtained, in which the zone of peripherical cells itself does not accumulate. If along the side E—A we approach the limit of solubility, defects in the film itself begin to manifest themselves.

We see again the splitting phenomena, though not so frequently as along side E—B. The same kind of defects are also met with near the curved line in the triangle (circular cracks).

Defects in the film and lesions of the cells are not present or limited

to a minimum in the neighbourhood of point 2. Here we often find that 95—100 % of the cells strongly accumulate toluidinblue.

When investigating the influences of other variables we therefore always used emulsions, in which the solvent was optimal for quality, i.e. ether : amylalcohol : benzene = 2 : 1 : 1.

A 3. *Concentration of the nitrocellulose.*

It is recommendable to use a relatively low concentration of the nitrocellulose, as in doing so films of greater diameter result, the right spread emulsion remains longer fluid, so that the emulsified drops get a better chance of arranging themselves to well ordered groups. Next table gives an example (nitrocellulose "C", ether + amylalcohol + benzene = 2 : 1 : 1, temperature 14°, distance from point of the pipette to the water surface = 3 cm, quantity delivered from the pipette = 0.33 cm, diameter of the dish = 30 cm, the latter being refilled with fresh tapwater for each experiment).

Concentration nitrocellulose "C" %	Diameters of the film after [6]			Boundary of the cell-groups	Percentage of accumulating cells
	1 min.	5 min.	10 min.		
2	8.8	7.2	7.2	diffuse	± 80 %
1	9.0	8.7	8.6	little diffuse	± 100 %
0.5	12.1	11.8	11.8	sharp	± 95—100 %
0.25	15.8	15.6	15.6	sharp	± 100 %

This table shows that the nitrocellulose concentration has a pronounced influence on the diameter and other properties of the film. If one plots in a diagram the second power of the diameter against the reciproke value of the concentration a straight line results. Thus the surface of the film is here a linear function of the dillution.

When we lower the concentration of "C", the "quality" (last column) increases, while the transition from diffuse cell boundaries to sharp ones progresses in the same ratio. Moreover, the shape of the individual cells within the cell groups grows more ideal as well, that is, it tends to assume the shape of regular 5, 6 or 7 sided polygons. The film obtained with 0.25 %, however, was very brittle, and could not be tested in the ordinary way on accumulation (instead of on the cuvette, the film was brought on the surface of a toluidineblue solution, on which it floated). The best results in the table were obtained with 1 % C, in which case only small cells did not accumulate at the edge of the cell groups, while the bigger central cells were all undamaged.

The film obtained with 0.5 % C, on the contrary, showed loss of the

[6]) As the circumference of the films is often not regular, we always mean by "diameter" here and in the following the mean of four diameters, measured at angles of 45°.

power to accumulate in some of the bigger central cells. So we see, that in decreasing the concentration of the nitrocellulose, an optimum is passed, which will, of course, be located elsewhere for other kinds of nitrocellulose.

A 4. *Volume ratio emulsifying liquid/hydrosol.*

A favourable value for this ratio is 4 : 1. If one should take more aqueous phase, the emulsion becomes too thick, which affects the easy spreading badly and consequently diminishes quality, too. As the emulsifying liquid itself dissolves some water the above ration cannot be altered too much in the reverse direction.

A 5. *Technique of emulsifying.*

This technique has been described in 1. A variable not yet mentioned is the degree of filling of the tube in which the emulsification is accomplished. If the column of air is relatively larger, the emulsified drops — and as a result the mean size of the cells in the cell groups — become smaller. We have not got the impression that this size in itself causes a change in the quality of the ensuing film.

A 6. *Substances added to the emulsifying liquid.*

There are a great many organic substances, which, when added to the emulsifying liquid in a relatively small concentration exert a great influence. As a rule the diameter of the film is increased and the bundles of folds between the cell groups disappear. Very often this does not cause an improvement of the percentage of the accumulation, on the contrary, the effect is very often adversely (e.g. with diamylphtalate).

In case of somewhat greater additions we often see that serious damages are the results. Diphenylmethane is an example that compares favourably with many others, the cell groups are not affected, though the fold systems between the cell groups disappear. In this example a strong retardation of the rate of accumulation was observed, which indeed was also the case with some other additions (e.g. triolein). When oleic acid is added to the emulsifying liquid in low concentration we first see a reduction of the film diameter, when of higher concentration an increase. As a result of the oleic acid contents, the film itself was stained blue by toluidinblue. A remarkable influence was excerted when part of the benzene in the emulsifying liquid is replaced by chlorbenzene. The ordinary system of folds connecting the cell groups makes place for another type of folds which run perpendicular or obliquely to the lines connecting the cell groups, and which do not end, therefore, in the cell groups themselves.

A 7. *Additions to the gum arabic solution.*

No influence is excerted from 0.1 N acetic acid in the gum arabic sol, however, 0.1 N HCl or NaOH is harmful. In the two latter cases the regular prismatic form of the cells is affected and the central cells of the groups show no longer or only feebly accumulation power.

In order to study complexcoacervation in the tissue cells a mixture of gum arabic and gelatine sols is emulsified. Such emulsions, if fresh give excellent results, but if kept a longer time at room temperature they form films in which the cell walls are often seriously damaged. This effect is caused by the gelating of the emulsion drops. The gelated contents may impede here the necessary flattening of the originally spherical drops in the cell groups to form the typical prismatic cells of the tissue. See for further details a previous communication 2)

B 1. *Distance from point of the pipette to the water surface.*

This factor has not been investigated in detail. In all experiments the distance was 3 cm. With a distance of 13 cm, the diameter of film was greater, but the number of non accumulating cells was strongly increased.

B 2. *Quantity of the emulsion spread.*

This variable has no striking influence on the diameter of the film. Below a very small quantity (0.15 cc on a dish of 20 cm diameter) no manageable film (brittle) is formed, above that quantity the films have approximately the same diameter (or the diameter passes through a flat maximum).

The quality, on the contrary, does change strikingly, and is optimal at a certain small quantity of the emulsion spread (e.g. 0.2 cc on a water-surface of 20 cm, 0.3 cc on a surface of 30 cm).

Now the shapes of the cells come near to that of a regular polygon, the cell groups have a sharp boundary and the accumulation percentage rises to 95—100 %.

When the quantity spread is increased to 0.7 cc, the boundaries of the cell groups become less sharp and the accumulation percentage diminishes. The character of the system of folds connecting the cell groups, also changes in this case. With a very small quantity the number of folds bundles attached to one cellgroup, is small, (e.g. 2) this number augments (4—6—8) when the quantity spread is increased, and at last these fold-bundles do not longer end very close to the boundary of the cell groups, but at a certain distance already. For a dish of 30 cm diameter the most favourable quantity spread was 0.33 cc for the usual emulsifying liquid (ether—amylalcohol—benzene = 2 : 1 : 1), containing 2 % nitrocellulose "C" or 2.5 % celloidin.

B 3. *Diameter of the water surface.*

We have repeatedly observed that the average diameter of the film is nearly proportional to the diameter of the dish used. The next table gives

Diameter watersurface (cm)	Diameter film after 15 min. (cm)			Average diameter film (cm)	Diam. film / Diam. dish
	1st film	2nd film	3rd film		
9.3	6	6.1	—	6.05	0 651
15.2	10	9.8	9.4	9.7	0.638
20.8	12.8	12.6	12.8	12.7	0.611
26.1	15.8	15.2	15.5	15.5	0.594
30.9	19	18.4	18.6	18.7	0.605

an example, referring to 1 % C under normal conditions, except that the quantity of emulsion spread was 0.9 cc now.

An approximate constant ratio was also found with emulsifying liquids in which benzene had been replaced by toluene or xylene.

Too small a water surface (in the above table 9.3 cm) gives films with relatively poor properties. They do not contain small circular cellgroups, but irregular fields of cells, the latter having irregular forms, while their accumulating properties were not very good. Moreover, they are of no use, as their surfaces are undulated by deep and broad folds, so that they cannot be used on the cuvette.

When the diameter of the water surface is increased, we get once more the same changes in the film as we saw in A 3. already as a consequence of a decrease of the nitrocellulose concentration, and in B 2. as a consequence of a decrease of the quantity of emulsion spread.

So we find: a change of the irregular cell fields into small circular cell groups; a change of diffuse boundaries of the latter into sharp ones; a change of the type and a decreased number of the fold systems connecting the cell groups; a change of irregular cell shapes into nearly regular polygons, an increase of the percentage of accumulating cells.

Summarizing we may say that in actual practice this variable is the simplest in order to realize films of good quality, when we start from a given emulsion. Of course the practical improvement by increasing the diameter of the water surface comes to an end at a certain diameter, because the film becomes too thin then and therefore fragile, so that it is no longer manageable.

B 4 and 5. *Depth and temperature of the water in the dish.*

We have not found any influence on the morphological features nor on the quality of the film by varying the depth from 0.6—6 cm. By varying the temperature of the water from 14—21° C, practically no influence was found on the morphology or quality of the film, all other factors being favourable. The diameters of the films were only smaller at a higher temperature.

B 6. *Influence of not refreshing the tapwater in the dish.*

This influence has already been discussed in 2. The usual changes in morphology etc. manifest themselves concurrently with the decrease of the film diameter.

B 7. *Sagging of the emulsion.*

The emulsion kept in the stoppered measuring flask, must be shaken very gently, just before filling the pipette, if one aims at reproducible diameter of the film. The emulsion-drops, having a larger specific weight than the emulsifying liquid — sag after some time to the bottom (though they do not coalesce, because they are surrounded by a film of nitrocellulose). In case partial sagging the upper layers of the emulsion give films of larger diameter, the bottom layers films of smaller diameter (in a

particular case e.g. before sagging = 13.4 cm, after partial sagging upper layer = 14.1 cm and bottom layer = 12.6 cm). This partial sagging had, however, little influence on the morphology of the cellgroups or on the accumulation percentage.

B 8. *Influence of a monomolecular layer on the water surface.*

If one puts a small drop of oleic acid on the water surface before spreading the emulsion, the monomolecular oleic acid layer formed has a great influence on the diameter of the film (e.g. 10.7 → 15.7; 12.4 → 18.6; 15 → 21.9). This effect may be due to a retardation of the velocity of spreading of the very thin nitrocellulose film discussed in 4, or, maybe, to any retardation of the solidification of the latter film. We often found that the circumference of the thick film is very much nearer to an ideal circle than without oleic acid on the water surface.

We found no influence of the monomolecular oleic acid film on the water on the morphological features nor on the quality of the film, provided the conditions were already optimal for spreading on a clear water surface.

Triolein on the water also increased the diameter of the film, though to a smaller degree than oleic acid.

This in accordance with the view that the retardation of the velocity of spreading of the very thin nitrocellulose film is due to the opposing film pressure of the monolayer, this pressure being greater for oleic acid than for triolein.

B 9. *Influence of pH of the water in the dish.*

The diameter of films on destilled water was the same as on tapwater. On 0.01 N HCl the diameter was smaller (e.g. 20 %). Little influence on the morphological features and quality of the film could be found when tapwater was replaced by destilled water or by 0.01 N HCl.

B 10. *Time between spreading of the film and lifting the film from the water surface with a microslide.*

This factor has not yet been investigated in details. It was observed a few times, that films which had floated on the water surface a long time before they were used, were inferior in quality (e.g. lesions in the peripheral cells of the cell groups) to films which were lifted from the water-surface just before the shrinking (of the film spread) sets in. As the systems of folds around the cell groups are formed during the very shrinking process, and then tensions are excerted on the cell groups, the difference mentioned above might be connected with any modification of the shrinking process, if this process takes place when the film lies stretched on a micro slide.

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