THE CHEMICAL COMPOSITION OF ISOLATED CHROMOSOMES

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PLATE 3
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The isolated chromosomes, whose chemical composition is to be described in this paper, are derived from the lymphocytes of calf thymus. The composition of these chromosomes has been investigated with reference only to their nucleic acid and protein contents, simply because these are quantitatively the major constituents. The procedure for isolating chromosomes has already been given in a previous paper (1).

By far the larger part of present knowledge of chromosomes comes not from chemistry but from cytology and genetics. Any information concerning the chemistry of chromosomes is accordingly at once placed against a background of chromosome morphology and cytogenetics. In an investigation of the chemistry of isolated chromosomes the chemical basis of chromosome structure will be sought; and in the present investigation this aspect of chromosome chemistry is considered. The results obtained may be briefly summarized: isolated lymphocyte chromosomes can be separated into two parts, of which one (some 90 per cent of the mass of the chromosome) is mainly deoxyribose nucleohistone and the other a coiled, thread-like structure containing non-histone protein combined with relatively small quantities of ribose and deoxyribose nucleic acids; this thread still shows the main characteristics of chromosomes, such as coiling, subdivision into heterochromatic and euchromatic sections, and constrictions; it appears to be imbedded in a mass of nucleohistone; both the thread and the material in which it is imbedded contain nucleoproteins, but of entirely different kinds.

Elementary Composition.—The phosphorus content of isolated lymphocyte chromosome (after extraction with alcohol and ether, to remove lipids) is 3.7 per cent and the nitrogen content 15.6 per cent. It can be shown, in a manner to be described below, that nearly all the phosphorus in such a preparation is present as deoxyribose nucleic acid, which accordingly constitutes close to 37 per cent of the mass of these lipid-free chromosomes.

Histone Content.—Thymus histone has been known for many years, and there has been little doubt that this highly characteristic basic protein is located in the chromosomes of the thymus. That this is in fact true, can be demonstrated by releasing histone from isolated thymus chromosomes. Kos-
sel's procedure for extracting histone was to treat the histone-containing tissue with an excess of dilute hydrochloric or sulfuric acid (2). When an excess of acid is added to isolated chromosomes, histone is released; indeed, little in addition to histone passes into solution. There is no difficulty in identifying as a histone the protein in solution. The basic character of this protein becomes apparent when dilute sodium hydroxide is added to the acid solution, for the protein remains dissolved until the medium becomes definitely basic, maximum precipitation occurring in the neighborhood of pH 10. If the base used is ammonium hydroxide, the protein precipitate obtained does not redissolve when an excess of this weak base is added. Insolubility in an excess of ammonium hydroxide was observed by Kossel at the time he discovered the histones and has ever since been regarded as one of their important characteristics.

Another property of the protein released by acid from isolated chromosomes which serves to identify it as a histone is its non-precipitability by divalent mercury (3). In the presence of dilute sulfuric acid, divalent mercury is known to be a general protein precipitant, acting rapidly if the solution is warmed to 60°C. Under these conditions histones, unlike other proteins, are not precipitated. When sulfuric acid in which a divalent mercury salt is dissolved is added to isolated chromosomes, histone is released and passes into solution.

The amount of histone released by a strong acid from a nucleoprotein complex can be estimated, and it has frequently been supposed that the histone so released represents the total amount present in the nucleoprotein complex. We have estimated the quantities of histone released from isolated chromosomes by acid and also by acid in which divalent mercury is dissolved. Slightly more histone is released by the latter procedure. It might appear as if the amount of histone released did indeed represent the total quantity present in the chromosomes, for the quantities so estimated are reproducible and repeated extraction releases no more histone. We find, however, that much of the histone fails to be released by acid; indeed, a third of the total histone may remain attached to the nucleoprotein complex. If the quantity of non-histone protein in isolated chromosomes or nuclei is calculated by subtracting from the total protein present the amount of histone released by acid, the non-histone content of chromosomes or nuclei will be grossly exaggerated.

Before describing experiments which make possible a more effective fractionation of the proteins present in isolated chromosomes than has heretofore been accomplished, another distinctive property of histones should be mentioned. Histones contain relatively little tryptophane. In some histones such as those of calf liver there is as much as 0.4 per cent tryptophane (and even this can be considered a rather low tryptophane content for a protein); other histones, such as those of the fowl erythrocyte, contain practically no trypto-
phane. The histone released by hydrochloric acid from isolated thymus chromosomes contains 0.14 per cent tryptophane.¹

Since the generality of proteins contain far more tryptophane, in many cases 1 per cent or more, the low tryptophane content of histones means that finding a considerable quantity of tryptophane in a protein fraction of isolated chromosomes indicates that some other type of protein, a non-histone protein, may be present. In a previous investigation the existence of such a tryptophane-containing protein in chromosomes was recognized (4). Since then a far better procedure has been found for separating the tryptophane-containing protein fraction from the histone fraction, and at the same time the significance of these two protein fractions for the structure of the chromosome has been recognized.

Fractionation of Chromosomes.—Isolated chromosomes are fractionated by placing them in a neutral solution of 1 M sodium chloride. In this medium they at once disperse to form an opalescent, highly viscous suspension. When this striking transformation occurs, one has the impression that perhaps the chromosomes have dissolved, and much material has indeed passed into solution; but when a drop of the viscous opalescent fluid is examined under the microscope, formed structures can still be seen. It might be supposed that the striking increase in viscosity is due to swelling of chromosomes, but centrifugation shows that this is not the case.

To sediment the chromosomes, rapid centrifugation is required because of the exceedingly high viscosity of the medium in which they are suspended. For this purpose we use a centrifuge running at 18,000 to 19,000 R.P.M., with a rotor 30 cm. in diameter, holding 12 tubes, each of 75 cc. capacity. After a run of 1 to 2 hours, the centrifuged material consists of a slightly opalescent but highly viscous supernatant and a scanty, tightly packed sediment. In the supernatant a few chromosomes are visible, but practically all of them are in the sediment. These bodies, which will be referred to as residual chromosomes, are resuspended in 1 M NaCl and again sedimented, and this time a far lower speed suffices. After several more washings with 1 M NaCl, no more material is present in the supernatant. The residual chromosomes in 1 M NaCl are stored in the cold.

Under the microscope these threads still look like chromosomes, but they are a good deal smaller. They exhibit longitudinal differentiation into thicker, tightly coiled heterochromatic sections and more loosely coiled euchromatic regions. As in whole chromosomes, constrictions are frequently visible. (Compare Fig. 1 with Figs. 2 to 4.)

¹ More than one kind of histone is present in the thymus. The evidence for this is that the tryptophane content of histone released by hydrochloric acid is less than the tryptophane content of the total thymus histone, 0.4 per cent.
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The staining characteristics of residual chromosomes are, when compared with original chromosomes, grossly changed. Before being treated with $\text{m} \ \text{NaCl}$ the chromosomes stain intensely with the Feulgen reagent, aceto-orcein, crystal violet, and hematoxylin: (staining with pyronine-methyl green will be described below). The residual chromosomes stain only faintly with these reagents. Loss of staining properties is not due to some change in physical state of the chromosomes; failure to stain is caused by the removal of material from the chromosomes. The substances responsible for staining are present in the supernatant. $\text{m} \ \text{NaCl}$ has, accordingly, separated the chromosomes into two parts: a residue which retains the essential form of the chromosome; and a soluble extract which contains the substances with the characteristic staining properties of chromosomes. The composition of each of these chromosome fractions will now be described.

$\text{m} \ \text{NaCl}$ was used by us some time ago to extract a nucleoprotein complex, chromatin, from the cell nucleus (4). At that time the significance for chromosome structure of the different components in the nucleoprotein complex removed from the nucleus was not yet understood.

The Residual Chromosome.—The material that remains insoluble when chromosomes are extracted with $\text{m} \ \text{NaCl}$ consists, as has already been said, of microscopic bodies with forms resembling those of the intact chromosomes. After thorough extraction with $\text{m} \ \text{NaCl}$, these residual chromosomes are extracted with alcohol and ether to remove any lipids present, and are then weighed. It is found that they represent only 8 to 10 per cent of the mass of the original (lipid-free) chromosomes from which they were prepared.

By far the larger part of nucleoprotein in the isolated chromosome dissolves in $\text{m} \ \text{NaCl}$, but some nucleoprotein, although of a different kind remains in the residue. The first sign that nucleoprotein may be present is provided by the phosphorus and nitrogen analyses of the lipid-free residue. Phosphorus content varies in different preparations from 1.0 to 1.8 per cent. The nitrogen content is 13.8 per cent. It will soon be seen that all of the phosphorus is in the form of nucleic acid. Presence of protein is indicated by positive tests for a number of amino acids—arginine, cystine, tyrosine, and tryptophane.

Nucleic acid can be removed from a nucleoprotein by heating at 90°C. for 15 minutes in 0.36 $\text{m}$ trichloracetic acid, after first treating with cold trichloracetic acid, to remove any nucleotides present (5). When this is done with residual chromosomes, no phosphorus is removed by cold trichloracetic acid and all of the phosphorus passes into the solution of hot trichloracetic acid. The absorption spectrum in the ultraviolet of the dissolved material differs slightly from nucleic acid, but the same difference occurs in the absorption spectrum of nucleic acid when it is heated in trichloracetic acid. Considering the phosphorus content of the trichloracetic acid extract the extinction coefficient at the wave length of maximum absorption is very close to that of
nucleic acid of the same phosphorus content. A characteristic of nucleic acid, as compared with simpler nucleotides, is its relatively high molecular weight, rendering it non-diffusible through a cellophane membrane. The material extracted from residual chromosomes by hot trichloracetic acid readily passes through a cellophane membrane, but so also does nucleic acid after the same treatment with trichloracetic acid. It should be noted that a mixture of purines and pyrimidines like those linked together in nucleic acid would have the same absorption spectrum, but if the relative proportions of the four bases were considerably different from the equimolar ratios found in nucleic acid, the difference would alter the absorption spectrum.

Pentose and desoxypentose were estimated in the trichloracetic acid extract by their reactions with orcinol and diphenylamine. The sum of the two sugars was equal to what would be expected if the phosphorus present were all in the form of nucleic acid, part of it as ribose and the rest as desoxyribose nucleic acid. In a nucleic acid the ribose of only the purine nucleosides reacts with orcinol, the ribose of the pyrimidine nucleosides remaining inactive (6, 7); and, similarly, the desoxyribose of only the purine nucleosides reacts with diphenylamine (3). The phosphorus content and extinction coefficient of a nucleic acid are over-all properties of both purine and pyrimidine nucleotides; the orcinol and diphenylamine reactions estimate, on the other hand, constituents of the purine nucleotides only. By combining all these analytical data, the ratio of purines to pyrimidines can be deduced, and in a large number of both pentose and desoxypentose nucleic acids they have been found to be present in a 1:1 ratio. In the trichloracetic acid extract of residual chromosomes, measurements of extinction coefficients and estimates of phosphorus contents and of pentose and desoxypentose contents all indicate that in this material too the purine pyrimidine ratio is close to 1:1. This is strong evidence that the substances extracted from chromosome residues by hot trichloracetic acid are nucleic acids.

Nucleic acid can also be separated from residual chromosomes by allowing them to remain for some time in neutral 0.14 M NaCl. This process will be described below but here it is mentioned because the evidence that it is indeed nucleic acid that is released is more complete than when hot trichloracetic acid is used. In this case the material extracted has precisely the same absorption spectrum as that characteristic of nucleic acid, and furthermore most of it resembles nucleic acid in not diffusing through a cellophane membrane. All of this nucleic acid is of the ribose type, for the desoxyribose nucleic acid present in residual chromosomes is not removed in 0.14 M NaCl. The quantity of pentose determined in this nucleic acid is the same as that found in a sample of ribose nucleic acid of the same phosphorus content prepared from yeast, indicating that in the ribose nucleic acid of residual chromosomes as in that of yeast there is a 1:1 ratio of purine to pyrimidine nucleotides.
The nucleic acid content of residual chromosomes represents only about 4 per cent of the total amount present in whole chromosomes. The rest is extracted with \( m \) NaCl. The nucleic acid content of the residual chromosome is mainly of the ribose type, although some desoxyribose nucleic acid is also present. This is in sharp contrast to the distribution of nucleic acids in the chromosome as a whole, in which some 95 per cent of all the nucleic acid is of the desoxyribose type, so that there is some difficulty in detecting that ribose nucleic acid is present. Once the mass of nucleic acid has been removed from chromosomes by extraction with \( m \) NaCl, there is no difficulty in detecting ribose nucleic acid in the residues. In different preparations the ribose nucleic acid content of residual chromosomes varies from 7.5 to 14 per cent, and the desoxyribose nucleic acid from 1.5 to 2.6 per cent.

It may be supposed that the small quantity of desoxyribose nucleic acid in the residual chromosomes is merely a "contamination" remaining after removal of nucleohistone with \( m \) NaCl. All that can be said at present is that every preparation of residual chromosomes contains some desoxyribose nucleic acid. Most of it is released from the residual chromosomes by desoxyribose nucleopolymerase.

The staining properties of residual chromosomes can be correlated with what is known of their nucleic acid contents. They are Feulgen-positive, but only faintly so. When one considers that residual chromosomes contain no more than 2.6 per cent of desoxyribose nucleic acid, a positive reaction shows how sensitive the Feulgen technique is. Stained with the pyronine-methyl green mixture of Unna-Pappenheim, residual chromosomes are red, whereas the same dye mixture stains the original isolated chromosomes purple-blue. Brachet has shown that in general the pyronine-methyl green mixture stains desoxyribose nucleoproteins blue-green and ribose nucleoproteins red. The staining of chromosomes and residual chromosomes with pyronine-methyl green is, then, what would be expected, considering their respective nucleic acid contents. The staining of residual chromosomes with pyronine-methyl green shows that the ribose nucleic acid present in a preparation of these bodies is actually in the residue itself and not merely in some other material present in the preparation. This is worth noting because it is known that nucleoli contain ribose nucleic acid and in preparations of isolated chromosomes some nucleoli can indeed be seen. It cannot be said how significant the ribose nucleic acid content of nucleoli is for the over-all determination of ribose nucleic acid in a suspension of residual chromosomes but staining shows that by far the larger part of this nucleic acid is present in the residual chromosomes themselves. It should be noted that by staining with pyronine-methyl green Brachet has already provided evidence that in some chromosomes ribose nucleic acid is present (8).
Even after removing the nucleic acids of residual chromosomes with hot trichloracetic acid or by allowing them to remain in 0.14 M NaCl, these bodies still retain their structure. The protein component of residual chromosomes is, accordingly, responsible for their structure. This protein will be referred to as the residual protein of the chromosome. Residual protein represents some 8 per cent of the mass of the isolated lymphocyte chromosome. Histone, on the other hand, accounts for 55 per cent of the mass of these chromosomes. Residual protein and thymus histone differ in numerous other respects: histone contains 18 per cent of nitrogen, residual protein close to 13 per cent; histone is soluble in a HgSO₄ -- H₂SO₄ mixture, whereas residual protein, like the generality of proteins, is insoluble in the HgSO₄ -- H₂SO₄ reagent; histone is soluble in water and in dilute acid, but residual protein has so far not been soluble in any medium in which proteins remain intact; residual protein contains 1.36 per cent tryptophane whereas thymus histone contains 0.14 per cent. Investigation of the properties of residual protein has only just begun, but it is already clear that it is quite different from histone, not possessing those properties of histone which distinguish this type of protein from other proteins.

Both histone and at least a part of the residual protein occur as nucleoproteins in the chromosome. Among the numerous differences between these nucleoproteins the nature of the linkage between nucleic acid and protein should be considered. In nucleohistone there is at present no reason to think that there is any more than a salt-like linkage between the two components. In the residual protein it seems likely that nucleic acid and protein are in something more than salt-like combination, for concentrated neutral salt does not dissociate this nucleoprotein, as it does a nucleohistone.

It will be of interest to know which of the enzymes present in chromosomes are located in the residual chromosome. At present it may be said that all of the alkaline phosphatase is located in the residual chromosome.

In a previous investigation of the constituents of chromosomes a non-histone, tryptophane-containing protein was isolated. It seems apparent that this protein is the same as what is now recognized as the protein component of the residual chromosome.

The chromosome fraction soluble in 1 M NaCl; i.e., the supernatant obtained when a suspension of chromosomes in M NaCl is centrifuged at high speed. This solution, it will now be seen, consists largely of nucleohistone.

When the supernatant is diluted with six volumes of water, a beautiful fibrous precipitate forms. This precipitate readily redissolves in M NaCl. Nuclear material with this property has been encountered before (3, 4), and it is now obvious that previous preparations contained some residual chromosomes in addition to nucleohistone. Combining the information we now have with that obtained previously, it is apparent that when a suspension of chromosomes in M NaCl is centrifuged at high speed, residual chromosomes sediment...
first, leaving nucleohistone in solution. More prolonged centrifugation (as carried out in previous work) leads to a fractionation of nucleohistone because nucleic acid sediments faster than histone, so that the top layer of fluid after prolonged centrifugation consists almost entirely of histone. One sign of this fractionation is that when the top layer, containing histone, is diluted with six volumes of water, no precipitate forms. A fibrous precipitate appears under these conditions only when both histone and highly polymerized deoxyribose nucleic acid are present. The quantity of precipitate formed from a given volume of the m NaCl supernatant after sedimentation of residual chromosomes is found by washing the precipitate with hot alcohol, ether, and determining the dry weight. The phosphorus content of this material is 4.4 per cent, equivalent to a nucleic acid content of 45 per cent.

Nucleic acid in the m NaCl supernatant can be detected spectrophotometrically. The absorption spectrum of such a solution is compared with that of a nucleic acid solution of the same phosphorus content. The curves are identical down to 2450 A.u. The divergence at shorter wave lengths is due to presence of histone in the supernatant. The equality of the extinction coefficients at 2600 A.u. shows that the same quantity of nucleic acid is present in both solutions and, therefore, that all of the phosphorus in the m NaCl supernatant is in the form of nucleic acid.

Practically all of this nucleic acid is of the deoxyribose type. This is shown by carrying out the diphenylamine reaction for deoxyribose on the supernatant and on a sample of pure deoxyribose nucleic acid of the same phosphorus content. The same color intensities are obtained. This agreement, as has been explained above, also shows that the purine pyrimidine ratio in the m NaCl supernatant is 1:1, as in nucleic acid itself. The phloroglucinol reaction (9) for ribose shows that a small quantity of ribose nucleic acid is probably present in the m NaCl supernatant.

To estimate the histone content of the m NaCl supernatant, a given quantity is added to six volumes of water, and histone is released by treating the fibrous precipitate so obtained with a HgSO₄ – H₂SO₄ mixture. When sodium nitrite is added to histone in this solution a derivative is formed with an absorption band at 3540 A.u.. The histone concentration is determined by measuring the extinction coefficient of 3540 A.u. and comparing it with that given by a solution of known histone content. This is the Millon reaction. A new feature in the procedure that has just been described is that whereas the familiar red pigment produced in the Millon reaction has an absorption maximum at 5000 A.u., another absorption maximum at 3540 A.u. with much higher extinction coefficient has been found.

The histone released and estimated in this way is equal to 47 per cent of the mass of the fibrous precipitate. Since 45 per cent of this precipitate consists of nucleic acid, 8 per cent would still be unaccounted for. This, as will now be
shown, is histone that is not released from nucleic acid by either hydrochloric or sulfuric acid. That some protein still adheres to nucleic acid after treatment with the \(\text{HgSO}_4 - \text{H}_2\text{SO}_4\) reagent is apparent from the red color of the precipitate when sodium nitrite is added.

A method is needed for removing and collecting all the protein from the nucleic acid in the \(\text{m NaCl}\) supernatant. We have already used such a procedure in our previous work \((4)\). Just sufficient sodium hydroxide is added to the supernatant so that no precipitate is formed when a sample is added to six volumes of water. The alkaline supernatant is shaken vigorously or mixed at high speed with a chloroform-octyl alcohol solution, as used by Sevag. When this mixture is centrifuged, three layers are obtained. The top layer consists of a solution of nucleic acid, nearly free of protein, and the middle layer consists of protein, quite free of nucleic acid.

The protein in the middle layer can be collected without loss, washed free of salt, dried, and weighed. It represents 59 per cent of the mass of chromosomes from which it was prepared. This, added to the 37 per cent of nucleic acid present in the chromosomes, accounts for 96 per cent of the total chromosome mass. Practically all of the protein collected in the middle layer is of the histone type.\(^2\) One indication of this is that when the \(\text{HgSO}_4 - \text{H}_2\text{SO}_4\) reagent, which precipitates most proteins but dissolves histones, is added to a suspension of this material, almost all of it dissolves, the insoluble residue being insignificant. If the quantity of histone in solution is estimated by measuring the extinction coefficient at 3540 A.u. of the derivative formed by adding sodium nitrite, this is found to be the same as the total quantity of protein in the suspension as determined by dry weight.

Further evidence that nearly all the protein collected in the middle layer is of the histone type is that all of it is basic. The protein collected in the middle layer can be dissolved by adding a little dilute hydrochloric acid. Practically all the protein dissolves at a pH close to 4.0. When sodium hydroxide is added to this solution, no precipitate appears until a pH of over 9 is reached and maximum precipitation occurs between pH 9.3 and 10.0. Of the total protein, 92 per cent precipitates, and this must be considered to be definitely basic protein. The protein that remains in solution is also basic, because it combines with nucleic acid at pH 8.0 to form a fibrous precipitate.

**Instability of Chromosomes.**—Both whole isolated chromosomes and residual chromosomes show marked instability under certain conditions. A suspension of residual chromosomes is quite stable at 0° in \(\text{m NaCl}\). But when the salt concentration is reduced to 0.14 \(\text{m}\) (physiological saline) and the pH is adjusted

\(^1\) In recent experiments on the chemical composition of isolated liver chromosomes, we find that a considerable part of the protein collected in this way is of a non-histone type. It is likely that a small quantity of this non-histone type of protein is also present in lymphocyte chromosomes.
to 6.8, a change occurs, slowly in the cold and exceedingly rapidly at room temperature. This change is demonstrated by centrifuging portions of the suspension of residual chromosomes and examining the clear supernatant solution. It contains ribose nucleic acid and a somewhat smaller quantity of protein. In the course of time, several days in the cold, nearly all of the ribose nucleic acid (and none of the desoxyribose nucleic acid) is split off from the residual chromosomes. The long time course of the reaction and the fact that it does not occur, or does so very slowly, in the presence of 2 M NaCl at pH 6.8 make it appear unlikely that a mere acid-base dissociation of nucleic acid takes place, for in the presence of concentrated electrolyte such dissociation would be increased. The change in composition of the residual chromosomes does not affect their microscopic appearance.

Since this change occurs in whole chromosomes, as well as in residual chromosomes, it must take place while chromosomes are being isolated. This means that the quantity of ribose nucleic acid found in residual chromosomes is always less than that present in chromosomes within an intact nucleus. The variations in composition found in different preparations of residual chromosomes are probably due, at least in part, to differences in the quantities of ribose nucleic acid split off while chromosomes are being isolated.

The instability of whole chromosomes is far more striking than is that of residual chromosomes. In saline at a pH of 7.0, and more rapidly at higher pH, chromosomes in a suspension tend to stick to each other. At room temperature stickiness increases with time far more rapidly than in the cold. The change is obvious in a flask of chromosomes, because larger and larger clumps are formed until finally the chromosomes stick together in one gelatinous mass.

Accompanying this gross transformation are finer morphological and chemical changes. In the fluid in which the chromosomes are suspended, protein and nucleic acid appear. Analysis shows that this is largely tryptophane-containing protein and ribose nucleic acid—the constituents of the residual chromosomes. These chemical transformation indicate that the substance of the residual chromosome is passing into solution, and this is borne out by microscopic observation. In the course of time fewer and fewer chromosomes can be seen until finally almost none are left. The gelatinous material dissolves in 1 M NaCl, but on high speed centrifugation only a small part of the original mass of residual chromosomes is found; their substance has passed into solution. The nucleohistone in 1 M NaCl appears to be much the same as that formed from a suspension of unchanged chromosomes, and on dilution with six volumes of water it forms the fibrous precipitate characteristic of nucleohistone. Apparently the changes that occur are limited essentially, though not entirely, to the materials of the residual chromosomes, and when their structure undergoes dissolution, no chromosome structure remains. The process of autolysis in the residual chromosome while it still is imbedded in the whole chromosome goes much farther than it does in the separated residual chromosomes.
The autolytic changes that take place in isolated chromosomes are of interest partly because it is possible that the substances discharged from the chromosomes play a part in interactions within the nucleus and in interactions between nucleus and cytoplasm.

**Chromosomes Other Than Those Isolated from Lymphocytes.**—Some preliminary observations on other chromosomes will be described. Chromosomes isolated from fish (carp) erythrocytes have been studied. Their nucleic acid content, 41 per cent, is somewhat higher than that of lymphocyte chromosomes. In carp erythrocyte chromosomes the residual chromosome represents an even smaller fraction than it does in lymphocyte chromosomes. In mammalian liver chromosomes, on the other hand, the residual chromosome fraction seems to be relatively larger than it is in lymphocyte chromosomes. Residual chromosomes can probably be seen in the nucleus of the trout sperm after treatment with \( m \) \( \text{NaCl} \), but a quantitative study of them has not yet been made. What appears to be the equivalent of residual chromosomes can be seen in grasshopper spermatocytes and in the salivary gland nuclei of Sciara after treatment with \( m \) \( \text{NaCl} \).

These observations indicate that residual chromosomes are constituents of many, if not of all, chromosomes. The relative size of this component of a chromosome will to a considerable extent determine its appearance and especially its staining properties. It is also apparent from the examples that were mentioned in the preceding paragraph and from a host of other possibilities that can be culled from the great amount of literature on chromosomes that recognition of the dual nature of the chromosome (consisting as it does of residual chromosome and of nucleohistone) should be of value in understanding the physiological functions of chromosomes. If for example lymphocyte, liver, erythrocyte, and sperm chromosomes differ in their composition, these differences may be correlated with special properties of the cells in which they are located.

Since Heidenhain, cytologists have often distinguished between basichromatin and oxychromatin, a distinction based on reactions towards basic and acid dyes. It appears likely now that these two chromatins are indeed different substances, oxychromatin corresponding to the residual chromosome, basichromatin to nucleohistone. If whole chromosomes are stained with safranin and light green, they stain bright red. Residual chromosomes, however, appear greenish red, taking only little of the basic safranin and more of the acid light green.

**SUMMARY**

By means of 1 \( m \) \( \text{NaCl} \) isolated lymphocyte chromosomes can be separated into two fractions, each of which contains nucleoprotein. The fraction soluble in \( m \) \( \text{NaCl} \) consists largely of deoxyribose nucleohistone, and constitutes 90 to 92 per cent of the mass of the chromosome. The insoluble residue (the residual
chromosome is a coiled thread containing some 12 to 14 per cent of ribose nucleic acid and about one-fifth as much deoxyribose nucleic acid; the residual chromosome accounts for 8 to 10 per cent of the mass of the chromosome. The staining of chromosomes—whether by the Feulgen procedure, by hematoxylin, orcein, or by basic dyes such as crystal violet—is due to the nucleohistone fraction which contains about 96 per cent of the nucleic acid of the chromosome. The form of the chromosome is due primarily to the protein thread of the residual chromosome. This thread is the only linear structure of microscopic dimensions in the chromosome that is not readily dispersed. When chromosomes are broken, it must be supposed that a break is made in the protein thread of the residual chromosome. The foregoing provides evidence for considering the residual chromosome to be the basis of the linear order of the genes. This would mean either that the residual chromosome is a structure around which the genes are organized or that the genes form part of its substance.

BIBLIOGRAPHY
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Magnification of all figures 3000.

Fig. 1. Isolated thymus chromosome. Aceto-orcein.

Figs. 2 to 4. Thymus residual chromosomes, fixed in acetic-alcohol, stained with pyronin.
(Mirsky and Ris: Chemical composition of isolated chromosomes)