THE STATE OF THE CHROMOSOMES IN THE INTERPHASE NUCLEUS

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PLATES 1 AND 2

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The present knowledge of chromosomes is derived mainly from a study of cells in mitosis, where they are well defined individual structures. Much less is known about the chromosomes in the non-dividing nucleus where they appear to lose their characteristic structure and individuality. In the living resting nucleus there is usually nothing visible except nucleoli. After fixation, however, a great variety of images are produced—finely or coarsely granular structures or a network of fibers with smaller or larger clumps of chromatin. Even in the same nucleus the structure can vary greatly according to pretreatment and mode of fixation. Therefore very little has been learned about interphase chromosomes from a study of fixed preparations. Since individual chromosomes cannot be recognized in the resting nucleus even the evidence that they persist from one mitosis to the next is mainly indirect. It is based on the fact that chromosomes usually do not change their position in the resting nucleus so that they reappear in prophase in the same place as they were seen at preceding telophase (5, 9).

Recently we have isolated chromosomes from resting nuclei of mammalian tissues (33). They are bodies of characteristic size and shape, visibly double, with tightly spiraled and more or less unspiraled regions. This is a direct confirmation of the view that chromosomes persist as individual structures in the interphase nucleus and this method makes it possible to study cytologically chromosomes from resting nuclei. It is at first surprising that chromosomes from interphase nuclei look so much like mitotic chromosomes, since it is usually assumed that chromosomes unravel and become completely dispersed at telophase. In mammalian tissues like thymus, liver, pancreas, kidney, this is, however, not the case. Only certain parts of the mitotic chromosomes are unraveled in the resting nucleus, others remain tightly spiralized. These thick and darkly staining regions correspond to the heterochromatic lumps seen in fixed nuclei. Structures similar to isolated chromosomes can be recognized in fixed nuclei, or nuclei isolated in citric acid, but since so many chromosomes are packed together in a nucleus they are all tangled up and cannot be seen as individual structures. The comparison of isolated chromosomes with isolated citric acid nuclei (or otherwise fixed nuclei) shows that they have not been changed essentially by the techniques of isolation, except perhaps by fragmenta-
Such fragmentation cannot be common, since morphologically characteristic types of chromosomes are found repeatedly.

In the living uninjured nucleus, however, no structures can be seen as has been pointed out by many investigators of living cells. What, then, are the changes which the chromosomes undergo during isolation in physiological saline or during fixation? What is their structure in the living interphase nucleus? We find in the literature two main points of view. One places emphasis on the apparent lack of structure in the living nucleus and holds that the living nucleus does not contain formed chromosomes but two colloidal substances, chromatin and karyolymph, which are evenly dispersed. Upon fixation a separation of the two phases takes place resulting in the familiar fixation images. We may call this the colloid hypothesis (16, 19, 37, 40, 45). The other viewpoint emphasizes the genetic continuity of the chromosomes and holds that chromosomes exist in the living nucleus very much as they are seen in fixed preparations. They are not visible because, it is supposed, chromosomes and surrounding karyolymph have the same refractive index (21, 30, 34, 41). The best evidence for this hypothesis of structural equivalence of the living and the fixed nucleus comes from photographs of living cells taken with ultraviolet light of wave length 2600 Å, the absorption maximum of nucleic acid (14, 29, 43, 44). These photographs seem to show that in living cells the distribution of chromatin (i.e., desoxyribonucleic acid = DNA) is the same as in fixed cells. Such photographs are, however, pertinent only if it is certain that the cells were alive and uninjured at the time when the photograph was taken. The best evidence that a cell is alive is a continued normal mitosis. Another criterion is the appearance of the nucleus in visible light, for it is well known that uninjured nuclei are usually without visible structure but that upon injury structural elements appear. Such a change in the appearance of nuclei is caused by ultraviolet radiation (4, 7). Therefore, it is necessary to demonstrate that during the exposure to ultraviolet light the appearance of the nucleus in visible light has not changed. The ultraviolet photographs of "living cells" published so far lack such a proof that the cells were not injured during preparation and exposure to the ultraviolet light. It is apparent then that the ultraviolet photographs hitherto published have given no decisive evidence on the structure of the living interphase nucleus. This has also been pointed out recently by Brumberg and Larionow (11). Using undoubtedly living cells they reached entirely different conclusions than had previous workers. Their results will be discussed later in this paper.

1. Ultraviolet Photographs of Living Cells.—In studying the chromosomes in living nuclei with ultraviolet photographs it is important to choose cells which can be demonstrated clearly to be alive and which, furthermore, have large chromosomes rich in desoxyribonucleic acid, since the absorption of chromosomes at 2600 Å depends mainly on the presence of this substance. Spermato-
cytes of grasshoppers have large chromosomes and can be followed through normal divisions in suitable preparations.

Testis follicles of a grasshopper (*Melanoplus femur-rubrum*) were teased in a drop of Belar's solution\(^1\) (6) and the cells mounted in a hanging drop preparation. Suitable cells were then photographed at 2537 Å using a G. E. germicidal lamp (4 watt) with quartz condensing lens and a Bäckström filter\(^2\) (1).

In such a preparation one finds among the prophase cells (pachytene) two types of different appearance. In some cells the chromosomes are highly refractile definite structures as in fixed cells, in others, on the other hand, no definite chromosomes are visible. In Fig. 2 are shown three cells form the same cyst and therefore in the same stage of prophase. In two of them the chromosomes are distinct, in the third cell only hazy shadows can be seen. A photograph at 2537 Å gives essentially the same picture, two cells with clearly visible, absorbing chromosomes, one with diffuse absorption throughout the nucleus (Fig. 1). These three cells were then irradiated with ultraviolet light (2537 Å) for 20 minutes and photographed again in visible light (Fig. 4) and at 2537 Å (Fig. 3). We see that as a result of the irradiation the nucleus which absorbed diffusely now shows distinct chromosomes both in the visible and the ultraviolet light. From a comparison of these figures it is evident that photographs at 2537 Å show definite chromosomes only when they are already distinct in visible light. Nuclei appearing homogeneous in visible light show diffuse absorption in the ultraviolet. Distinct chromosomes can be seen only in cells which are clearly injured, either mechanically during teasing or through ultraviolet irradiation. Such cells do not continue to divide. In pachytene cells which are alive and continue mitosis the nuclei appear homogeneous and absorb diffusely at 2537 Å.

Another material often used for the study of living cells is the epidermis of onion bulb scales. The epidermis can easily be stripped free and mounted on a slide in a drop of tap water. In freshly prepared epidermis only nucleoli, but no chromosomes are visible. A photograph at 2537 Å shows the diffuse absorption of the nucleus (Fig. 5). If the preparation has been standing for a while or if dilute acetic acid is added to the water, a change takes place in the nucleus. A mass of thin coiled threads becomes visible. Upon fixation with 45 per cent acetic acid the nucleus shrinks and the chromosome threads become thicker and more highly refractile. A photograph at 2537 Å gives the same picture, the absorption being localized in definite chromosome structures (Fig. 6).

Ultraviolet photography of grasshopper spermatocytes prophase and inter-

\(^1\) NaCl (9 per cent) 20 cc.; KCl (1 per cent) 4 cc.; CaCl\(_2\) (1 per cent) 4 cc.; NaHCO\(_3\) (10 per cent) 0.4 cc. Distilled water up to 200 cc.

\(^2\) The filter used with the germicidal lamp consists of a quartz cell 5 cm. in length filled with an aqueous solution of 28 per cent NiSO\(_4\) and 8 per cent CoSO\(_4\).
phase nuclei of onion epidermis thus gives entirely different pictures when the
cells are alive than when they are injured or dead. Furthermore, ultraviolet
photographs do not reveal chromosomal structures in living cells where none
are seen in visible light. The difference in appearance of the nucleus when
alive or dead is therefore not primarily due to a change in refractive index, but
to a change in the distribution of the material with a high absorption at 2537 Å.
This is the desoxyribonucleic acid (DNA) of the chromosomes. When it is dis-
tributed evenly through the nucleus as in the living cell we shall refer to such
a nucleus as being in the extended state. When the DNA is localized in typical
chromosomal structures as in fixed cells it will be referred to as the condensed
state of the nucleus.

2. The Effect of Electrolytes and Non-Electrolytes on the Structure of the Inter-
phase Nucleus.—That the extended state of the interphase (and also prophase)
nucleus is extremely labile has been demonstrated by a number of authors since
the reversible appearance and disappearance of chromatin structures was de-
scribed by Lewis (27) and van Herwerden (23). Almost any interference with
the cell causes the appearance of visible structures in the nucleus. In order to
study the state of the chromosomes in the living nucleus and the nature of the
changes upon injury, conditions must be found which will make the state of the
living nucleus more stable. Cohen (17) described experiments in which nuclei
of onion scale epidermis were dissected out in various media. He found that in
sucrose, glycerin, and distilled water nuclei looked structureless as in the unin-
jured cell. Bancher (2) reported the same for glucose. On addition of salts
or acid Cohen saw the chromatin structures appear as in fixed nuclei.

In order to determine whether the structure of nuclei teased in sucrose cor-
responds to that in living cells, grasshopper spermatocytes and onion epidermis
cells were teased in 10 per cent sucrose and photographed at 2537 Å. Figs. 7
and 8 compared with Figs. 1 and 5 demonstrate the identity in appearance of
living nuclei and dead nuclei in sucrose solution. If such nuclei are teased out
in physiological salt solutions they have the same structure as after fixation.

If some methyl green is added to the sucrose solution the nuclei stain diffusely
green. Upon the addition of acetic acid the chromosomes appear and the green
stain is now limited to these structures. Since it has been shown that methyl
green stains specifically the DNA of the nucleus, this is further evidence that
the DNA is evenly distributed in the extended state and localized in the visible
chromosomes in the condensed state of the nucleus.

Next we isolated nuclei from mammalian tissues by teasing a small piece of
a drop of 10 per cent sucrose on a slide. Fig. 9 shows a rat liver nucleus photo-
grahed at 2537 Å. In sucrose the nucleus looks perfectly homogeneous. If
now a drop of 0.8 per cent NaCl is added to the slide the chromatin structure
appears and the nucleus shrinks (Fig. 10). If the salt is washed out with su-
crose the nucleus becomes homogeneous again (Fig. 11). This process can be
repeated several times. Figs. 12 and 13 show the appearance of calf thymus nuclei in sucrose and after fixation. Figs. 14 and 15 represent beef liver nuclei in sucrose and after fixation.

The nuclei of salivary glands in Diptera are of special interest because of their peculiar banded appearance and their significance in cytogenetic work. They are rather unusual as interphase nuclei since the chromosomes are clearly visible as individual structures after fixation. If glands are dissected out in 10 per cent sucrose only the nucleolus is visible in the nucleus. By gently pressing on the cover glass the nuclei can be squeezed out of the cell. Even so they retain their homogeneous appearance. If methyl green is added to the sucrose the nuclei stain evenly green, only the nucleolar area being unstained (Fig. 23).

These observations then lead to the conclusion that the extended state of the nucleus is not peculiar to the living cell. Even in dead nuclei the chromatin can exist either in an extended state or condensed into chromosomal structures depending on the medium. In non-electrolytes (glucose, sucrose, glycerin) the chromatin is extended, in electrolyte solution it condenses.

3. The Effect of Electrolytes and Non-Electrolytes on Isolated Chromosomes.—What is the nature of this reversible change in the distribution of DNA in the nucleus? Does the DNA go on and off the chromosomes or do the chromosomes themselves swell and contract?

To answer this question chromosomes from calf thymus resting nuclei were isolated in 0.8 per cent NaCl and suspended in sucrose. Even in the test tube the difference in appearance of the chromosomes in salt and sucrose is striking. The suspension of chromosomes in sucrose is much less opaque than in saline and greater centrifugal force is necessary to spin them down. Under the microscope individual chromosomes are almost invisible. If some methyl green is added they stain green and one can see now that they have the same general shapes but are greatly swollen (Fig. 17). Upon addition of 0.8 per cent NaCl they shrink and look again as they did originally (Fig. 16). As with nuclei the change is reversible. This change in volume of the chromosomes can be measured directly. Equal amounts of isolated chromosomes were suspended in 0.8 per cent NaCl and in 30 per cent sucrose. They were then centrifuged down until no further change in volume of the chromosome mass took place. In sucrose the volume of the precipitate is four to five times greater than in saline. The swelling and shrinking which were observed on nuclei in sucrose and salt solutions (Figs. 9–11) is therefore the result of the volume changes in the chromosomes. When the chromosomes condense the nucleus shrinks and the nuclear membrane often looks shrivelled (Fig. 10). If the chromosomes are made to extend again they fill the nucleus and expand it so that the nuclear membrane becomes tight and smooth. Analysis of chromosomes in saline and after standing in sucrose overnight shows that no DNA comes off the chromosomes when
the state of chromosomes in interphase nucleus they swell (Table I). Some pentose phosphorus is lost, caused by slight autolysis of the residual chromosome (33).

The behavior of the chromosomes in sucrose was then compared with that of residual chromosomes (cf. reference 33), that is to say chromosomes from which the histone and practically all the DNA have been removed. These residual chromosomes look the same whether they are suspended in sucrose or saline. This is further evidence that the DNA is responsible for the change in the state of the chromosomes.

These experiments on isolated chromosomes demonstrate clearly that the change observed in the appearance of nuclei is actually a change in the state of the individual chromosomes themselves. The highly polymerized DNA which makes up a large part of most chromosomes forms a gel-like structure which can reversibly extend and condense.

4. The Effect of Salt Concentration on Interphase Chromosomes.—If interphase chromosomes isolated from mammalian tissues are suspended in 1 M NaCl a thick gel is formed. Microscopic study reveals that this gel is due to the swelling of the individual chromosomes. Upon rapid stirring the gel breaks and the DNA and histone go into solution. Nuclei of onion scale epidermis behave similarly. If teased out in 1 M NaCl the nuclei become homogeneous, they swell until the membrane breaks, and the nucleolus floats out into the cytoplasm. Sometimes the nucleus swells considerably before bursting. The nuclear contents then separate into two fractions, a cap-like mass of threads and a clear area (Kappenplasmolyse, cf. references 3, 40). The fibrous mass consists most likely of the residual chromosomes, while in the clear vacuole the DNA and histone are in solution.

If nuclei are fixed in acetone followed by 95 per cent alcohol the DNA condenses, but in 1 M NaCl the nucleus becomes again homogeneous, staining diffusely with methyl green. After several hours in 1 M NaCl in the cold the nuclei no longer stain with methyl green, which indicates that the DNA has gone into solution.

The effect of salts on nuclei and chromosomes then depends on the concentration of the salts. In very low concentrations the chromosomes are in the ex-
tended state, in physiological concentration they condense. In higher concentration they extend again until finally the DNA goes into solution. The behavior of nuclei and chromosomes thus parallels the behavior of isolated nuclerohistone (32).

5. Fixation of Nuclei in the Extended State.—After treatment with most common fixatives the interphase nuclei show definite structure; the chromatin is fixed in the condensed state. But it has been observed many times that the structure of interphase nuclei varies with different fixatives. After fixation with formalin and osmic acid for instance the nuclei were often found to be quite homogeneous. Formalin and osmic acid thus preserve the extended state of the uninjured living nucleus. To prevent occasional condensation after formalin fixation we found the following method most useful. Before fixation it must be made certain that the nuclei are in the extended state, either in uninjured cells or, with dead material, in sucrose. The material is then fixed in 20 per cent formalin (1 part neutral formalin plus 4 parts water). Time of fixation depends on the size of the material. The formalin is replaced with 0.2 M lanthanum acetate for several hours. After careful washing with water the nuclei can be stained with Feulgen.

Fig. 18 shows a nucleus of onion scale epidermis fixed with formalin and stained with Feulgen. The nucleus stains diffusely and only the nucleoli are unstained. The cytoplasm and the nucleoli are perfectly clear. Fig. 19 represents a nucleus which was in the condensed state before fixation, treated in the same fashion. The chromosome threads are clearly visible. In Fig. 21 we see a pachytene of the grasshopper fixed in the extended state and stained with Feulgen. The Feulgen picture is identical with the ultraviolet photograph of a living nucleus (Fig. 1). The substance absorbing diffusely at 2537 Å in a living nucleus is therefore DNA. Isolated chromosomes in sucrose can be fixed in the extended state by adding formalin to the sucrose suspension.

6. Distribution of Protein in the Extended State.—After the nuclei are fixed in the extended state a cytochemical test for proteins can be performed (35). Only the distribution of total protein can be shown, since after fixatives which preserve the extended state the histone can no longer be removed with acids.

Fig. 20 shows a nucleus of the onion scale epidermis, fixed in the extended state, treated with trichloracetic acid–Millon reagent and photographed at 3650 Å. A marked general absorption of the nucleus and the absence of structure are apparent. Therefore, in the extended state we find not only the DNA but also protein, diffusely distributed throughout the nucleus.

7. The Behavior of Chromosomes with Low DNA Concentration.—If the reversible extension and condensation of chromosomes are due to their DNA content, then we should expect that chromosomes with a very low concentration of DNA would behave differently from the chromosomes discussed so far. The lampbrush chromosomes in the oocyte of the frog contain so little DNA that
they hardly stain with Feulgen or methyl green. Germinal vesicles of frog eggs were dissected out in 10 per cent sucrose. The chromosomes are faintly visible as delicate threads forming the characteristic loops. If the nuclei are dissected out in sucrose to which a little pyronin is added, the chromosomes begin to stain red and stand out most clearly (Fig. 24). Since pyronin in high concentration precipitates nucleic acid, the solution used was tested on a piece of calf liver. The liver nuclei, teased in this solution, remained completely extended. The pyronin, therefore, did not cause the appearance of chromosome threads. Germinal vesicles dissected out in sucrose were also fixed with 20 per cent formalin. The lampbrush chromosomes were as clearly and distinctly visible as after fixation in Carnoy (alcohol-acetic acid). Furthermore, the fixation did not distort the appearance of the chromosomes, they looked no different from those in sucrose.

Chromosomes poor in DNA thus do not show reversible condensation and their structure is not visibly altered through handling or fixation. They behave in the same way as residual chromosomes prepared from isolated calf thymus chromosomes.

We can now summarize the evidence that the DNA is responsible for the reversible extension and condensation of chromosomes. In living nuclei we have found a diffuse absorption throughout the nucleus at 2537 Å. Nuclei isolated in sucrose stain evenly with methyl green. After fixation with formalin or osmic acid the nuclei stain diffusely with Feulgen. Thus in the extended state the DNA is distributed throughout the nucleus. Finally residual chromosomes and lampbrush chromosomes which contain very little DNA do not show the reversible condensation.

8. The Nuclei of Chick Fibroblasts in Tissue Culture.—In 1946 Brumberg and Larionow (11) published some very interesting ultraviolet photographs of living and dead cells in tissue culture. Using a reflecting objective they did not have to expose the cells to the ultraviolet except in taking the photograph. They found that the nuclei of living cells absorb very little at 2600 Å. Only after the cells were killed by longer exposure to the ultraviolet did nuclear structures become visible which appeared to absorb at 2600 Å. They concluded that the DNA either did not absorb in the living nucleus or that it was differently distributed.

We have repeated and extended these observations on cultures of chick embryo fibroblasts. In order not to expose the cells unnecessarily to the ultraviolet the focussing was done in visible light and then adjusted for 2537 Å by moving the fine adjustment a definite number of units which had been determined empirically. We found that after taking a photograph at 2537 Å the cells went through normal mitosis and were therefore alive at the time of exposure. A group of cells were then killed by prolonged exposure to the ultraviolet and photographed again. The pictures of living cells were found to be

These cultures were kindly prepared for us on quartz slides by Dr. Ruth Hoffman.
strikingly different from those of cells killed with ultraviolet. In living fibroblasts the cytoplasm appears dark, the nucleus much lighter and without structure except for nucleoli. In the dead cells, however, the cytoplasm is very light, but the nucleus now stands out clearly with definite membrane and chromosomal structure. So far then our observations agree with those of the Russian workers. But it must now be determined whether the darker appearance of the dead nucleus is due to specific absorption at 2537 Å as Brumberg and Larionow assumed or caused by an increase in structural light loss due to a change in refractive indexes. Nucleic acids can be removed in cytological preparations by heating at 90°C. in 0.3% trichloroacetic acid for 15 minutes (35). A culture of chick fibroblasts was therefore fixed in acetic-alcohol and a group of cells photographed at 2537 Å. The nucleic acids were then removed in hot trichloroacetic acid and the same cells photographed again at 2537 Å. After the trichloroacetic acid treatment the nucleoli and the cytoplasm were markedly less dark, but the appearance of the chromatin had not changed. It follows that some of the dark appearance of the cytoplasm and nucleoli was due to nucleic acid. The appearance of chromatin structures in the dead nucleus, however, is not caused by specific absorption, but by structural light loss due to a change in refractive indexes. This means that the nucleus of these cells contains little DNA, while the cytoplasm is rich in ribonucleic acid. Therefore, the nucleus is lighter than the cytoplasm in ultraviolet photographs of living cells. When a cell is killed with ultraviolet the ribonucleic acid leaks out of the disintegrating cytoplasm and the refractive index of nuclear membrane and chromatin increases over that of the surrounding medium. Therefore, in ultraviolet photographs the nucleus appears now darker than the cytoplasm and with definite internal structure. Brumberg and Larionow were certainly correct in their observations that ultraviolet photographs of living and dead cells are strikingly different. But the cells they chose contain so little DNA that they are unsuited for the study of the nature of any changes in the distribution of nucleic acids. Nuclei with a higher concentration of DNA had to be used for that.

9. The State of Chromosomes during Mitosis.—During nuclear division, when the chromosomes are moved about in the cell, they occupy only a small volume of the nucleus. Since they fill the entire nucleus during interphase, they must decrease in volume in addition to the spiralization which occurs during prophase. Such a decrease in volume can be effected easily through a condensation of the DNA during mitosis. In living cells the chromosomes are usually visible during metaphase and anaphase. The change in appearance during fixation is small compared with that of the resting nucleus. This indicates that indeed a condensation of the DNA has taken place. But it is only partial, since a further condensation can still occur in mitotic chromosomes with weak acids, or through asphyxiation, without killing the cell. In fresh hanging drop preparations of grasshopper spermatocytes the chromosomes are faintly visible.
After some time the chromosomes become more refractile and sharply outlined. The same effect is observed if the pH of the medium is lowered with CO₂ or dilute acetic acid (pH 5–6). Such cells can still finish mitosis normally.

Fig. 22 shows a metaphase of the first spermatocyte division fixed with formalin-lanthanum acetate and stained with Feulgen. It comes from the same preparation as Fig. 21 which shows a pachytene nucleus in the extended condition. The metaphase chromosomes appear somewhat swollen and with hazy outlines compared with similar chromosomes fixed in acid fixatives.

In prophase chromosomes therefore a partial condensation of the DNA takes place together with the coiling of the chromonemata. During telophase this is reversed again into the maximally extended state of the interphase nucleus.

DISCUSSION

The discoverer of the nucleus, Robert Brown, described it as a clear vesicle within every cell. Since then many investigators studying living cells in both animals and plants found the nucleus to be without visible structure except for the nucleoli. The use of fixatives and dyes allowed a detailed analysis of the morphology and the complicated behavior of the chromosomes during cell division. But only conflicting results were obtained with regard to the structure of the resting nucleus, especially since little was known about the changes which take place in it during fixation. Engelmann (20) and Flemming (21) already knew that even unfixed nuclei could look quite differently depending on the medium. The reversible appearance and disappearance of chromosome structures in the living nucleus were first clearly described by Lewis (27) and van Herwerden (23) and since then a large number of papers have been published describing the various conditions under which structure appears in previously homogeneous nuclei. It was thus established that structures seen in fixed preparations are invisible in uninjured nuclei. This situation has been explained mainly in two ways: (1) The colloid hypothesis assumes that the chromatin exists in the resting nucleus in colloidal dispersion and not in individually persisting chromosomes (16, 19, 36, 40, 45). The evidence for this view is as follows: Agents causing the appearance of nuclear structures are also coagulants of nucleoproteins. The nucleus can be fixed in the extended state with osmic acid or formalin and stained with Feulgen. Such preparations show the DNA in these nuclei to be evenly distributed throughout the nucleus (17, 31, 42). In concentrated salt solutions the nuclear content is separated into two phases, karyolymph (sol) and chromatin (gel) (microdissection experiments of Strugger (40) and Bancher (3)). Microdissection shows that most nuclei are filled with a highly viscous substance, but not with chromosomal bodies (4, 26). (2) The equivalence hypothesis on the other hand holds that chromosomes exist in the living nucleus as individual structures in a more or less despiraled state and similar to the way they are seen in fixed and stained cells. It is based mainly on the evidence of genetic continuity of chromosomes and observations
on the position of chromosomes in telophase and following prophase (5, 9). Ultraviolet photographs of assumedly living cells showing typical chromatin structures absorbing at 2600 Å were thought to be definite proof for this point of view.

There can hardly be any serious doubt today that chromosomes are persistent structures and that changes in their individuality are rare events caused by spontaneous breakage or under experimental conditions (x-ray, etc.). But the cytological evidence has been indirect only, since it is usually impossible to recognize individual chromosomes in the non-dividing nucleus. With the preparation of morphologically distinct chromosomes from mammalian tissue cells the direct cytological evidence for the individual persistence of chromosomes has been produced. The problem now was no longer whether chromosomes persist during interphase, but in what state they exist in the living nucleus and what changes they undergo upon fixation. Ultraviolet photographs of uninjured cells and the Feulgen staining of formalin-fixed nuclei show that the colloid hypothesis was correct in assuming an even distribution of DNA in the nucleus. But the behavior of isolated chromosomes in electrolyte and non-electrolyte solutions demonstrates that this does not contradict the assumption of individual persistence of the chromosomes. Depending on the state of the highly polymerized DNA each chromosome exists either in an extended or condensed form. Nucleohistone itself behaves in a similar fashion towards electrolytes and non-electrolytes as was pointed out by Jeener (24) who therefore suggested that the properties of nucleohistone might explain the various aspects of nuclei and chromosomes. But the nucleus and the chromosomes are not simply gels of nucleohistone. Chromosomes consist of a complex system of non-histone protein, DNA, and histone with a definite structure. Properties of chromosomes can therefore be studied only on intact chromosomes and not on nucleohistone gels or nucleohistone fibers. Thus, even though the behavior of chromosomes in the living cell can be imitated with nucleohistone in various concentrations of inorganic ions, we know nothing as yet about the conditions in the living nucleus which cause the chromosomes to extend or condense, nor about the possible meaning of the extended state for the functioning of the chromosomes in the metabolic nucleus.

During mitosis, when the chromosomes become tightly coiled, a partial condensation of the DNA takes place. Therefore, chromosomes are generally visible in living dividing cells. Agents which cause condensation in interphase nuclei can cause a further condensation and therefore an increase in refractility of mitotic chromosomes. This explains the observations of cytologists who found that the visibility of chromosomes changes with pH, tonicity of the medium, mechanical injury, etc. (8, 12, 36). In telophase, together with the despiralization, the chromosomes extend again, so that they all touch each other and can no longer be seen individually. This process has been observed many times in living cells (cf. reference 28) and the chromosomes were said to swell
into separate vesicles. In some cases, especially during cleavage, such separate chromosome vesicles or karyomeres are clearly visible during interphase and some authors concluded from this that in every resting nucleus the chromosomes exist as vesicles, even if no membranes can be seen (25, 28). Karyomeres in prophase, however, show clearly that the karyomere membrane behaves like a nuclear membrane and not like part of the chromosome. The chromosome condenses and spiralizes inside the karyomere membrane which breaks down in later stages of mitosis (cf. reference 18). Whether a membrane is formed around a chromosome group or around individual chromosomes probably depends on whether the chromosomes are close together or widely separate at telophase. In any case the chromosomes of the resting nucleus are not vesicles surrounded by a membrane.

The state of the chromosomes in the interphase nucleus naturally determines its viscosity. In most tissue nuclei with a high concentration of DNA the chromosomes fill the entire nucleus. Therefore, nuclei were found to be filled with a highly viscous mass which could be pulled into fibers with the micro-maneipulator (4, 26). Egg nuclei, however, were described as containing a liquid of low viscosity (22). These are nuclei with a very low concentration of DNA and where the chromosomes fill only a small part of the nuclear volume.

Stedman and Stedman (38) have recently broached the hypothesis that DNA exists mainly in the nuclear sap and does not form an integral part of the chromosome. Their view was based on an unusual interpretation of the Feulgen reaction. With regard to this they have been answered adequately (10, 13, 15, 39). But it must be pointed out here that the even distribution of the DNA in the living nucleus demonstrated in this paper is entirely different from Stedman's assumption. We have shown that the DNA forms an integral part of the chromosomes and cannot be dislodged from them without breaking chemical bonds, and that the uniform distribution is the result of the swelling of the individual chromosomes, so that they fill the entire volume of the nucleus.

**SUMMARY**

In the living interphase nucleus no chromosomal structures are visible. Yet in the injured cell and after treatment with most histological fixatives chromatin structures become apparent. Under certain conditions this appearance of structure in the living interphase nucleus is reversible.

We have found that this change in the interphase nucleus is the result of a change in the state of the chromosomes. In the living nucleus the chromosomes are in a greatly extended state, filling the entire nucleus. Upon injury the chromosomes condense and therefore become visible. At the same time the nuclear volume decreases. This behavior of the chromosomes is connected with their content of deoxyribonucleic acid (DNA). This view is based on the following observations:

(a) **Distribution of DNA in the Nucleus.**—(1) The living interphase nucleus
of uninjured cells absorbs diffusely at 2537 Å. No chromosomal structures are visible in ultraviolet photographs unless they are also distinct in ordinary light. If the chromosomes are made to condense they become visible and the absorption at 2537 Å is now localized in these structures. (2) After fixation with formalin and osmic acid interphase nuclei stain diffusely with Feulgen. These fixatives preserve the extended state of the chromosomes. (3) If nuclei are teased out in non-electrolytes (sucrose, glycerin) the chromosomes are extended. Such nuclei stain homogeneously with methyl green. On adding salts the chromosomes condense and the methyl green is now restricted to the visible structures.

(b) Extension and Condensation of Isolated Chromosomes.—When chromosomes isolated from interphase nuclei of calf thymus are suspended in sucrose, their volume is four to five times larger than in saline, but they retain their characteristic shapes. Chromosomes from which DNA and histone have been removed do not show this reversible extension and condensation, neither do lampbrush chromosomes of frog oocytes which contain very little DNA.

During mitosis a partial condensation of the DNA occurs in prophase, so that the mitotic chromosomes now occupy a much smaller volume of the nucleus. At telophase the chromosomes swell again to fill the entire nucleus.

BIBLIOGRAPHY

18. Cooper, K. W., Chromosoma, 1939, 1, 51.

**EXPLANATION OF PLATES**

**PLATE 1**

_Figs. 1 and 2._ Grasshopper spermatocytes in prophase, photographed at 2537 Å and 436 μ. One uninjured and two injured cells. Zeiss 2.5 mm. quartz objective, 10 × quartz ocular. 900 × (2537 Å); 600 × (436 μ).

_Figs. 3 and 4._ Same cells, after 20 minutes' irradiation with ultraviolet (2537 Å).

_Figs. 5 and 6._ Nucleus of onion epidermis cell, photographed at 2537 Å, living and fixed with acetic acid. Zeiss 2.5 mm. quartz objective, 10 × quartz ocular. 900 ×.

_Fig. 7._ Grasshopper spermatocyte prophase, killed with ultraviolet radiation (2537 Å) suspended in 10 per cent sucrose. 2537 Å, Zeiss 2.5 mm. quartz objective, 10 × quartz ocular. 900 ×.

_Fig. 8._ Onion epidermis nucleus, teased out in 10 per cent sucrose. 2537 Å, Zeiss 2.5 mm. quartz objective, 10 × quartz ocular. 900 ×.

_Figs. 9, 10, and 11._ Rat liver nucleus, teased free in 10 per cent sucrose (9); suspended in 0.8 per cent NaCl (10); salt washed out with 10 per cent sucrose (11). Reversible condensation of chromosomes in electrolyte solution. 2537 Å, Zeiss 2.5 mm. quartz objective, 10 × quartz ocular. 1800 ×.

_Figs. 12 and 13._ Calf thymus nuclei, suspended in 10 per cent sucrose and after fixation in Carnoy. 2537 Å, Zeiss 2.5 mm. quartz objective, 10 × quartz ocular. 1800 ×.

_Figs. 14 and 15._ Beef liver nuclei, suspended in 10 per cent sucrose and after fixation in Carnoy. 2537 Å, Zeiss 2.5 mm. quartz objective, 10 × quartz ocular. 1800 ×.
(Ris and Mirsky: State of chromosomes in interphase nucleus)
PLATE 2

Figs. 16 and 17. Isolated chromosomes from calf thymus, stained with methyl green in 0.8 per cent NaCl (16) and in 30 per cent sucrose (17). 630 mm, Zeiss 2 mm. objective, 15 × ocular. 1200 ×.

Fig. 18. Onion epidermis nucleus, fixed in extended state with formalin and lanthanum acetate, stained with Feulgen. 546 mm, Zeiss 2 mm. objective 15 × ocular. 1200 ×.

Fig. 19. Onion epidermis nucleus treated as in Fig. 18, but nucleus was in condensed state before fixation. 546 mm, Zeiss 2 mm. objective, 15 × ocular. 1200 ×.

Fig. 20. Onion epidermis nucleus, fixed in extended state with formalin and lanthanum acetate, treated with trichloroacetic-Millon to show distribution of protein. 365 mm, Zeiss 2 mm. objective, 10 × ocular. 860 ×.

Fig. 21. Grasshopper spermatocyte prophases, fixed with formalin and lanthanum acetate, stained with Feulgen. 546 mm, Zeiss 2 mm. objective, 15 × ocular. 1200 ×.

Fig. 22. Grasshopper spermatocyte, metaphase of first meiotic division. Fixed in formalin and lanthanum acetate, stained with Feulgen. 546 mm, Zeiss 2 mm. objective, 15 × ocular. 1200 ×.

Fig. 23. Drosophila pseudoobscura, salivary gland nucleus teased out in 10 per cent sucrose, stained with methyl green. 630 mm, Zeiss 2 mm. objective, 15 × ocular. 1200 ×.

Fig. 24. Frog oocyte lampbrush chromosome. Germinal vesicle isolated in 10 per cent sucrose, stained with pyronin in sucrose. 546 mm, Zeiss 2 mm. objective, 15 × ocular. 2400 ×.
(Ris and Mirsky: State of chromosomes in interphase nucleus)