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THE CHEMICAL COMPOSITION OF CHROMOSOMES*

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THE chromosome theory of heredity has great implications for biochemistry and in recent years, due in large measure to the work on neurospora, biochemists have become more interested in genetics. Investigations on neurospora by a group of brilliant and imaginative workers have shown how much the concepts and methods of genetics can contribute to biochemistry.

If, however, we consider the contributions of biochemistry to genetics (rather than the contribution of genetics to biochemistry), it is striking how slight has been the influence of chemistry on the fundamentals of the chromosome theory of heredity. This situation is bound to change, and indeed it already has been changing. In this lecture some chemical investigations of chromosomes and nuclei will be described.

One of the rudiments of the chromosome theory of heredity is that, in general, although with some exceptions, equal quantities of germinal material, the material of which genes are made, come from the egg and sperm and after fertilization this combined or double quantity is present in each cell of the body. The idea that some materials are present in equal quantity in all cells of the body is not inconsistent with any principles of biochemistry, but the general experience of biochemists is that substances such as hemoglobin, myosin, arginase, and ATP are present in very different quantities in different cells of the organism. In studying the chemistry of chromosomes the biochemist, considering both the principles of genetics and the experience of his own science, should ask concerning each component of a chromosome: is it a constant or a variable component, is it pres-

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ent in a constant or variable amount in the different cell nuclei of an organism?

The first chromosomal component that we shall consider is desoxyribonucleic acid—DNA. This substance was discovered by Miescher only a few years after the publication of Mendel's great paper. Staining by means of the Feulgen procedure has shown that DNA is present in practically all chromosomes and generally absent from the cytoplasm. The presence of DNA in chromosomes can also be demonstrated by the use of basic dyes, which tend to combine with DNA and so stain the chromosomes. The material in chromosomes which combines with basic dyes has been called chromatin and this is essentially DNA.

It has been known for many years that intensity of staining varies greatly in different nuclei of the same organism and so it seemed obvious that the chromatin content, or DNA content as we would now say, per nucleus is a variable. This point of view was expressed by Strasburger,[1] one of the founders of the chromosome theory of heredity, when he said, "In the stages preliminary to their division, the chromosomes become denser and take up a substance which increases their staining capacity; this is called chromatin. This substance collects in the chromosomes and may form the nutritive material for the carriers of hereditary units which we now believe to be enclosed in them. The chromatin cannot itself be the hereditary substance, as it afterwards leaves the chromosomes, and the amount of it is subject to considerable variation in the nucleus, according to its stage of development." More recently the term, "nucleic acid charge," has been introduced and this expresses accurately the idea that the quantity of DNA attached to the chromosomes varies under different physiological conditions.

In none of the work that has been mentioned were measurements made of the DNA content per nucleus. Conclusions were drawn from observations of staining intensity of a heterogeneous field, and yet it would hardly be claimed that the eye is able to integrate quantitatively the amount of pigment in such a field or that the quantity of pigment is always proportional to the quantity of DNA.

In the past few years the first determinations of DNA per nucleus were made independently by two groups of workers—by Boivin and Vendrely[2] in France and by Mirsky and Ris[3] at the Rockefeller Institute—and these determinations showed that at least in many instances the DNA content per nucleus is a constant for different cells of the same organism. These measure-

TABLE I

ANIMAL	DESOXYRIBONUCLEIC ACID (mgm. x 10^{-9}) in:			NUMBER IN PREVIOUS COLUMN x 2
	NUCLEUS OF ERYTHROCYTE	NUCLEUS OF HEPATIC CELLS	SPERM	
Domestic fowl	2.34	2.39	1.26	2.52
Shad	1.97	2.01	0.91	1.82
Carp	3.49	3.33	1.64	3.3
Brown trout	5.79		2.67	5.34
Frog	15.0	15.7		
Toad	7.33		3.70	7.40
Green turtle	5.27	5.12		

ments were made on suspensions of cells (such as sperm or nucleated erythrocytes) and on suspensions of nuclei isolated from tissue cells. In such suspensions the DNA per ml. was determined chemically; the number of cells or nuclei per ml. was counted; and so the DNA per nucleus was readily computed.

In Table I the DNA contents per nucleus are given for the hepatic and erythrocyte nuclei of a number of different animals and in Table II values are given for a number of different nuclei of one animal, the fowl. The data in these tables show that the quantity of DNA per nucleus is a constant for different somatic cells of an animal and that this constant has a characteristic value for each species. From the data in Table I it can be seen that DNA content of a sperm nucleus is one-half that of a somatic nucleus of the same animal. Since sperm cells have one set of chromosomes and somatic cells have two sets it may be said that the DNA is a constant for each set of chromosomes. It will soon be shown that this relationship holds for a wider range of material than is given in Table I. A substance that is part of

the chromosomes, that is present in constant quantity in the different somatic cells of an organism and in one-half the quantity in its germ cells is surely part of the germinal material. As an addition to the chromosome theory of heredity it may accordingly be said that DNA is part of the hereditary substance.

There are cells, the DNA content of which cannot be measured

TABLE II

DNA Content of Various Nuclei of the Fowl Expressed as mg. x 10^{-9} per Nucleus

	ERYTH-ROCYTE	LIVER	KIDNEY	SPLEEN	HEART	PAN-CREAS	SPERM
Determinations by Mirsky and Ris, 1949	2.34	2.39					1.26
Determinations by Davidson, Leslie, Smellie, and Thomson, 1950	2.49	2.56	2.20	2.54	2.45	2.61	

by the methods that have been described, but once the results mentioned have been obtained, it is possible by means of them to devise a less direct but reliable method for other cells. In this method microscopic preparations are stained by the Feulgen procedure and the light absorbed by a single stained nucleus is measured with a microscope equipped with a photocell. Some investigators[4] have attempted from such a measurement to compute the DNA content of the nucleus, but such determinations are worthless because of light scattering and other factors that are difficult to evaluate. It can, however, be shown that under certain conditions the *relative* values are correct.[5]

For a biochemist it need hardly be said that when a colorimetric method of analysis is proposed, evidence must be adduced that the results obtained have a relative quantitative validity. And when the colorimetric measurements are made not on a water-clear solution but on the turbid suspension in a microscopic preparation, the biochemist will be sceptical about the relative quantitative value of determinations. This is said because the cytologist

equipped with a microscope, photocell and galvanometer now finds that he can make reproducible measurements of the stained preparations that he has heretofore merely looked at, and he too often supposes that from these measurements, expressed in "arbitrary units," he can tell what the relative quantities of various substances are in the preparations examined. For the quantitative analyst, and the biochemist has inherited this tradition, certain

TABLE III

Intensity of Feulgen Reaction and DNA Content in Liver Nuclei with Even Distribution of DNA. Nuclei Isolated in Sucrose and Fixed in 20 Per Cent Formalin

	CARP	BULL FROG	GREEN TURTLE	CHICKEN
E x area n = 20	3.8 ± 0.05	18.3 ± 0.02	5.3 ± 0.1	2.9 ± 0.04
DNA per nucleus mg. x 10^{-9} chemical determination	3.3	15.7	5.1	2.4
DNA calculated from Feulgen using carp as standard	3.3	15.8	4.6	2.5

requirements must be satisfied before the presence of relative quantities of a substance can be inferred from photometric determinations. One of these requirements is that satisfactory analyses of known quantities of a substance should be made under conditions similar to those encountered when unknown quantities are to be determined.

How can this requirement be met when the substances to be analyzed are present in cytological preparations? For determinations of DNA on nuclei stained by the Feulgen procedure a whole series of "knowns" are provided by the work on DNA content of counted suspensions of cells and isolated nuclei. Microphotometric determinations on a series of Feulgen-stained nuclei can be compared with the values of DNA found by chemical analysis on the same nuclei. For nuclei of different DNA contents the relative values found photometrically are, as seen in Table III, in good agreement with those known from chemical analyses.

Under certain definite conditions, therefore, relative values by the cytochemical method are correct. This gives us the opportunity to measure the DNA contents of some nuclei that are not accessible to direct chemical methods. Polyploid nuclei, for example, can now be examined.[5] The polyploid nuclei which we have studied are those in mammalian livers. Since Jacobi's[6] measurements of the sizes of hepatic nuclei, it has been supposed that

TABLE IV

Size of Nuclei (Polyploidy) and Intensity of Feulgen Reaction in Rat Liver. Fixation 10 Per Cent Formalin.

SIZE OF NUCLEI	E X AREA n = 10	RATIO
Smallest nuclei	5.5 ± 0.1	1
Medium sized nuclei	10.4 ± 0.1	1.9
Largest nuclei	19.9 ± 0.2	3.6

in mammalian liver there are tetraploid and octaploid as well as diploid nuclei. We would expect that the quantity of DNA in a nucleus would be proportional to the number of sets of chromosomes and that nuclei with four and eight sets of chromosomes would contain two and four times as much DNA as is found in a nucleus with two sets of chromosomes. The microphotometric data in Table IV show that this is indeed so.

Another nucleus, the DNA of which can be determined cytochemically and not otherwise at present, is the nucleus of the ovum.[7] Analyses by chemical methods have made it appear that in the sea urchin egg there is thirty times as much DNA as in sea urchin sperm. If this egg nucleus had in fact so much DNA it would be expected from the known dimensions of the nucleus that it would stain intensely by the Feulgen procedure. In fact, it scarcely stains at all. This is what would be expected if the same quantity of DNA present in the sperm nucleus were also present in the egg nucleus, for although this amount compressed in the small sperm nucleus renders it intensely Feulgen-positive, when diffused in the far larger volume of the egg nucleus this amount of DNA would not give a visible stain. In the sea urchin egg there is, therefore, at present a discrepancy between the faint-

ness of Feulgen staining and the large quantity of DNA determined chemically.

To learn whether egg and sperm nuclei contain equal quantities of DNA it is necessary to choose an egg nucleus which is smaller

TABLE V

DNA Content Per Cell of Some Invertebrates

ANIMAL	TYPE OF CELL	WEIGHT OF CELL IN SPONGES. IN OTHERS WEIGHT OF SPERM HEAD	DNA PER NUCLEUS MG. $\times 10^{-9}$
Sponges:			
Tube sponge	diploid	1.04×10^{-8} mg.	0.12
Orange sponge, *Dysidea crawshagi*	diploid	1.13×10^{-8} mg.	0.11
Coelenterate:			
Jellyfish, *Cassiopeia*	Sperm, haploid	1.43×10^{-9} mg.	0.33
Echinoderms:			
Sea urchin, *Echinometria*	Sperm, haploid	3.23×10^{-9} mg.	0.98
Sea urchin, *Lytechinus*	Sperm, haploid	3.50×10^{-9} mg.	0.90
Sea cucumber, *Stichopius Diabole*	Sperm, haploid		0.99
Annelid:			
Nereid worm	Sperm, haploid		1.45
Molluscs:			
Limpet, *Fissurella barbadensis*	Sperm, haploid	1.71×10^{-9} mg.	0.50
Snail, *Tectarius muricatus L*	Sperm, haploid	3.54×10^{-9} mg.	0.67
Chiton tuberculatus	Sperm, haploid	2.2×10^{-9} mg.	0.63
Squid	Sperm, haploid		4.5

than that of the sea urchin egg so that the concentration of DNA will be sufficiently high to be Feulgen-positive. The egg of *Ascaris megalocephala* has a small, Feulgen-positive nucleus. Following van Beneden's classical observations, the egg nucleus can best be compared with the sperm nucleus after fertilization, when the sperm nucleus has already penetrated into the egg and has enlarged, just before fusing with the egg nucleus, at a time when the two nuclei are of the same size and also have the same structure. A Feulgen preparation made at this time shows the two nuclei indistinguishable from each other; the two haploid nuclei, therefore, have identical quantities of DNA.

All of the data that have been given provide considerable support for the rule that in the cells of an organism there is a characteristic and constant quantity of DNA for each haploid set of chromosomes. It should be noted that in their original work on

TABLE VI

DNA Content and Mass of Erythrocytes of Various Vertebrates. DNA Expressed as mg. x 10^{-9} per Cell and Mass as mg. x 10^{-8} per Cell

ANIMAL	DNA	MASS
Dipnoan:		
African lungfish, *Protopterus*	100	161
Amphibians:		
Amphiuma	168	368
Necturus	48.4	40.5
Frog	15.0	27
Toad	7.33	13.7
Reptiles:		
Green turtle	5.27	18.4
Wood turtle	4.92	14.1
Snapping turtle	4.97	
Alligator	4.98	14.9
Water snake	5.02	13.7
Pilot snake	4.28	13.3
Black racer snake	2.85	10.2
Birds:		
Domestic fowl	2.34	4.39
Guinea hen	2.27	4.58
Duck	2.65	5.44
Goose	2.92	7.37
Mammals:		
Man—Lymphocytes	5.84	
Granulocytes	6.25	
(Data of Davison and Osgood)		
Rat—lymphocytes	6.1	
(Data of Cunningham, Griffin, and Murray)		

this subject Mirsky and Ris[3] presented data which purported to show that nuclei of the somatic tissues of cattle contain somewhat more DNA than would be expected from twice the haploid value for this species. These values appeared to form an exception to the rule of DNA constancy. More recent experiments

by Swift[8] show that the nuclei of cattle tissues do not form an exception to the rule.

A deeper insight into the relationship of DNA to the gene and to the cell as a whole is had by considering the DNA contents of nuclei in their evolutionary setting.[7] This has been made possible by determinations of DNA per nucleus in a number of different invertebrates (Table V) and in a wide variety of vertebrates (Table VI).

Beginning with relatively simple organisms the DNA content of several sponges was measured. The value found was about one-sixtieth of what it is in man. Phylogenetically a more advanced group, the coelenterates, have more DNA per nucleus and coming to the molluscs a further increase is found. Within the molluscs there seems to be an increase of nuclear DNA in the course of evolution, for primitive forms such as the limpet, snail and chiton have far less than does the squid, a very highly developed mollusc. In the invertebrates there is evidence for a rise in DNA per nucleus in the course of evolution and one might even suspect that the number of genes per cell is correlated with the DNA per cell. When, however, the phylogeny of the vertebrates is considered in relation to DNA content it can be seen that such simple relationships do not hold.

In Figure I a plan of vertebrate phylogeny is given. When working with these animals one must remember that descent in each case is not from existing species, but from ancestral ones. Experiments are made with living species but the inferences drawn concern ancestral species. This difficulty can, to some extent, be overcome by examining a number of diverse species in each group and on the basis of these data one is probably justified in inferring what the ancestral form was. The reptilian ancestors of the birds are, for example, extinct, but by examining living turtles, alligators and snakes a probable value can be reached for the DNA per nucleus of the extinct form. When this is done, it can be seen that in the descent of birds from reptiles there was probably a considerable drop in the DNA per nucleus, from approximately 5 for reptiles to 2.5 for birds. In the evolution of mammals from reptiles there was no considerable change in

DNA per nucleus. There probably was a fall in DNA per nucleus in the descent of reptiles from amphibians, and this trend is made more likely still when the exceedingly high value for lung

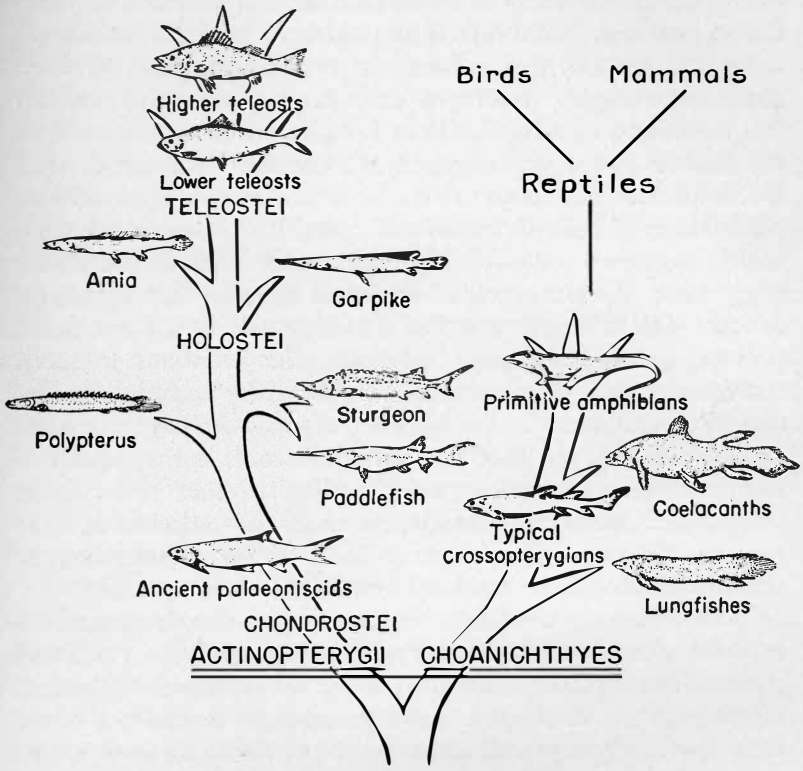

FIG. 1. This illustration is reproduced through the courtesy of Romer, A. S.: *The Vertebrate Body,* Philadelphia, W. B. Saunders Co., 1949.

fishes is considered, for these fish are close relatives of the Crossopterygii, the ancestors of the amphibians. It may be said, therefore, that the ancestors of the amphibians and the amphibian ancestors of the reptiles had far higher DNA contents per cell than did the reptiles; that the reptilian ancestors of the birds had more DNA per nucleus than do the birds; and that over a long period of vertebrate evolution there probably was a decline in DNA per cell.

DNA is a part of the germinal material. What changes in the nature of the germinal material are associated with the large differences in DNA content per cell observed in vertebrates? Comparing the largest and one of the smallest examples among vertebrates, one finds that a cell of amphiuma, a urodele, contains 70 times as much DNA as is found in a cell of the domestic fowl, a far more highly developed animal. It seems most unlikely that amphiuma contains 70 times as many different genes as does the fowl or that a gene of amphiuma contains 70 times as much DNA as does one in the fowl. To make a somewhat different comparison; a cell of amphiuma contains 170 times as much DNA as does a cell of a relatively closely related animal, the trigger fish, whereas a cell of the latter contains only nine times as much DNA as does a cell of a sponge, which is far removed phylogenetically from any vertebrate. The variations in DNA content per cell in vertebrates would hardly seem to be due simply to difference in the number of genes. Perhaps variations in DNA per cell are associated with differences in the number of strands in the chromosomes. According to this view, where polyploidy is not a factor and where enormous variations in numbers of different genes seem unlikely, DNA content may be some indication of the number of strands in a chromosome.

In vertebrates there does not appear to be a simple relationship between quantity of DNA per cell and the number of different genes. It seems possible, however, that in some primitive organisms the number of DNA molecules represents the number of genes. In a haploid sponge cell there are 40,000 molecules of DNA, if a molecular weight of a million is assumed. But if in each chromosome of a vertebrate there are many strands containing DNA, the same may be true in invertebrates. There may, therefore, be more than one DNA molecule for each gene, even in the sponge.

The relationship between DNA and the size or number of genes is obscure, but the relationship between the DNA content of a cell and the size of the cell is clear: in general, when homologous cells are compared the greater the DNA content, the larger the cell. In the nucleated red cells of vertebrates, a series

of homologous cells, there is an approximately direct relationship between cell mass and DNA content, and considering the physiological variations in quantity of hemoglobin per cell, no more than an approximate relationship would be expected. Of all the diploid cells which we have examined the sponge cell weighs the least, and it also contains the smallest amount of DNA. In the course of evolution there have been great changes, both increases and decreases, in cell size and in DNA content.

A relationship between DNA content and cell size is but another aspect of the relationship between number of sets of chromosomes and cell size. The classical experiments on the subject are those of Boveri.[9] In experiments on sea urchin eggs he was able to vary the number of chromosomes in several different ways, and in every case cell size was found to depend upon the number of chromosomes present. When in different animals DNA per cell varies, it does not mean, of course, that there is a variation at the same time in chromosome number. What can be said, however, is that when DNA per cell increases, whether due to an increase in number of chromosomes or to an increase in the number of strands per chromosome, an increase in cell size follows.

Protamines and histones have been known for a long time as proteins associated with DNA. Protamines were discovered by Miescher[10] in fish sperm and histones were discovered by Kossel[11] in bird erythrocytes. Histones were subsequently found in calf thymus and other mammalian tissues. Histones and protamines certainly are constituents of chromosomes and, furthermore, they have not yet been found in other parts of the cell. Concerning these chromosomal constituents the same question arises as for DNA: are they constant or variable components, are they present in constant or variable amounts in the different cell nuclei of an organism?

To answer this question a comparison was made of the basic proteins in different nuclei of the same organism. The data for this comparison were not available in the work of Miescher and Kossel. The first step of an investigation along these lines was indeed made by Miescher when he found that he could not iso-

late protamine from immature salmon testes, but he did not succeed in isolating another basic protein from this tissue. Although Kossel studied many protamines and histones, preparations were made from many different organisms rather than from different tissues of the same organism. Protamines, for example, were frequently prepared from salmon sperm and histone from fowl erythrocytes, but which basic protein was present in salmon erythrocytes or fowl sperm was not known. These gaps have now been filled by the isolation of a histone from salmon erythrocytes and of a protamine from fowl sperm.[12] In both the salmon and fowl it is, therefore, now known that distinctly different basic proteins are present in sperm and erythrocytes.

TABLE VII

AMINO ACID	MOLES AMINO ACID PER 100 MOLES N		GM. AMINO ACID RESIDUE PER 100 GM. PROTEIN	
	FOWL ERYTHROCYTE HISTONE	GALLIN	FOWL ERYTHROCYTE HISTONE	GALLIN
Leucine	5.81	}	8.68	
Isoleucine	3.68	0.35	5.50	
Phenylalanine	1.63	}	3.16	
Valine	3.94	0.68	5.16	1.18
Methionine	0.0	0.0	0.0	0.0
Tyrosine	1.62	1.58	3.49	4.49
Proline	2.68	1.94	3.44	3.28
Glutamic acid	5.75	0.45	9.81	1.01
Alanine	7.30	1.22	6.86	1.51
Threonine	3.39	0.72	4.52	1.28
Aspartic acid	3.49	0.28	5.30	0.56
Serine	4.54	3.89	5.23	5.91
Glycine	5.79	2.80	4.37	2.79
Ammonia	5.13	1.17		
Arginine	6.35	19.1	13.1	51.9
Lysine	6.29	0.0	10.6	0.0
Histidine	1.17	0.51	2.06	1.23
Total recovery	96.2	92.0	91.3	
Nitrogen content per cent	18.5	24.4		

Gallin, the protamine of fowl sperm, and fowl erythrocyte histone differ in many respects. They have, for example, quite different molecular weights, for histone is retained by a cello-

phane membrane through which gallin diffuses. They are both basic proteins but the amino acid compositions are altogether different and this holds even with respect to the basic amino acids (Table VII). Protamines and histones, in marked contrast to the DNA to which they are attached, are variable components of chromosomes.

Histones and especially protamines are in some respects unlike most other proteins. Protamines, especially, are exceedingly simple proteins. As one works with histones and protamines there arises the question of whether other proteins are present in chromo-

TABLE VIII

CHROMOSOMES OF:	DNA PER CENT	RESIDUAL PROTEIN PER CENT	PART OF TOTAL CELL MASS FORMED BY NUCLEI PER CENT
Calf thymus	39	8.5	61
Calf liver	26	39	19
Calf kidney	28	33	20
Beef pancreas	28	29	9

somes. One reason for considering this question is that it is well known that chromosome structure is dependent in part, at least, on protein, for the structure of a chromosome is destroyed by trypsin. Some investigators have maintained that histone is a structural protein in chromosomes, but the evidence given for this is altogether inadequate. Information about the non-histone protein fraction of chromosomes and about the significance of this protein and of histone for chromosome structure has come from the study of isolated chromosomes.

Isolated chromosomes, essentially free of non-chromosomal material, have been prepared from a number of mammalian tissues—thymus, liver, pancreas and kidney.[13] In the course of isolation these chromosomes surely have been changed somewhat morphologically and also in their chemical composition, for materials have probably been extracted from them, and some adsorption of contaminants may have occurred. And, yet, imperfect as these preparations are, if they are indeed chromosomes, much can be learned from them.

These bodies have the staining properties and chemical com-

position characteristic of chromosomes. They take basic dyes and they are intensely Feulgen-positive. They contain from 26 to 40 per cent DNA (Table VIII), the amount depending upon the cells from which they are derived, and a high concentration of histone. Are they in fact chromosomes or are they merely threads of chromatin or fragments of drawn-out nuclei?

Careful microscopic study shows that most of this material consists of chromosomes. They are helically coiled and have characteristic longitudinal differentiation into thicker, more tightly coiled and thinner, more or less despiralized segments. In many cases they can clearly be seen to be double. A comparison with isolated interphase nuclei shows that the chromosomes still within nuclei are quite similar to those that have been isolated from fragmented nuclei. In his classical work on chromosomes Boveri laid great emphasis on the individuality of the chromosome and the importance of this characteristic of a chromosome has been recognized ever since. In preparations of isolated chromosomes several well defined types can be recognized and it is possible to recognize the same types in preparations from different tissues. One such type is the nucleolus-organizing chromosome. It is well known that nucleoli are attached at definite points to some chromosomes and in these cases nucleoli serve as tags which mark particular chromosomes. In preparations isolated from liver and pancreas it was possible to identify one of the nucleolus-organizing chromosomes and it could be seen clearly that this chromosome has the same morphological structure whether isolated from liver or from pancreas. From the standpoint of chromosome individuality there can, therefore, be no doubt that there are chromosomes in these preparations. It would be a mistake to suppose that a preparation of isolated chromosomes contains a few chromosomes in a mass of nondescript material. If the time is taken to study those bodies that are not entangled with others, in most cases it can be seen that they are fairly well formed chromosomes, not unlike the chromatin-containing bodies that are seen within the nuclei from which they were derived.

Using preparations of isolated chromosomes it has been found that chromosomes contain a non-histone protein fraction and, fur-

thermore, that this protein is an essential part of the structure of a chromosome. The ground was cleared for this investigation by finding that all the histone in a suspension of chromosomes can be removed without any marked change in their microscopic appearance. This is done by extracting the chromosomes with 1 M NaCl at pH 2.9. The histone-free chromosomes contain practically all of their DNA and associated with it is a non-histone protein. After removal of histone, the salt concentration is reduced and the pH is raised so that the chromosomes are now suspended in physiological saline at pH 7.3. Under these conditions DNA is readily soluble and yet in the histone-free chromosomes it still remains attached to protein. In the intact chromosome DNA is probably attached to this protein as well as to histone.

Extraction of histone from chromosomes causes no marked change in their microscopic appearance, but when DNA is subsequently removed there is a striking change. DNA can be removed from histone-free chromosomes by treating them with desoxyribonuclease. As the DNA is depolymerized it passes into solution leaving behind a mass of tiny coiled protein threads which do not resemble chromosomes in microscopic appearance. Nor do these threads have the staining properties of chromosomes. This thread-like protein material can be distinguished from histone in several ways. Histones, unlike the generality of proteins, are soluble in a $HgSO_4$-H_2SO_4 medium, but in this the thread-like protein of chromosomes, like other proteins, is insoluble; histones do not contain tryptophane, whereas the non-histone protein fraction of chromosomes contains somewhat more than 1 per cent of tryptophane. This protein has, accordingly, been referred to as the tryptophane-containing protein of chromosomes. It has also been called the residual protein of chromosomes because it is the residue that remains when histone and DNA are removed.

Both DNA and residual protein are essential for the morphological integrity of chromosomes. When DNA is removed from a histone-free preparation nothing remains of chromosomal structure but a mass of minute protein threads; and when

the residual protein of a histone-free preparation is disintegrated by trypsin, polymerized DNA is liberated to form a viscous gel in which nothing can be seen under the microscope. Residual protein does not re-combine with DNA. The morphological configuration of the chromosome as seen under the microscope is due to the combination of DNA with residual protein and once these components are separated, neither the combination nor the configuration can be restored.

The quantity of residual protein in a suspension of chromosomes is determined by first extracting histone with 1 M NaCl at pH 2.9, then removing DNA enzymatically and finally dehydrating and weighing the protein residue. In Table VIII, the residual protein contents of some chromosome preparations are given. The relative amounts of DNA and residual protein vary considerably in chromosomes isolated from different tissues. Since it is known that the DNA per nucleus is a constant for different cells of the same organism, it follows that the quantity of residual protein varies in different nuclei. There must be a lower limit, for without some residual protein there would be no chromosomal structure, and this minimum quantity may also have some genetic significance.

As the residual protein contents of chromosomes from beef thymus, liver, pancreas and kidney are compared it can be seen that the amount in the thymus is far less than in the others. Thymus cells also have far less cytoplasm than is found in cells of liver, kidney and pancreas. The nuclear mass, that part of the mass of the cell which is due to the nucleus, has been determined for these tissues and is given in Table VIII. A correlation between the quantity of cytoplasm in a cell and the quantity of residual protein in its chromosomes would be an indication that the cytoplasm may influence the composition of chromosomes and presumably their behavior.

To summarize briefly, three chemical components of chromosomes have been considered: DNA, histone and residual protein. Both protein fractions are combined with DNA. The morphological configuration of chromosomes as seen under the microscope is dependent upon the combination of DNA with

residual protein. The quantity of DNA in different cells of the same organism is a constant for each set of chromosomes. DNA is, therefore, from the standpoint of the chromosome theory of heredity part of the germinal material. Histone and residual protein are variable components.

Both constant and variable chromosomal components are important for an organism. The constant components of the chromosome insure its genetic continuity. The presence of variable components in the chromosome, components that are influenced by the cytoplasm, show that the chromosomes, though enclosed in the nucleus are not isolated and they play a part in the physiological adaptations of the cell.

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