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On the Action of Sodium Glycocholate on Nuclei and Chromosomes

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While KOSSEL (1891)[1], HANSTEEN-CRANNER (1919), GRAFE (1925), LEPESCHKIN (1926), GUTSTEIN (1927), and some other authors who deal with the problem of the chemical side of the nucleus, recognize the existence of lipoid in the nuclear constituents, ZACHARIAS (1909)[2] and CIACCIO (1926)[3] claim that the nucleus contains little or none of that substance. In connection with this problem, BOAS' finding that the nuclei in leaves of *Sambucus nigra* and *Vicia Faba* (BOAS, 1930), were dissolved by sodium glycocholate seems to be very interesting, because alkaline salts of glycocholic acid are known to have the property of dissolving lipoid (BANG, KNOOP, (ABDERHALDEN, Handlexikon Bd. 3. 1911); comp. also BAYER, 1907; and FÜRTH and SCHOLL 1930). In the hope of obtaining some results which may have a bearing on this line of inquiry, the action of sodium glycocholate on chromosomes was studied with pollen-mother-cells of *Tradescantia reflexa* and cells in root tips of *Vicia Faba*.

I. Pollen-mother-cells of *Tradescantia reflexa*

When a few drops of a 1 % solution of sodium glycocholate, or more dilute solutions, even as weak as a 0.03125 %, is put on a mass of fresh anther contents, including pollen-mother-cells, no coarse structures, but only starch grains, can be seen in these cells, the nuclei

1. 2. 3. Cited from KIESEL.

and chromosomes all having disappeared. By this manner of treatment of material, one can hardly determine either in what stage of mitosis the cells in question are, or to what extent these solutions act on the chromosomes or nuclei. If, however, we treat the material previously with certain dye-solutions such as acetocarmine, a 0.005 % neutral red solution, containing a trace of $CaCl_2$, or a 0.005 % solution of neutral red, in which as solvent a buffer solution whose pH value is 3 is used instead of distilled water, we can distinctly observe nuclei and chromosomes, and readily determine the stage of mitosis in the cell in question. Also, if we use any one of these dye-solutions once again after applying the test solutions, we can discriminate the extent to which the latter has affected the chromosomes and nuclei (comp. Fig. 1). The results obtained with and without the previous treatment are just the same. In the tests hereinafter mentioned, previous treatment with the first named dye-solution, and after treatment with one of these dye-solutions was always applied.

When a 1% sodium glycocholate solution is applied, chromosomes in the heterotype metaphase, which have previously been contracted by the $CaCl_2$-containing neutral red solution, immediately disappear, and, when the second dye-solution is added, they soon reappear as individually distinct chromosomes, if the addition is made one minute after the test solution has been added. If, however, the test solution is allowed to act on the chromosomes for 3 minutes, they no longer reappear in their original shapes. If, as the second dye-solution, acetocarmine is used, they form an aggregated mass of red-stained chromosome substance, which occupies the central region of the cell (Fig. 5b), and, if one of the other dye-solutions is used instead of acetocarmine, the chromosome substance does not show any clear localization, but is diffuse, and the cell is found stained more red in the peripheral than in the inner region, where the substance appears homogeneous. The chromosomes have here completely been disorganized, and the constituent substance of each chromosome has been rendered confluent with that of the others. When a 0.5 % sodium glycocholate solution is used, it takes about 10 minutes to bring the chromosomes to this extent of disorganization, and when a 0.25 % is used, it takes more than 30 minutes. The more dilute the solution is, the longer is the time which is necessary for the complete disorganization of the chromosomes. If a 0.125 % solution is used, it takes more than 1 hour, and if a 0.0625 % or 0.03125 % one is used, the chromosomes are no longer disorganized—at least not within a time which is long

enough for them to be completely disorganized when the 0.125 % solution is used,—but reappear in their own shapes when the second dye-solution is added.

Similar behaviour of chromosomes towards sodium glycocholate is also observed in pollen-mother-cells of *Lilium tigrinum*.

The process of the disorganization of chromosomes can best be traced when a 0.25 % solution of sodium glycocholate is used as the test solution. When chromosomes which have disappeared under the action of the test solution are caused to reappear by the addition of $CaCl_2$-neutral red solution, applied 10 minutes after the test solution, there appears in the chromosomes, in which the matrix is obscure, a very clear spiral band stained red (Fig. 2 b) with such clearness that it presents a keen contrast to the cases where the dye-solution is merely applied to fresh pollen-mother-cells (Fig. 2 a). When the test solution is allowed to act for 30 minutes, individual chromosomes can no longer be made clearly distinguishable by the addition of the second dye-solution; although their spiral portion is still visible, it is now very irregular in its coiling (Fig. 2 c); and, when the solution is allowed to act for 1 hour, even that is lost, and the cell is now stained more red in the periphery than in the central region.

In anaphase, telophase and interkinesis, the effect of the test solution is the same as in metaphase with a seeming exception, which is due to the fact that in these stages the chromosomes have been divided into two polar groups. As the chromosomes swell under the influence of the test solution, and the groups become larger, the red stained cytoplasm which surrounds them is centrifugally dislocated, and the part between them appears in an optical section as a red-stained band at the equatorial region of the cell (Fig. 3). This band becomes thinner as the influence increases. In the last premeiotic interphase, and the meiotic prophase, the effect is also the same as in these stages (Fig. 4); when the effect is strong enough, the nucleus disappears, and the cell is stained more red in the peripheral region than in the central (Fig. 4b). The latter region appears to be homogeneous, and the longer the test solution is allowed to act, the larger it grows, until the peripheral region is made into a thin layer. If, in these experiments, acetocarmine is used instead of the $CaCl_2$-neutral red solution for the second staining, the disorganized chromosomes are contracted into an irregular mass, and naturally in anaphase and the succeeding stages, where two chromosome groups are found, no such stained band as that just mentioned above comes into view in the equatorial region.

In accordance with BOAS' result, it is found that the disorganization process is accelerated if sodium glycocholate is used together with a neutral salt in a certain concentration. For example, while, if merely a 0.25 % sodium glycocholate solution is used, it takes more than 30 minutes to disorganize the metaphasic chromosomes, only 10 minutes is long enough, if 0.4 mol. KCl is added to the solution. No individual chromosomes reappear when acetocarmine is added, but only an irregular mass of chromosome substance, which occupies the central region of the cell. The time necessary for bringing the chromosomes or nuclei to the disorganized or swollen state, is more or less different in different pollen-mother-cells. But the range of variation is so small that it is possible to draw a sharp line of demarcation between cases in which KCl is used and those in which it is not.

A series of experiments was made in which some other salts such as NaCl, NaBr, Na_2SO_4, LiCl, $CaCl_2$ etc. were added. These salts do not appear to form a definite series according to the intensity of their influence upon the action of the sodium glycocholate solution, but seem to have almost the same intensity except $CaCl_2$, which is less intense than any of the other salts. For instance, when a 0.25 % sodium glycocholate solution containing 0.4 mol. $CaCl_2$ is used, chromosomes can reappear in their individual forms showing a clear spiral structure (Fig. 5 a) on the addition of acetocarmine, if it is added 10 minutes after the solution has been applied, while with the same mode of treatment they reappear only as an irregular, aggregated mass of chromosome substance (Fig. 5 b) when the test solution used contains one of the other salts instead of $CaCl_2$. In the former case, it takes about 15 minutes after the solution is added for the chromosomes to reach the disorganized state. That the action of sodium glycocholate is not perceptibly influenced by the previous treatment of the material with the neutral red solution containing a trace of $CaCl_2$, was shown by a control experiment.

Observations were also made under dark field illumination by means of a paraboloid condenser. In this method of observation, the region of a cell occupied by the swollen or disorganized chromosomes, is at first optically empty (Fig. 6 b), but after a few minutes, a cloudy or turbid appearance which can not be observed with the ordinary method of illumination, becomes visible in this region (Fig. 6 c), if a neutral salt is contained in the test solution. Not only NaCl, but also some other salts such as KCl, $CaCl_2$ bring about the same appearance, and the phenomenon is observed to take place irrespective of the

stage of division. The time necessary for bringing about the turbidity, depends on the concentration of the sodium glycocholate, and not on that of the salts contained, as is seen from the following table, although the lower the concentration of the latter is, the weaker is the turbidity.

Concentration of glycocholate solution in %	Concentration of NaCl in mols.	Time necessary for turbidity in minutes
0.25	0.5	3—4
0.25	0.25	3—4
0.25	0.125	3—4
0.125	0.125	10—15
0.0625	0.125	20—30

This fact shows that the salts are responsible for bringing about the turbidity, and also that, before they act on the chromosome substance, the latter must undergo a certain change produced by the sodium glycocholate.

A 1 % sodium glycocholate solution is alkaline in reaction, and hence, there is a question whether the hydrogen ion concentration is alone responsible for the chromosome swelling or disorganization mentioned above. We have, on the other hand, a datum which shows that this is not the case. If we use a 0.01 N HCl solution of sodium glycocholate in the concentration of 1 % (pH 3.4) instead of the ordinary water solution in the same concentration, the chromosomes gradually swell as usual, and entirely disappear after a few minutes (5′–10′ or more). They may be contracted by acetocarmine into an irregular mass, but no individual chromosomes can be distinguished.

Another bile salt, sodium cholate, was also found to act on the chromosomes and nuclei just in the same way as sodium glycocholate.

II. Root-tip-cells of *Vicia Faba*

In this experiment, root tips of *Vicia Faba* grown in sawdust were used as material. Roots of 3 to 4 cms. in length were washed, and then immersed in the test solution for a time. In one series of experiments, the root tips were immersed in a 2 % sodium glycocholate solution for 4 hours, 5 hours, 10 hours, 24 hours, 30 hours, 50 hours and 120 hours respectively, and were immediately fixed with CIACCIO's lipoid fixative[1], after they had just been washed with water. In an-

1. CIACCIO's fixing mixture: 5 % potassium bichromate solution 80 c.c.; 40 % formalin 20 c.c.; glacial acetic acid 5 c.c. For details, see ROMEIS (1928), p. 269.

other series, the root tips were immersed in a 2 % sodium glycocholate solution containing 0.5 mol. NaCl for 10 minutes, 20 minutes, 30 minutes, 1 hour, 2 hours, 3 hours and 5 hours respectively, and then immediately fixed in the same way as in the case of the first series. Sections, 7 micra thick, were cut, and doubly stained with Sudan III and HEIDENHEIN'S iron haematoxylin, or with the latter only, for comparison.

In those root tips, which were, as control, directly fixed with CIACCIO'S method without receiving any previous treatment with the test solutions, chromosomes and nuclei are stainable by HEIDENHEIN'S haematoxylin, but by Sudan III only very faintly. The nucleolus takes both haematoxylin and Sudan III. The cytoplasm is stained to a certain extent with Sudan III, but hardly with haematoxylin.

In the material in the first series fixed after immersions of 1 hour, 2 hours, and 3 hours respectively, no recognizable change was found in either nuclei or chromosomes. In the root tips, fixed after 4 and 5 hours' immersion, the nucleus disappears in the dermatogen, and many drops, stained with Sudan III, are found in the cytoplasm. In the region of the periblem just below the dermatogen, the nucleus is very meagre in reticulum, but contains many droplets within it. These droplets are thickly stained with haematoxylin, while the ground substances of the nucleus are faintly stained only with Sudan III (Fig. 7 a). In this case, the cytoplasm takes Sudan III more intensly than in the case where the material has not been treated with the test solution. In the next inner region of the periblem, a rather large spherical body, which appears to be the nucleus, is seen (Fig. 7 b). This body looks homogeneous, and takes Sudan III evenly, except for a spherical region, which is homogeneous as well, but remains unstained. The part of the body which takes Sudan III is positive to FEULGEN'S thymonucleic acid reaction, and it seems that this part has been derived from the nucleus by its deformation. In the region of the periblem, very near the plerome, and in the latter too, no marked effect of the sodium glycocholate is recognizable, the stainability of the chromosomes and nuclei by haematoxylin and Sudan III being the same as in the case of the control.

In material fixed after immersion for 10 hours, nuclei and chromosomes can be seen neither in the dermatogen nor in a large part of the periblem. In the dermatogen, and a few outer layers of the periblem, an irregular mass of substances, stained with Sudan III, or such masses loosely grouped together are found in the cytoplasm, which is faintly stained with the dye. In the outer region of the middle

layers of the periblem, no such masses are observable, but a number of drops of various sizes, stained with Sudan III, are found distributed in the cytoplasm, which is stained as well, though to a less degree (Fig. 8 a). These drops do not take much haematoxylin in a single staining, while the cytoplasm containing them may be deeply stained. This region of the periblem corresponds to the region where in the material fixed after 5 hours' immersion, a spherical body, stained with Sudan III, was observed. In the next inner region, the whole cytoplasm is stained to a certain extent with Sudan III, but, in most cases, contains no particular structures or visible products worth special mention, although there may sometimes be found a few small droplets, stained with Sudan III. In a few deep inner layers of the periblem, next the plerome, nuclei appear to remain almost intact, but somewhat swollen, and chromosomes are found showing a ladder, or spiral structure (Fig. 8 b). In the plerome, too, nuclei remain in a more or less swollen state, and show an internal vacuolization.

In material immersed for 24 hours, the effects of the test solution are almost the same in the dermatogen and in a large part of the periblem as in the material immersed for 10 hours, the irregular mass or masses of substances, stained with Sudan III, being also found in the cytoplasm (Fig. 9 a). These masses are negative to FEULGEN's thymonucleic acid reaction, while the cytoplasm containing them shows a weakly positive reaction. If the effect is not very strong, the nuclear boundary may be seen in the outer layers of the periblem, and the substances stained with Sudan III are found surrounding the outside of the nuclear boundary, within which the rest of the nuclear reticulum can be seen, making a lump on one side of the nucleus (Fig. 9 b). This remnant of the reticulum, when doubly stained with Sudan III and HEIDENHEIN's haematoxylin, does not show much affinity to these dyes, especially to the former, and is positive to FEULGEN's reaction. In the middle layers of the periblem, no trace of nuclei and chromosomes can be observed, but drops, which are stained with Sudan III, are found in the cytoplasm (Fig. 9 c). The substance of these drops is negative to FEULGEN's reaction. In a few deep inner layers of the periblem, just outside the plerome, both nuclei and chromosomes still exist in a swollen state, as in the materials immersed for a shorter time. The chromosomes are found stained with haematoxylin, and show a spiral structure which is more or less broken (Fig. 10). In the plerome, on the other hand, there can no longer be seen nuclei and chromosomes, but in the cytoplasm, such small drops or masses,

stained with Sudan III, are found as those which have been seen in the middle layers of the periblem.

Material fixed after an immersion of 30 hours and that after 50 hours, show no considerable difference in the results from that immersed for 24 hours. While in all the tissues except the deep inner layers of the periblem, the nuclei and chromosomes disappear, the cells being found stained uniformly with Sudan III, they still show, in the exceptional layers, a certain strong resistance to the test solution. In this region, the chromosomes lose distinctness of contour, but their spirals still remain in a more or less clear form. The nuclei also remain in a swollen state.

In material fixed after 120 hours' immersion, the influence seems to be stronger, but the results are largely the same as in the case of 30 or 50 hours' immersion. In this material a plasmolysis-like appearance is found in the cells. In the other materials, mentioned above, this is also recognizable, but to a less extent. Though it remains undetermined whether or not it represents a shrinkage of the protoplasm, due to the influence of the fixative, such has not been observed in the control, i. e. the non-treated material fixed with the same fixative. In short, so far as the present investigation is concerned, the longer the time of immersion, the clearer the plasmolysis-like appearance. In the deep inner layers of the periblem, the nuclei still remain in the swollen state, as was mentioned above in the cases of the shorter immersions, but here, the reticulum, which takes more Sudan III than haematoxylin, is very thin. The chromosomes present the appearance of the "chromosome-negative" in NĚMEC's sense, showing no trace of the spiral element within (Fig. 11). In the plerome, the nuclei completely disappear, and the cells are stained with Sudan III uniformly.

The nucleolus also disappears under the action of the test-solution. When the action is not strong enough, it remains, and takes both haematoxylin and Sudan III, to a certain extent. In the dermatogen, it can not be seen in material which has been immersed in the solution for 4 and for 5 hours. In most periblem layers, and the plerome, it remains intact even in material immersed for 10 hours, but in those of material immersed still longer it disappears. In the deep inner layers of the periblem, where chromosomes and nuclei show the strongest resistance, it also remains intact, irrespective of the length of the time of immersion, so far as the present investigation is concerned.

To sum up the results obtained, nuclei and chromosomes first swell and then disappear under the action of sodium glycocholate. In

the transition stage, drops or irregular masses of a substance giving a lipoid reaction appear in the cytoplasm, the former usually being distributed here and there, and the latter being loosely grouped together in the central region of the cell. In the case of chromosomes, the matrix swells, and disappears first, and the spiral part remains for a time in a more or less affected state, before it completely disappears. Another remarkable fact, found in the experiments mentioned above, is that, in a few deep inner layers of the periblem next the plerome, the nuclei and chromosomes, and also the nucleoli, show an apparent resistance to the sodium glycocholate solution which is stronger than in any of the other parts of that tissue, or indeed of other tissues.

In the material of the second series, which was fixed after 10 minutes' immersion, the nucleus disappears in the dermatogen, and the cell is stained with Sudan III uniformly. In the outer large part of the periblem, the nucleus exists, but swells, and is vacuolized. In a few inner layers, it swells as well, but is not so extensively vacuolized. In all the region of the periblem, it is stained with Sudan III heavily, and with haematoxylin only faintly. The cytoplasm takes both Sudan III, and haematoxylin, to a certain extent, but seems to take them more in the inner layers of the tissue than in the outer. The chromosomes in the inner layers are stained with Sudan III very faintly, and with haematoxylin slightly more deeply, so that they almost present the appearance of the "chromosome-negative". In the plerome, the nucleus stains deeply with Sudan III, but the cytoplasm only rather faintly. The plasmolysis-like appearance is marked in this material (Fig. 12 a), and the cells show it less markedly the longer it is immersed (Fig. 12 b). The influence of sodium glycocholate seems to be more intense when the "plasmolysis" is less marked. This seems, at first sight, to be inconsistent with the conclusion drawn from the experiments in which sodium glycocholate was used without NaCl, but it becomes comprehensible if it is assumed that NaCl has an effect on the permeability (cf. BOAS, 1921). In material fixed after 20 and 30 minutes' immersion, the results are almost the same, in all respects, as those just mentioned above. In material fixed after immersion for a longer time the nuclei disappear in the dermatogen, as well as in the outer layers of the periblem, droplets stained with Sudan III being found in the cytoplasm. In this root tip, "plasmolysis" is hardly observable. In the inner region of the periblem, and in the plerome, the nucleus exists, but the reticulum can not be seen in it, the whole nucleus being stained with Sudan III homogeneously. In the deep

inner layers of the periblem, the reticulum can be seen, more or less clearly, and the chromosomes as well, but they present the appearance of negative figures, not taking any dye. In material fixed after 2 hours' immersion, the nuclei and chromosomes disappear in the dermatogen, in most parts of the periblem, and in the plerome; the cells are found stained with Sudan III uniformly except in the periphery, where it stains more deeply. In the deep inner layers of the periblem, the chromosomes still retain their shapes, but appear as negative figures. Neither in the material fixed after 3, nor in that fixed after 5 hours, is there found any difference in any respect from the case of materials immersed for 2 hours, and the effect in these materials is comparable in its intensity with that found in the material of the first series which was treated with the test-solution for 24 hours.

The nucleolus disappears in the dermatogen in all the materials of the second series. In most periblem layers, and in the plerome, on the contrary, it may persist, and rather clearly, if the material is immersed for lengths of time not longer than 1 hour. In material fixed after 2 and 3 hours' immersion, it can no longer be observed in most of the layers of the periblem. In the plerome, it continues to exist, but loses the property of taking much Sudan III. In material immersed for 5 hours, in almost all cases, both in the periblem and in the plerome, except the few deep inner layers of the former, where it can always be found irrespective of the length of time of immersion, it hardly persists.

The results obtained, like those obtained from the pollen mother cells in *Tradescantia reflexa*, point to the conclusion that the action of a sodium glycocholate solution is accelerated by the addition of NaCl.

Conclusion

In the present tests, made with sodium glycocholate, the following results were obtained :— 1) When fresh material is used, the nuclei and chromosomes are, under the action of the test solution, brought to such an extremely swollen state, that they can no longer be observed under the microscope. If, in this case, the material has been treated for only a short time, they can be contracted and brought back again to their original state, in which they present their characteristic shapes, by the use of certain contracting agents such as acetic acid or $CaCl_2$. If on the other hand, the treatment is continued for over an hour, they can no longer be brought back to their individualized forms, but either remain in a diffuse state, or are contracted into a mass

of irregular shape, according to the contracting agent used. 2) When material treated with the test solution is fixed with CIACCIO's lipoid fixative, the nucleus and chromosomes can be seen only in tissues on which the test solution can hardly, or only weakly, act, and in tissues where the action is strong enough, they disappear with transition stages, in which drops or irregular masses of a substance with a lipoid reaction appear in the cytoplasm. These results we obtained are significant from the point of view that lipoid may be a constituent substance of the nucleus and chromosomes.

From the results of a series of experiments, OES came to the conclusion that cells capable of division contain a chromatin-dissolving enzyme, "nuclease", which, on addition of toluol, chloroform, phenol etc. with, or without, a certain percentage of NaCl, or some other neutral salt, can dissolve the mitotic figures, and especially quickly, those in metaphase and anaphase. NĚMEC (1910), has not positively disagreed with this theory of OES, but has, at the same time, not fully accepted his view of the existence of "nuclease" as convincingly demonstrated.

OES, who seems not to have considered lipoid as one of the main constituents of chromosomes, has not made any statement about the rôle which is played by toluol, chloroform, and phenol in his experiments. If, on the other hand, we recognize the existence of lipoid in the chromosome substance, the rôle these reagents played in OES' experiments will become conceivable, and the results we obtained with sodium glycocholate are comprehensible as being those of its direct action on lipoid, or, at least, its indirect action as an activator of a lipoid-dissolving enzyme (see WATERMAN, 1931).

We are at present unable to discuss the problem further, and, from the results of our investigation alone, no definite conclusion may be drawn as to whether or not a notable amount of lipoid is contained in the nucleus and chromosomes. If, on the other hand, there was other evidence to support the view that there is lipoid in the substance of the nucleus and chromosomes, our results might be taken as supplementary to that evidence.

KOSSEL (1891)[1], HANSTEEN-CRANNER (1919), GRAFE (1926), LEPESCHKIN (1924, '26), GUTSTEIN (1927), and some others are inclined to believe in the existence of lipoid in the nuclear substance, at least, to a certain extent. KOSSEL[1] shows no direct proof of it, but GUTSTEIN

1. Cited from KIESEL.

(1927) comes to this conclusion on the basis of the results he obtained with haematain staining. YAMAHA (1929) mentions in his monograph that he obtained positive results with SCHUMACHER's method in the peripheral layer of chromosomes, and the membrane surrounding the nucleus. HANSTEEN-CRANNER (1919) and GRAFE (1926) are rather extreme in their views, taking lipoid as the principal constituent of the nucleus without laying much stress on nucleoproteids, commonly so regarded. In his monograph, LEPESCHKIN (1924) concludes that in the nucleus, proteins may not exist in the free form, but in an unstable combination with lipoids. Our further investigation[1], undertaken in conjunction with Mr. SHINKE, the senior collaborator, also shows that lipoid is one of the chief constituents of the nucleus and chromosomes.

Since the first chemical analysis by MIESCHER, it has been commonly believed that nucleoproteids are the most important constituents of the nucleus (KOSSEL, 1911; LILIENFELD[2], 1892, '93; STEUDEL[3], 1913, '14, etc.) and that the chromatin is richer in nucleic acid in mitosis than in the resting stage of the nucleus (NĚMEC, 1910; OES, 1908). In the present experiments, it has been shown that, under the influence of a sodium glycocholate solution, the matrix of the chromosome disappears first, and then the spiral part. In our joint investigation, it was found that, in heterotype metaphase, the spiral part of the chromosomes in pollen-mother-cells of *Tradescantia reflexa* is positive to FEULGEN's thymonucleic acid reaction. The results of the tests made with sodium glycocholate are, therefore, in accord with the view that the matrix consists mainly of lipoid probably in a loose combination with proteins, and the spiral part mainly of nucleic acids, or nucleoproteids.

As to whether the fact found by the present author, that the spiral part too finally disappears under the action of sodium glycocholate, is due to the protein-dissolving property of the reagent as assumed by BOAS (1930), or chiefly to solution of lipoids contained in the spiral part, we are at present not in a position to give any suggestion, but the latter alternative seems to be likely. Summarizing the results of his experiment, YAMAHA (1927) states that "lipoidlösliche organische Verbindungen zur Auflösung des Karyotins befähigt sind".

1. To be published later.
2. 3. Cited from KIESEL.

Summary

1. In pollen-mother-cells of *Tradescantia reflexa* and in root-tip-cells of *Vicia Faba*, nuclei and chromosomes swell extremely and disappear under the action of sodium glycocholate solutions.
2. The matrix of the chromosome swells and disappears first, while the spiral part remains distinct for a time before it gradually disappears.
3. The action of sodium glycocholate is accelerated by the addition of some neutral salts such as NaCl, KCl etc. In this case, a cloudy or turbid appearance is observable under dark field illumination in the nuclear, as well as the chromosome substance, after an interval of time. The length of the interval depends on the concentration of the sodium glycocholate solution, and the intensity of the turbid appearance on that of the salts added.
4. In root-tips, the nuclei, chromosomes, and nucleoli plainly show stronger resistance to the reagents in a few deep inner layers of the periblem lying next to the plerome, than in any of the other tissues.

In conclusion, the writer wishes to express his cordial thanks to Prof. Y. KUWADA for his kind guidance throughout this investigation.

Literature

ABDERHALDEN, E. 1911. Biochemisches Handlexikon. Bd. 3.
BAYER, G. 1907. Untersuchungen über die Gallenhämolyse. Bioch. Zeitschr. Bd. 5.
BOAS, F. 1921. Untersuchungen über die Mitwirkung der Lipoide beim Stoffaustausch der pflanzlichen Zelle. Bioch. Zeitschr. Bd. 117.
BOAS, F. 1930. Zur Kenntnis der Wirkung von Gallensalzen auf die Zelle. Protoplasma. Bd. 9.
FÜRTH, O. und SCHOLL, R. 1930. Über den Einfluss von gallensauren Salzen auf Diffusions- und Resorptionsvorgänge. Ein Beitrag zur Physiologie der Fettverdauung. Bioch. Zeitschr. Bd. 222.
GRAFE, V. 1925. Zur Physiologie und Chemie der Pflanzenphosphatide. Bioch Zeitschr. Bd. 159.
GRAFE, V. und MAGISTRIS, H. 1926. Über die Phosphatide aus Daucus Carota. Planta. Bd. 2.
GUTSTEIN, M. 1926. Zur Theorie der Hämatoxylinfärbungen. Ein Beiträge zum färberischem Nachweis der Zellipoide. Virchows Arch. f. patho. Anat. u. Physiol. Bd. 261.
HANSTEEN-CRANNER, B. 1919. Beiträge zur Biochemie und Physiologie der Zellwand und der plasmatischen Grenzschichten. Ber. d. dtsch. bot. Ges. Bd. 37.
KAWAMURA, R. 1911. Die Cholesterinesterverfettung. (Cholesterinsteatose) Jena.
KIESEL, A. 1930. Chemie des Protoplasmas. Leipzig.
KOSSEL, A. 1911. Über die chemische Beschaffenheit des Zellkerns. Münch. Med. Wochenschr. Bd. 58.
LEPESCHKIN, W. 1924. Kolloidchemie des Protoplasmas. Berlin.

LEPESCHKIN, W. 1926. Über physikalisch-chemische Ursache des Todes. Biol. Zentralblatt. Bd. 46.

NĚMEC, B. 1910. Das Problem der Befruchtungsvorgänge und andere zytologische Fragen. Berlin.

OES, A. 1908. Über die Autolyse der Mitose. Bot. Zeitung. Bd. 66.

ROMEIS, B. 1928. Taschenbuch der microskopischen Technik. München und Berlin.

WATERMAN, N. 1931. Zellstruktur, Strahlung und Ferment. Protoplasma. Bd. 12.

YAMAHA, H., 1927. Experimentelle zytologische Beiträge. Jour. of the Facult. of Sci. Imper. University of Tokyo. Sec. III. Vol. 1 . part 1.

YAMAHA, H. 1929. "Saibo" (Japanese). Tokyo.

Explanation of Plates

Pl. V

Fig. 1. Pollen-mother-cells in *Tradescantia reflexa* in I-metaphase; a and b. A pair of microphotographs from the same preparation. Pollen-mother-cells were first treated with 0.005 % neutral red solution containing a trace of $CaCl_2$ (a), and then with 1 % sodium glycocholate solution for 3 minutes, not followed by after-treatment with a dye-solution (b). (C×K 12)

Fig. 2. The same; a. With the previous treatment only. b and c. Treated with 0.25 % sodium glycocholate solution for 10 minutes (b), or 30 minutes (c), the previous and after-treatment with 0.005 % neutral red solution, containing a trace of $CaCl_2$, being applied in both cases. (a & b, 1/12×K 12; c, 1/12×periplan oc. ×8, "Makam")

Fig. 3. The same in various meiotic stages from I-metaphase to Interkinesis; a, a′ and b, b′ are two pairs of microphotographs; a and b. Taken after previous treatment with the dye-solution containing $CaCl_2$; a′ and b′. End-figures, restained with the dye-solution after having been treated with 0.5 % sodium glycocholate solution containing 0.5 mol. NaCl for 5 minutes. (DD×periplan oc. ×8, "Makam")

Pl. VI

Fig. 4. The same in a young stage; a and b. A pair of microphotographs; a. Taken after previous treatment with the neutral red solution containing $CaCl_2$; b. End-figure, treated with 0.5 % sodium glycocholate solution containing 0.5 mol. NaCl, for 5 minutes, and restained. (DD×periplan oc. ×8, "Makam")

Fig. 5. The same in I-metaphase. Photographed after application of test solution for 10 minutes. Acetocarmine has here been used as an after-staining dye-solution instead of the neutral red solution containing $CaCl_2$; a. Treated with 0.25 % sodium glycocholate solution, containing 0.5 mol. $CaCl_2$; b. With the same solution, containing 0.5 mol. KCl instead of $CaCl_2$. (1/12×Hu gens oc. 5).

Fig. 6. Microphotographs of the same taken with dark field illumination; a. Ph tographed after previous treatment with 0.005 % neutral red solution, containing $CaCl_2$; b. Photographed immediately after application of the test solution, 0.5 % sodium glycocholate c ntaining 0.5 mol. NaCl; c. Photographed a few minutes later. (C×periplan oc. ×8, "Makam")

Fig. 7. From a root tip of *Vicia Faba*, treated with 2 % sodium glycocholate solution for 5 hours; a. Periblem cell layers immediately below dermatogen; b. Inner periblem, (1/12×periplan oc. ×8, "Makam")

Fig. 8. a. The same treated for 10 hours; From periblem. (1/12×periplan oc. ×8, "Makam")

Pl. VII

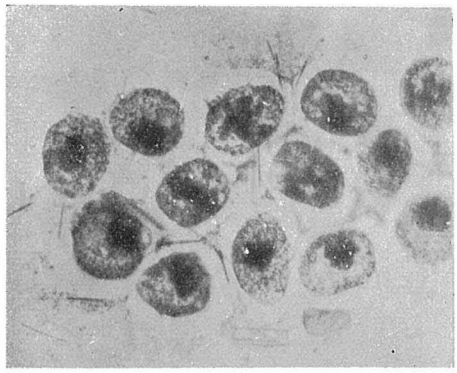
Fig. 8. b. The same. From the deep inner region of the periblem, showing chromosomes in metaphase. (Camera drawing: 1/12×K 12)

Fig. 9. The same treated for 24 hours; a and b. Periblem cell layer below dermatogen; c. Inner region of periblem. (1/12×periplan oc. ×8, "Makam")

Fig. 10. The same treated for 30 hours. A cell from the deep inner region of the periblem. (Camera drawing: 1/12×K 12)

Fig. 11. The same treated for 120 hours. From the same region as in Fig. 10. (1/12×periplan oc. ×8, "Makam")

Fig. 12. Periblem layers from the same, treated with 2 % sodium glycocholate solution containing 0.5 mol. NaCl; a. for 10 minutes; b. for 5 hours. (1/12×periplan oc. ×8, "Makam")



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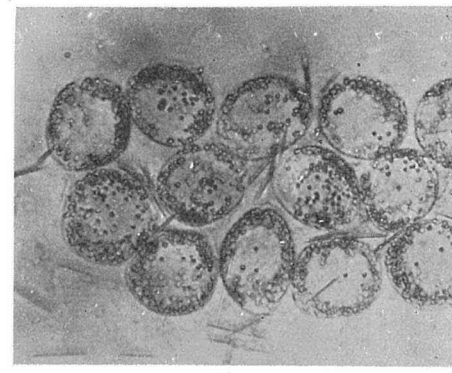

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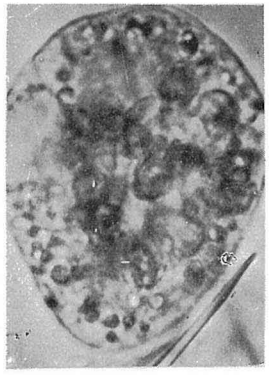

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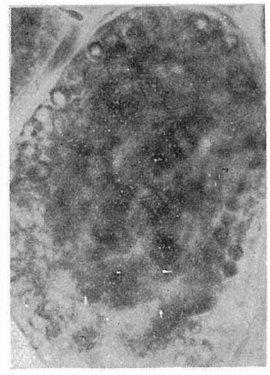

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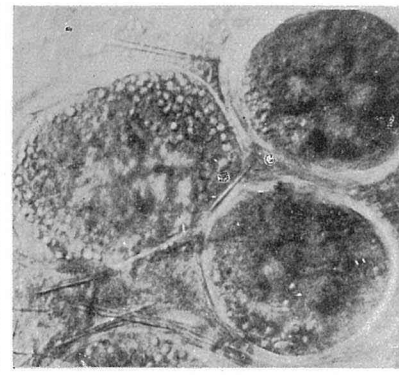

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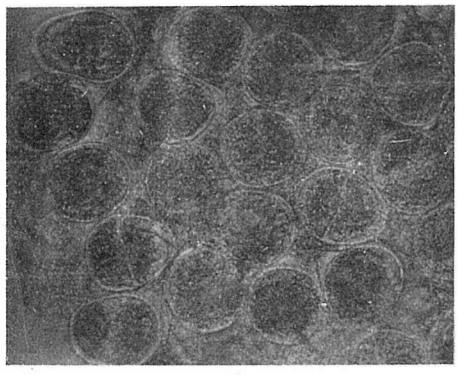

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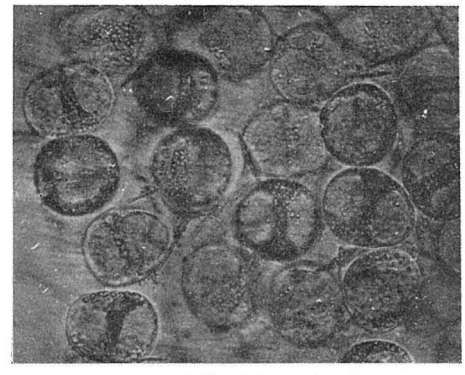

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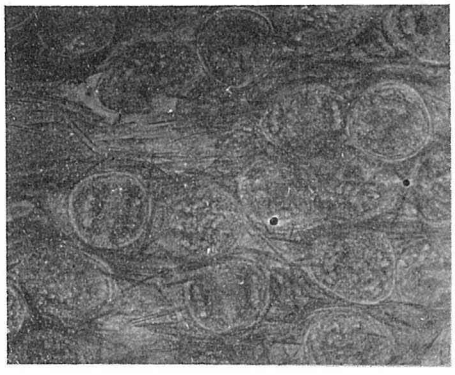

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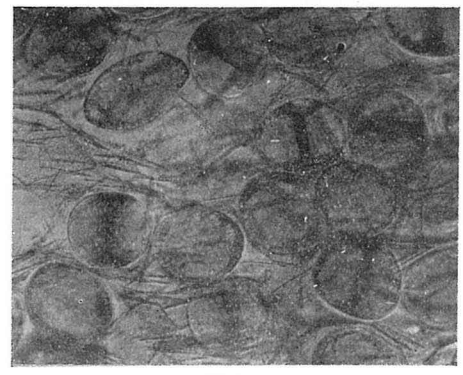
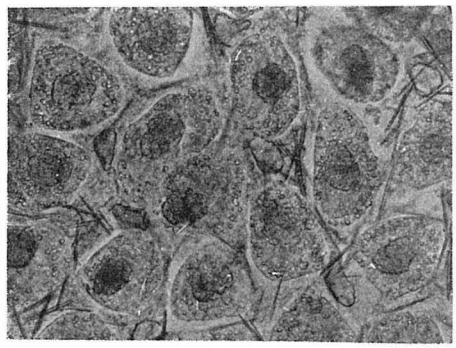


3b′

M. Shigenaga



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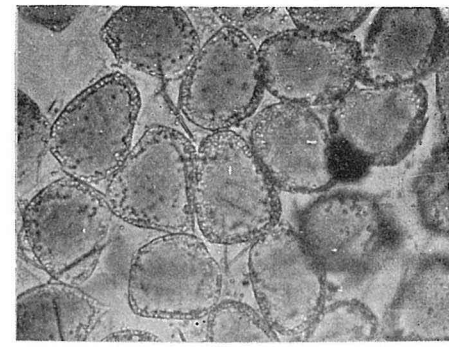

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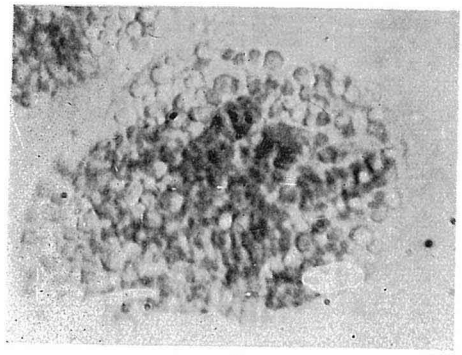

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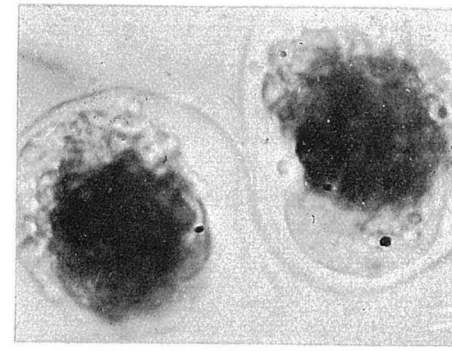

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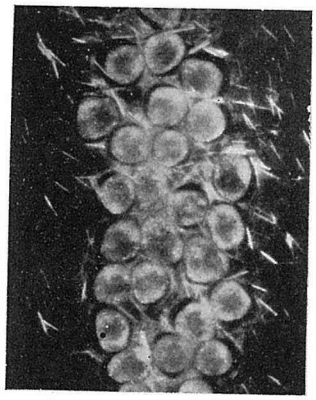

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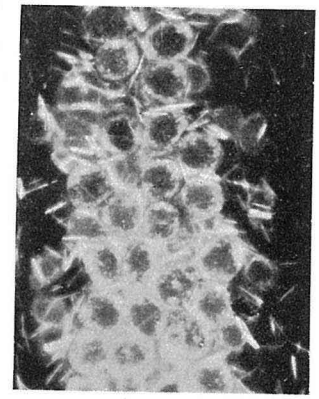

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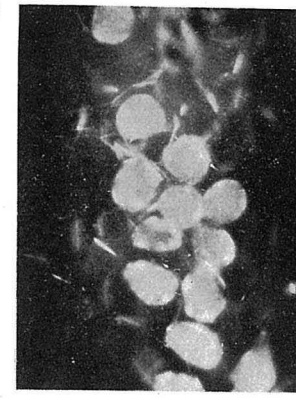

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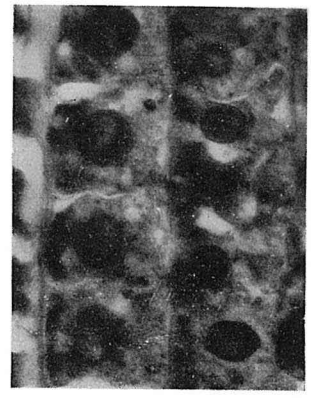

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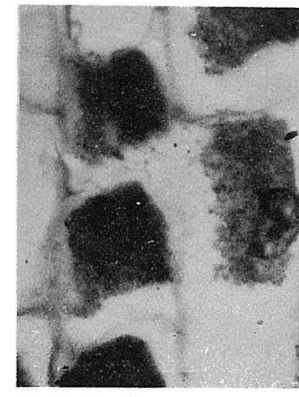

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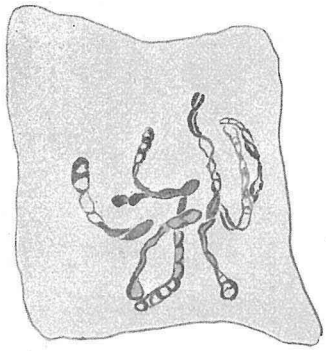

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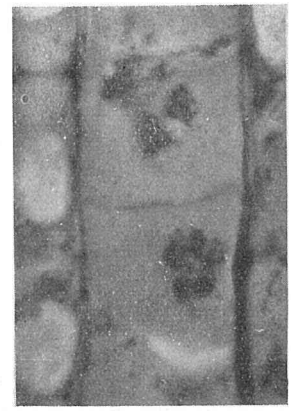

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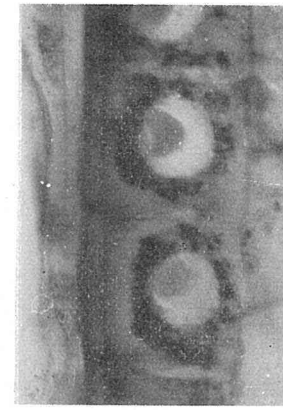

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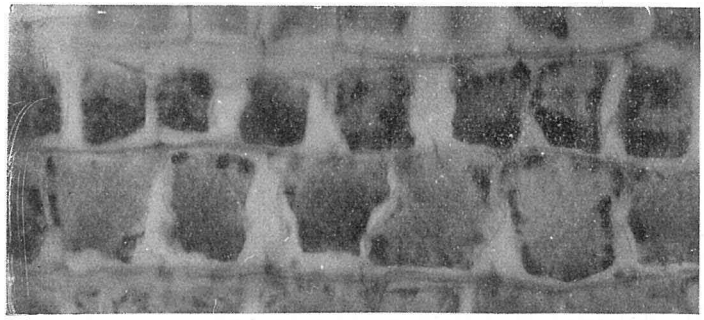

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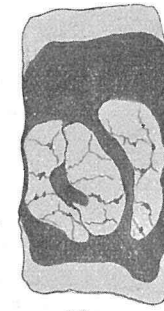

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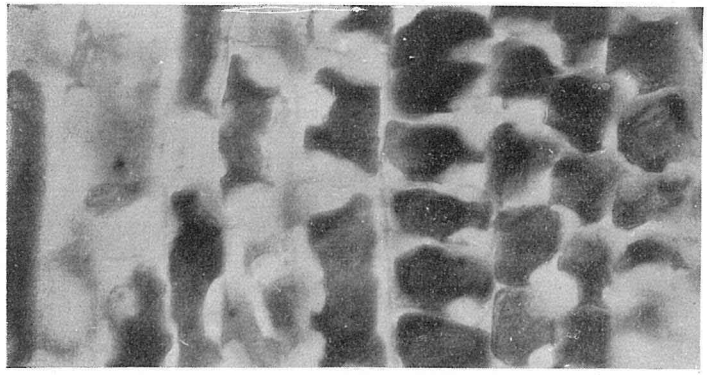

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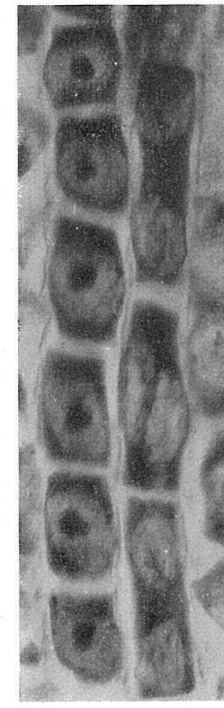

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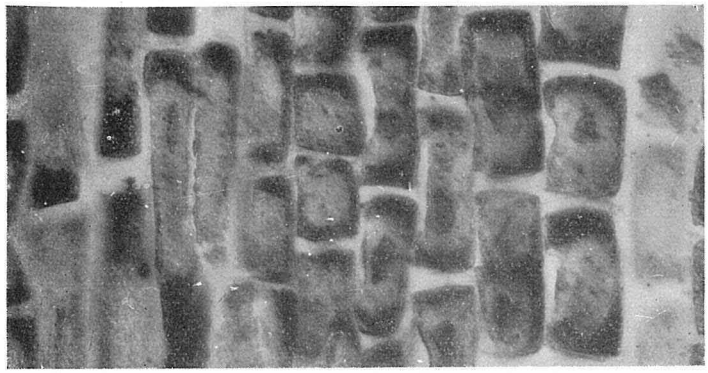

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