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Chemical Specificity of Nucleic Acids and Mechanism of their Enzymatic Degradation[1]

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I. *Introduction*

The last few years have witnessed an enormous revival in interest for the chemical and biological properties of nucleic acids, which are components essential for the life of all cells. This is not particularly surprising, as the chemistry of nucleic acids represents one of the remaining major unsolved problems in biochemistry. It is not easy to say what provided the impulse for this rather sudden rebirth. Was it the fundamental work of E. HAMMARSTEN[3] on the highly polymerized desoxyribonucleic acid of calf thymus? Or did it come from the biological side, for instance the experiments of BRACHET[4] and CASPERSSON[5]? Or was it the very important research of AVERY[6] and his collaborators on the transformation of pneumococcal types that started the avalanche?

It is, of course, completely senseless to formulate a hierarchy of cellular constituents and to single out certain compounds as more important than others. The economy of the living cell probably knows no conspicuous waste; proteins and nucleic acids, lipids and polysaccharides, all have the same importance. But one observation may be offered. It is impossible to write the history of the cell without considering its geography; and we cannot do this without attention to what may be called the chronology of the cell, i. e. the sequence in which the cellular constituents are laid down and in which they develop from each other. If this is done, nucleic acids will be found pretty much at the beginning. An attempt to say more leads directly into empty speculations in which almost no field abounds more than the chemistry of the cell. Since an ounze of proof still weighs more than a pound of prediction, the important genetical functions, ascribed —probably quite rightly—to the nucleic acids by many workers, will not be discussed here. Terms such as "template" or "matrix" or "reduplication" will not be found in this lecture.

II. *Identity and Diversity in High Molecular Cell Constituents*

The determination of the constitution of a complicated compound, composed of many molecules of a number of organic substances, evidently requires the exact knowledge of the nature and proportion of all constituents. This is true for nucleic acids as much as for proteins or polysaccharides. It is, furthermore, clear that the value of such constitutional determinations will depend upon the development of suitable methods of hydrolysis. Otherwise, substances representing an association of many chemical individuals can be described in a qualitative fashion only; precise decisions as to structure remain impossible. When our laboratory, more than four years ago, embarked upon the study of nucleic acids, we became aware of this difficulty immediately.

The state of the nucleic acid problem at that time found its classical expression in LEVENE's monograph[1]. (A number of shorter reviews, indicative of the development of our conceptions concerning the chemistry of nucleic acids, should also be mentioned[2].) The old tetranucleotide hypothesis—it should never have been called a theory—was still dominant; and this was characteristic of the enormous sway that the organic chemistry of small molecules held over biochemistry. I should like to illustrate what I mean by one example. If in the investigation of a disaccharide consisting of two different hexoses we isolate 0·8 mole of one sugar and 0·7 mole of the other, this will be sufficient for the

[1] This article is based on a series of lectures given before the Chemical Societies of Zürich and Basle (June 29th and 30th, 1949), the Société de chimie biologique at Paris, and the Universities of Uppsala, Stockholm, and Milan.

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[3] E. HAMMARSTEN, Biochem. Z. *144*, 383 (1924).

[4] J. BRACHET in *Nucleic Acid*, Symposia Soc. Exp. Biol. No. 1 (Cambridge University Press, 1947), p. 207. Cp. J. BRACHET, in *Nucleic Acids and Nucleoproteins*, Cold Spring Harbor Symp. Quant. Biol. *12*, 18. (Cold Spring Harbor, N.Y., 1947).

[5] T. CASPERSSON, in *Nucleic Acid*, Symp. Soc. Exp. Biol., No. 1 (Cambridge University Press, 1947), p. 127.

[6] O. T. AVERY, C. M. MACLEOD, and M. MCCARTY, J. Exp. Med. *79*, 137 (1944).

[1] P. A. LEVENE and L. W. BASS, Nucleic Acids (Chemical Catalog Co., New York, 1931).

[2] H. BREDERECK, Fortschritte der Chemie organischer Naturstoffe *1*, 121 (1938). – F. G. FISCHER, Naturwissensch. *30*, 377 (1942). – R. S. TIPSON, Adv. Carbohydrate Chem. *1*, 193 (1945). – J. M. GULLAND, G. R. BARKER, and D. O. JORDAN, Ann. Rev. Biochem. *14*, 175 (1945). – E. CHARGAFF and E. VISCHER, Ann. Rev. Biochem. *17*, 201 (1948). – F. SCHLENK, Adv. Enzymol. *9*, 455 (1949).

recognition of the composition of the substance, provided its molecular weight is known. The deviation of the analytical results from simple, integral proportions is without importance in that case. But this will not hold for high-molecular compounds in which variations in the proportions of their several components often will provide the sole indication of the occurrence of different compounds.

In attempting to formulate the problem with some exaggeration one could say: The validity of the identification of a substance by the methods of classical organic chemistry ends with the mixed melting point. When we deal with the extremely complex compounds of cellular origin, such as nucleic acids, proteins, or polysaccharides, a chemical comparison aiming at the determination of identity or difference must be based on the nature and the proportions of their constituents, on the sequence in which these constituents are arranged in the molecule, and on the type and the position of the linkages that hold them together. The smaller the number of components of such a high-molecular compound is, the greater is the difficulty of a decision. The occurrence of a very large number of different proteins was recognized early; no one to my knowledge ever attempted to postulate a protein as a compound composed of equimolar proportions of 18 or 20 different amino acids. In addition, immunological investigations contributed very much to the recognition of the multiplicity of proteins. A decision between identity and difference becomes much more difficult when, as is the case with the nucleic acids, only few primary components are encountered. And when we finally come to high polymers, consisting of one component only, e. g. glycogen or starch, the characterization of the chemical specificity of such a compound becomes a very complicated and laborious task.

While, therefore, the formulation of the tetranucleotide conception appeared explainable on historical grounds, it lacked an adequate experimental basis, especially as regards "thymonucleic acid". Although only two nucleic acids, the desoxyribose nucleic acid of calf thymus and the ribose nucleic acid of yeast, had been examined analytically in some detail, all conclusions derived from the study of these substances were immediately extended to the entire realm of nature; a jump of a boldness that should astound a circus acrobat. This went so far that in some publications the starting material for the so-called "thymonucleic acid" was not even mentioned or that it was not thymus at all, as may sometimes be gathered from the context, but, for instance, fish sperm or spleen. The animal species that had furnished the starting material often remained unspecified.

Now the question arises: How different must complicated substances be, before we can recognize their difference? In the multiformity of its appearances nature can be primitive and it can be subtle. It is primitive in creating in a cell, such as the tubercle bacillus, a host of novel compounds, new fatty acids, alcohols, etc., that are nowhere else encountered. There, the recognition of chemical peculiarities is relatively easy. But in the case of the proteins and nucleic acids, I believe, nature has acted most subtly; and the task facing us is much more difficult. There is nothing more dangerous in the natural sciences than to look for harmony, order, regularity, before the proper level is reached. The harmony of cellular life may well appear chaotic to us. The disgust for the amorphous, the ostensibly anomalous—an interesting problem in the psychology of science—has produced many theories that shrank gradually to hypotheses and then vanished.

We must realize that minute changes in the nucleic acid, e. g. the disappearance of one guanine molecule out of a hundred, could produce far-reaching changes in the geometry of the conjugated nucleoprotein; and it is not impossible that rearrangements of this type are among the causes of the occurrence of mutations[1].

The molecular weight of the pentose nucleic acids, especially of those from animal tissue cells, is not yet known; and the problem of their preparation and homogeneity still is in a very sad state. But that the desoxypentose nucleic acids, prepared under as mild conditions as possible and with the avoidance of enzymatic degradation, represent fibrous structures of high molecular weight, has often been demonstrated. No agreement has as yet been achieved on the order of magnitude of the molecular weight, since the interpretation of physical measurements of largely asymmetric molecules still presents very great difficulties. But regardless of whether the desoxyribonucleic acid of calf thymus is considered as consisting of elementary units of about 35,000 which tend to associate to larger structures[2] or whether it is regarded as a true macromolecule of a molecular weight around 820,000[3], the fact remains that the desoxypentose nucleic acids are high-molecular substances which in size resemble, or even surpass, the proteins. It is quite possible that there exists a critical range of molecular weights above which two different cells will prove unable to synthesize completely identical substances. The enormous number of diverse proteins may be cited as an example. *Duo non faciunt idem* is, with respect to cellular chemistry, perhaps an improved version of the old proverb.

III. *Purpose*

We started in our work from the assumption that the nucleic acids were complicated and intricate high-

[1] For additional remarks on this problem, compare E. CHARGAFF, in *Nucleic Acids and Nucleoproteins*, Cold Spring Harbor Symp. Quant. Biol., *12*, 28 (Cold Spring Harbor, N.Y., 1947).

[2] E. HAMMARSTEN, Acta med. Scand., Suppl. *196*, 634 (1947). – G. JUNGNER, I. JUNGNER, and L.-G. ALLGÉN, Nature *163*, 849 (1949).

[3] R. CECIL and A. G. OGSTON, J. Chem. Soc. 1382 (1948).

polymers, comparable in this respect to the proteins, and that the determination of their structures and their structural differences would require the development of methods suitable for the precise analysis of all constituents of nucleic acids prepared from a large number of different cell types. These methods had to permit the study of minute amounts, since it was clear that much of the material would not be readily available. The procedures developed in our laboratory make it indeed possible to perform a complete constituent analysis on 2 to 3 mg of nucleic acid, and this in six parallel determinations.

The basis of the procedure is the partition chromatography on filter paper. When we started our experiments, only the qualitative application to amino acids was known[1]. But it was obvious that the high and specific absorption in the ultraviolet of the purines and pyrimidines could form the basis of a quantitative ultra-micro method, if proper procedures for the hydrolysis of the nucleic acids and for the sharp separation of the hydrolysis products could be found.

IV. *Preparation of the Analytical Material*

If preparations of desoxypentose nucleic acids are to be subjected to a structural analysis, the extent of their contamination with pentose nucleic acid must not exceed 2 to 3%. The reason will later be made clearer; but I should like to mention here that all desoxypentose nucleic acids of animal origin studied by us so far were invariably found to contain much more adenine than guanine. The reverse appears to be true for the animal pentose nucleic acids: in them guanine preponderates. A mixture of approximately equal parts of both nucleic acids from the same tissue, therefore, would yield analytical figures that would correspond, at least as regards the purines, to roughly equimolar proportions. Should the complete purification—sometimes an extremely difficult task—prove impossible in certain cases, one could think of subjecting preparations of both types of nucleic acid from the same tissue specimen to analysis and of correcting the respective results in this manner. This, however, is an undesirable device and was employed only in some of the preparations from liver which will be mentioned later.

It is, furthermore, essential that the isolation of the nucleic acids be conducted in such a manner as to exclude their degradation by enzymes, acid or alkali. In order to inhibit the desoxyribonucleases which require magnesium[2], the preparation of the desoxypentose nucleic acids was carried out in the presence of citrate ions[3]. It would take us here too far to describe in detail the methods employed in our laboratory for the preparation of the desoxypentose nucleic acids from animal tissues. They represent in general a combination of many procedures, as described recently for the isolation of yeast desoxyribonucleic acid[1]. In this manner, the desoxypentose nucleic acids of thymus, spleen, liver, and also yeast were prepared. The corresponding compound from tubercle bacilli was isolated *via* the nucleoprotein[2]. The procedures leading to the preparation of desoxypentose nucleic acid from human sperm will soon be published[3]. All desoxypentose nucleic acids used in the analytical studies were prepared as the sodium salts (in one case the potassium salt was used); they were free of protein, highly polymerized, and formed extremely viscous solutions in water. They were homogeneous electrophoretically and showed a high degree of monodispersity in the ultracentrifuge.

The procedure for the preparation of pentose nucleic acids from animal tissues resembled, in its first stages, the method of CLARKE and SCHRYVER[4]. The details of the isolation procedures and related experiments on yeast ribonucleic acid are as yet unpublished. Commercial preparations of yeast ribonucleic acid also were examined following purification. As has been mentioned before, the entire problem of the preparation and homogeneity of the pentose nucleic acids, and even of the occurrence of only one type of pentose nucleic acid in the cell, urgently requires re-examination.

V. *Separation and Estimation of Purines and Pyrimidines*

Owing to the very unpleasant solubility and polar characteristics of the purines, the discovery of suitable solvent systems and the development of methods for their quantitative separation and estimation[5] presented a rather difficult problem in the solution of which Dr. ERNST VISCHER had an outstanding part. The pyrimidines proved somewhat easier to handle. The choice of the solvent system for the chromatographic separation of purines and pyrimidines will, of course, vary with the particular problem. The efficiency of different solvent systems in effecting separation is illustrated schematically in Fig. 1. Two of the solvent systems listed there are suitable for the separation of the purines found in nucleic acids, i. e. adenine and guanine, namely (1) n-butanol, morpholine, diethylene glycol, water (column 5 in Fig. 1); and (2) n-butanol, diethylene glycol, water in a NH_3 atmosphere (column 11). The second system listed proved particularly

[1] R. CONSDEN, A. H. GORDON, and A. J. P. MARTIN, Biochem. J. *38*, 224 (1944).

[2] F. G. FISCHER, I. BÖTTGER, and H. LEHMANN-ECHTERNACHT, Z. physiol. Chem. *271*, 246 (1941).

[3] M. MCCARTY, J. Gen. Physiol. *29*, 123 (1946).

[1] E. CHARGAFF and S. ZAMENHOF, J. Biol. Chem. *173*, 327 (1948).

[2] E. CHARGAFF and H. F. SAIDEL, J. Biol. Chem. *177*, 417 (1949).

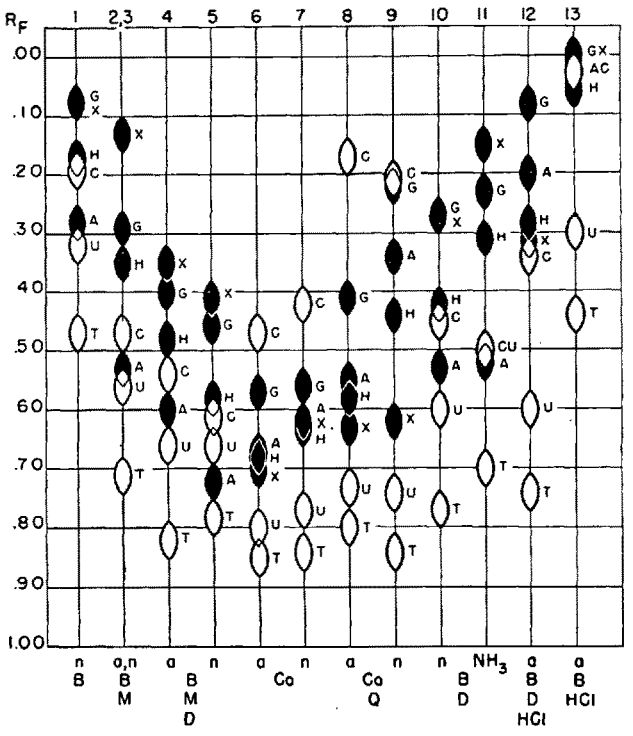
[3] S. ZAMENHOF, L. B. SHETTLES, and E. CHARGAFF, Nature (in press).

[4] G. CLARKE and S. B. SCHRYVER, Biochem. J. *11*, 319 (1917).

[5] E. VISCHER and E. CHARGAFF, J. Biol. Chem. *168*, 781 (1947); *176*, 703 (1948).

convenient. The separation of the pyrimidines is carried out in aqueous butanol (column 1).

Following the separation, the location of the various adsorption zones on the paper must be demonstrated. Our first attempts to bring this about in ultraviolet light were unsuccessful, probably because of inadequate filtration of the light emitted by the lamp then at our disposal. For this reason, the expedient was



Schematic representation of the position on the paper chromatogram of the purines and pyrimidines following the separation of a mixture. *A* adenine, *G* guanine, *H* hypoxanthine, *X* xanthine, *U* uracil, *C* cytosine, *T* thymine. The conditions under which the separations were performed are indicated at the bottom, *a* acidic, *n* neutral, *B* n-butanol, *M* morpholine, *D* diethylene glycol, *Co* collidine, *Q* quinoline.

(Taken from E. VISCHER and E. CHARGAFF, J. Biol. Chem. *176*, 704 [1948].)

used of fixing the separated purines or pyrimidines on the paper as mercury complexes which then were made visible by their conversion to mercuric sulfide. The papers thus developed served as guide strips for the removal of the corresponding zones from untreated chromatograms that were then extracted and analyzed in the ultraviolet spectrophotometer. The development of the separated bases as mercury derivatives has, however, now become unnecessary, except for the preservation of permanent records, since there has for some time been available commercially an ultraviolet lamp emitting short wave ultraviolet ("Mineralight", Ultraviolet Products Corp., Los Angeles, California). With the help of this lamp it is now easy to demonstrate directly the position of the separated purines and pyrimidines (and also of nucleosides and nucleotides[1]) which appear as dark absorption shadows on the background of the fluorescing filter paper and can be cut apart accordingly. (We are greatly indebted to Dr. C. E. CARTER, Oak Ridge National Laboratory, who drew our attention to this instrument[2].)

The extracts of the separated compounds are then studied in the ultraviolet spectrophotometer. The measurement of complete absorption spectra permits the determination of the purity of the solutions and at the same time the quantitative estimation of their contents. The details of the procedures employed have been published[3]. In this manner, adenine, guanine, uracil, cytosine, and thymine (and also hypoxanthine, xanthine, and 5-methylcytosine[4]) can be determined quantitatively in amounts of 2–40 γ. The precision of the method is $\pm 4\%$ for the purines and even better for the pyrimidines, if the averages of a large series of determinations are considered. In individual estimations the accuracy is about $\pm 6\%$.

Procedures very similar in principle served in our laboratory for the separation and estimation of the ribonucleosides uridine and cytidine and for the separation of desoxyribothymidine from thymine. Methods for the separation and quantitative determination of the ribonucleotides in an aqueous ammonium isobutyrate-isobutyric acid system have likewise been developed[5].

VI. *Methods of Hydrolysis*

It has long been known that the purines can be split off completely by a relatively mild acid hydrolysis of the nucleic acids. This could be confirmed in our laboratory in a more rigorous manner by the demonstration that heating at 100° for 1 hour in N sulfuric acid effects the quantitative liberation of adenine and guanine from adenylic and guanylic acids respectively[6]. The liberation of the pyrimidines, however, requires much more energetic methods of cleavage. Heating at high temperatures with strong mineral acid under pressure is usually resorted to. To what extent these procedures brought about the destruction of the pyrimidines, could not be ascertained previously owing to the lack of suitable analytical procedures. The experiments summarized in Table I, which are quoted from a recent paper[6], show that the extremely robust cleavage methods with mineral acids usually employed must have led to a very considerable degradation of cytosine to uracil. Uracil and also thymine are much more resistant. For this reason, we turned to

[1] E. CHARGAFF, B. MAGASANIK, R. DONIGER, and E. VISCHER, J. Amer. Chem. Soc. *71*, 1513 (1949).

[2] A similar arrangement was recently described by E. R. HOLIDAY and E. A. JOHNSON, Nature *163*, 216 (1949).

[3] E. VISCHER and E. CHARGAFF, J. Biol. Chem. *176*, 703 (1948).

[4] J. KREAM and E. CHARGAFF, unpublished experiments.

[5] E. VISCHER, B. MAGASANIK, and E. CHARGAFF, Federation Proc. *8*, 263 (1949). – E. CHARGAFF, B. MAGASANIK, R. DONIGER, and E. VISCHER, J. Amer. Chem. Soc. *71*, 1513 (1949).

[6] E. VISCHER and E. CHARGAFF, J. Biol. Chem. *176*, 715 (1948).

Table I

Resistance of pyrimidines to treatment with strong acid. A mixture of pyrimidines of known concentration was dissolved in the acids indicated below and heated at 175° in a bomb tube. The concentration shifts of the individual pyrimidines were determined through a comparison of the recoveries of separated pyrimidines before and after the heating of the mixture.

Experiment No.	Acid	Heating time min.	Concentration shift, per cent of starting concentration		
			Uracil	Cytosine	Thymine
1	HCl (10%)	90	+62	−63	+3
2	10 N HCOOH + N HCl (1:1)	60	+ 3	− 5	0
3		120	+24	−19	0
4	HCOOH (98 to 100%)	60	0	− 1	−2
5		120	0	+ 2	+1

the hydrolysis of the pyrimidine nucleotides by means of concentrated formic acid. For the liberation of the purines N sulfuric acid (100°, 1 hour) is employed; for the liberation of the pyrimidines, the purines are first precipitated as the hydrochlorides by treatment with dry HCl gas in methanol and the remaining pyrimidine nucleotides cleaved under pressure with concentrated formic acid (175°, 2 hours). This procedure proved particularly suitable for the investigation of the desoxypentose nucleic acids. For the study of the composition of pentose nucleic acids a different procedure, making use of the separation of the ribonucleotides, was developed more recently, which will be mentioned later.

VII. *Composition of Desoxypentose Nucleic Acids*

It should be stated at the beginning of this discussion that the studies conducted thus far have yielded no indication of the occurrence in the nucleic acids examined in our laboratory of unusual nitrogenous constituents. In all desoxypentose nucleic acids investigated by us the purines were adenine and guanine, the pyrimidines cytosine and thymine. The occurrence in minute amounts of other bases, e.g. 5-methylcytosine, can, however, not yet be excluded. In the pentose nucleic acids uracil occurred instead of thymine.

A survey of the composition of desoxyribose nucleic acid extracted from several organs of the ox is provided in Table II. The molar proportions reported in each case represent averages of several hydrolysis experiments. The composition of desoxypentose nucleic acids from human tissues is similarly illustrated in Table III. The preparations from human liver were obtained from a pathological specimen in which it was possible, thanks to the kind cooperation of M. FABER, to separate portions of unaffected hepatic tissue from carcinomatous tissue consisting of metastases from the sigmoid colon, previous to the isolation of the nucleic acids[1].

Table II[1]

Composition of desoxyribonucleic acid of ox (in moles of nitrogenous constituent per mole of P).

Constituent	Thymus			Spleen		Liver
	Prep. 1	Prep. 2	Prep. 3	Prep. 1	Prep. 2	
Adenine . .	0·26	0·28	0·30	0·25	0·26	0·26
Guanine . .	0·21	0·24	0·22	0·20	0·21	0·20
Cytosine . .	0·16	0·18	0·17	0·15	0·17	
Thymine . .	0·25	0·24	0·25	0·24	0·24	
Recovery . .	0·88	0·94	0·94	0·84	0·88	

[1] From E. CHARGAFF, E. VISCHER, R. DONIGER, C. GREEN, and F. MISANI, J. Biol. Chem. *177*, 405 (1949); and unpublished results.

Table III[2]

Composition of desoxypentose nucleic acid of man (in moles of nitrogenous constituent per mole of P).

Constituent	Sperm		Thymus	Liver	
	Prep. 1	Prep. 2		Normal	Carcinoma
Adenine . . .	0·29	0·27	0·28	0·27	0·27
Guanine . . .	0·18	0·17	0·19	0·19	0·18
Cytosine . . .	0·18	0·18	0·16		0·15
Thymine . . .	0·31	0·30	0·28		0·27
Recovery . . .	0·96	0·92	0·91		0·87

In order to show examples far removed from mammalian organs, the composition of two desoxyribonucleic acids of microbial origin, namely from yeast[3] and from avian tubercle bacilli[4], is summarized in Table IV.

Table IV[5]

Composition of two microbial desoxyribonucleic acids.

Constituent	Yeast		Avian tubercle bacilli
	Prep. 1	Prep. 2	
Adenine	0·24	0·30	0·12
Guanine	0·14	0·18	0·28
Cytosine	0·13	0·15	0·26
Thymine	0·25	0·29	0·11
Recovery	0·76	0·92	0·77

The very far-reaching differences in the composition of desoxypentose nucleic acids of different species are best illustrated by a comparison of the ratios of adenine to guanine and of thymine to cytosine as given in Table V. It will be seen that in all cases where enough material for statistical analysis was available highly significant differences were found. The analytical figures on which Table V is based were derived by comparing the ratios found for individual nucleic acid hydrolysates of one species regardless of the organ from which the preparation was isolated. This procedure assumes that there is no organ specificity with

[1] Unpublished experiments.

[2] From E. CHARGAFF, S. ZAMENHOF, and C. GREEN, Nature (in press); and unpublished results.

[3] E. CHARGAFF and S. ZAMENHOF, J. Biol. Chem. *173*, 327 (1948).

[4] E. CHARGAFF and H. F. SAIDEL, J. Biol. Chem. *177*, 417 (1949).

[5] From E. VISCHER, S. ZAMENHOF, and E. CHARGAFF, J. Biol. Chem. *177*, 429 (1949); and unpublished results.

Table V

Molar proportions of purines and pyrimidines in desoxypentose nucleic acids from different species.

Species	Number of different organs	Number of different preparations	Adenine/Guanine			Thymine/Cytosine		
			Number of hydrolyses[3]	Mean ratio	Standard error	Number of hydrolyses[3]	Mean ratio	Standard error
Ox[1]	3	7	20	1·29	0·013	6	1·43	0·03
Man[2]	2	3	6	1·56	0·008	5	1·75	0·03
Yeast.	1	2	3	1·72	0·02	2	1·9	
Avian tubercles bacillus	1	1	2	0·4		1	0·4	

[1] Preparations from thymus, spleen, and liver served for the purine determinations, the first two organs for the estimation of pyrimidines.

[2] Preparations from spermatozoa and thymus were analysed.

[3] In each hydrolysis between 12 and 24 determinations of individual purines and pyrimidines were performed.

respect to the composition of desoxypentose nucleic acids of the same species. That this appears indeed to be the case may be gathered from Tables II and III and even better from Table VI where the average purine and pyrimidine ratios in individual tissues of the same species are compared. That the isolation of nucleic acids did not entail an appreciable fractionation is shown by the finding that when whole defatted human spermatozoa, after being washed with cold 10% trichloroacetic acid, were analyzed, the same ratios of adenine to guanine and of thymine to cytosine were found as are reported in Tables V and VI. It should also be mentioned that all preparations, with the exception of those from human liver, were derived from pooled starting material representing a number, and in the case of human spermatozoa a very large number, of individuals.

Table VI

Molar proportions of purines and pyrimidines in desoxypentose nucleic acids from different organs of one species.

Species	Organ	Adenine/ Guanine	Thymine/ Cytosine
Ox	Thymus	1·3	1·4
	Spleen	1·2	1·5
	Liver	1·3	
Man	Thymus	1·5	1·8
	Sperm	1·6	1·7
	Liver (normal)	1·5	1·8
	Liver (carcinoma)	1·5	1·8

The desoxypentose nucleic acids extracted from different species thus appear to be different substances or mixtures of closely related substances of a composition constant for different organs of the same species and characteristic of the species.

The results serve to disprove the tetranucleotide hypothesis. It is, however, noteworthy—whether this is more than accidental, cannot yet be said—that in all desoxypentose nucleic acids examined thus far the molar ratios of total purines to total pyrimidines, and also of adenine to thymine and of guanine to cytosine, were not far from 1.

VIII. *Composition of Pentose Nucleic Acids*

Here a sharp distinction must be drawn between the prototype of all pentose nucleic acid investigations—the ribonucleic acid of yeast—and the pentose nucleic acids of animal cells. Nothing is known as yet about bacterial pentose nucleic acids. In view of the incompleteness of our information on the homogeneity of pentose nucleic acids, which I have stressed before, I feel that the analytical results on these preparations do not command the same degree of confidence as do those obtained for the desoxypentose nucleic acids.

Table VIII[1]

Composition of pentose nucleic acids from animal tissues.

Constituent	Calf liver	Ox liver	Sheep liver	Pig liver	Pig pancreas
Guanylic acid . . .	16·3	14·7	16·7	16·2	22·5
Adenylic acid . . .	10	10	10	10	10
Cytidylic acid . . .	11·1	10·9	13·4	16·1	9·8
Uridylic acid . . .	5·3	6·6	5·6	7·7	4·6
Purines: pyrimidines	1·6	1·4	1·4	1·1	2·5

Three procedures, to which reference is made in Tables VII and VIII, were employed in our laboratory for the analysis of pentose nucleic acids. In *Procedure 1*, the pentose nucleic acid was hydrolysed to the nucleotide stage with alkali, at p_H 13·5 and 30°, and the nucleotides, following adjustment to about p_H 5, separated by chromatography with aqueous ammonium isobutyrate-isobutyric acid as the solvent. Under these conditions, guanylic acid shares its position on the chromatogram with uridylic acid; but it is possible to determine the concentrations of the two components in the eluates by simultaneous equations based on the ultraviolet absorption of the pure nucleotides[2]. The very good recoveries of nucleotides obtained in terms of both nucleic acid phosphorus and nitrogen show the cleavage by mild alkali treatment of pentose nucleic acids to be practically quantitative.—In

[1] Unpublished results.

[2] E. VISCHER, B. MAGASANIK, and E. CHARGAFF, Federation Proc. *8*, 263 (1949). – E. CHARGAFF, B. MAGASANIK, R. DONIGER, and E. VISCHER, J. Amer. Chem. Soc. *71*, 1513 (1949).

Table VII[1]

Composition of yeast ribonucleic acid (in moles of nitrogenous constituent per mole of P).

Constituent	Preparation 1			Preparation 2			Preparation 3		
	Procedure 1	Procedure 2	Procedure 3	Procedure 1	Procedure 2	Procedure 3	Procedure 1	Procedure 2	Procedure 3
Adenylic acid	0·29	0·26	0·26	0·27		0·24	0·25	0·23	0·24
Guanylic acid	0·28	0·29	0·26	0·25		0·25	0·26	0·28	0·26
Cytidylic acid	0·18	0·17	0·24	0·20			0·21	0·21	
Uridylic acid	0·20	0·20	0·08	0·18	0·19		0·20	0·25	
Recovery	0·95	0·92	0·84	0·90			0·92	0·97	

[1] From E. VISCHER and E. CHARGAFF, J. Biol. Chem. *176*, 715 (1948). – E. CHARGAFF, B. MAGASANIK, R. DONIGER, and E. VISCHER, J. Amer. Chem. Soc. *71*, 1513 (1949); and unpublished results.

Procedure 2, the purines are first liberated by gaseous HCl in dry methanol and the evaporation residue of the reaction mixture is adjusted to p_H 13·5 and then treated as in Procedure 1. In this manner, uridylic and cytidylic acids, adenine and guanine are separated and determined on one chromatogram.—The determinations of free purines and pyrimidines in acid hydrolysates of pentose nucleic acids, following the methods outlined before for the desoxypentose nucleic acids, are listed as *Procedure 3*. It will be seen that it is mainly uracil which in this procedure escapes quantitative determination. This is due to the extreme refractoriness of uridylic acid to complete hydrolysis by acids, a large portion remaining partially unsplit as the nucleoside uridine. As matters stand now, I consider the values for purines yielded by Procedures 1 and 3 and those for pyrimidines found by Procedures 1 and 2 as quite reliable.

A survey of the composition of yeast ribonucleic acid is provided in Table VII. Preparations 1 and 2, listed in this table, were commercial preparations that had been purified in our laboratory and had been subjected to dialysis; Preparation 3 was isolated from baker's yeast by B. MAGASANIK in this laboratory by procedures similar to those used for the preparation of pentose nucleic acids from animal tissues and had not been dialyzed. It will be seen that the results are quite constant and not very far from the proportions required by the presence of equimolar quantities of all four nitrogenous constituents.

An entirely different picture, however, was encountered when the composition of pentose nucleic acids from animal cells was investigated. A preliminary summary of the results, in all cases obtained by *Procedure 1*, is given in Table VIII. Here guanylic acid was the preponderating nucleotide followed, in this order, by cytidylic and adenylic acids; uridylic acid definitely was a minor constituent. This was true not only of the ribonucleic acid of pancreas which has been known to be rich in guanine[1], but also of all pentose nucleic acids isolated by us from the livers of three different species (Table VIII).

In the absence of a truly reliable standard method for the isolation of pentose nucleic acid from animal tissue, generalizations are not yet permitted; but it would appear that pentose nucleic acids from the same organ of different species are more similar to each other, at least in certain respects (e. g. the ratio of guanine to adenine), than are those from different organs of the same species. (Compare the pentose nucleic acids from the liver and the pancreas of pig in Table VIII.)

IX. *Sugar Components*

It is deplorable that such designations as desoxyribose and ribose nucleic acids continue to be used as if they were generic terms. Even the "thymus nucleic acid of fish sperm" is encountered in the literature. As a matter of fact, only in a few cases have the sugars been identified, namely, *d*-2-desoxyribose as a constituent of the guanine and thymine nucleosides of the desoxypentose nucleic acid from calf thymus, D-ribose as a constituent of the pentose nucleic acids from yeast, pancreas, and sheep liver.

Since the quantities of novel nucleic acids usually will be insufficient for the direct isolation of their sugar components, we attempted to employ the very sensitive procedure of the filter paper chromatography of sugars[1] for the study of the sugars isolated from minute quantities of nucleic acids. It goes without saying that identifications based on behavior in adsorption or partition are by no means as convincing as the actual isolation, but they will at least permit a tentative classification of new nucleic acids. Thus far the pentose nucleic acids of pig pancreas[2] and of the avian tubercle bacillus[3] have been shown to contain ribose, the desoxypentose nucleic acids of ox spleen[4],

[1] E. HAMMARSTEN, Z. physiol. Ch. *109*, 141 (1920). – P. A. LEVENE and E. JORPES, J. Biol. Chem. *86*, 389 (1930). – E. JORPES, Biochem. J. *28*, 2102 (1934).

[1] S. M. PARTRIDGE, Nature *158*, 270 (1946). – S. M. PARTRIDGE and R. G. WESTALL, Biochem. J. *42*, 238 (1948). – E. CHARGAFF, C. LEVINE, and C. GREEN, J. Biol. Chem. *175*, 67 (1948).

[2] E. VISCHER and E. CHARGAFF, J. Biol. Chem. *176*, 715 (1948).

[3] E. VISCHER, S. ZAMENHOF, and E. CHARGAFF, J. Biol. Chem. *177*, 429 (1949).

[4] E. CHARGAFF, E. VISCHER, R. DONIGER, C. GREEN, and F. MISANI, J. Biol. Chem. *177*, 405 (1949).

yeast and avian tubercle bacilli[1] desoxyribose. It would seem that the free play with respect to the variability of components that nature permits itself is extremely restricted, where nucleic acids are concerned.

X. *Depolymerizing Enzymes*

Enzymes capable of bringing about the depolymerization of both types of nucleic acids have long been known; but it is only during the last decade that crystalline ribonuclease[2] and desoxyribonuclease[3] from pancreas have become available thanks to the work of KUNITZ. Important work on the latter enzyme was also done by MCCARTY[4].

Table IX[5]

Enzymatic degradation of calf thymus desoxyribonucleic acid.

	Digestion *hours*	Dialysis *hours*	Distribution of fractions % of original	Composition of fractions (molar proportions)			
				Adenine / Guanine	Thymine / Cytosine	Adenine / Cytosine	Pyrimidines / Purines
Original. . . .	0	0	100	1·2	1·3	1·6	1·2
Dialysate . . .	6	6	53	1·2	1·2	1·2	1·0
Dialysis residue	24	72	7	1·6	2·2	3·8	2·0

We were, of course, interested in applying the chromatographic micromethods for the determination of nucleic acid constituents to studies of enzymatic reaction mechanisms for which they are particularly suited. The action of crystalline desoxyribonuclease on calf thymus desoxyribonucleic acid resulted in the production of a large proportion of dialyzable fragments (53 per cent of the total after 6 hours digestion), without liberation of ammonia or inorganic phosphate. But even after extended digestion there remained a non-dialyzable core whose composition showed a significant divergence from both the original nucleic acid and the bulk of the dialyzate[6]. The preliminary findings summarized in Table IX indicate a considerable increase in the molar proportions of adenine to guanine and especially to cytosine, of thymine to cytosine, and of purines to pyrimidines. This shows that the dissymmetry in the distribution of constituents, found in the original nucleic acid (Table II), is intensified in the core. The most plausible explanations of this interesting phenomenon, the study of which is being continued, are that the preparations consisted of more than one desoxypentose nucleic acid or that the nucleic contained in its chain clusters of nucleotides (relatively richer in adenine and thymine) that were distinguished from the bulk of the molecule by greater resistance to enzymatic disintegration.

In this connection another study, carried out in collaboration with S. ZAMENHOF, should be mentioned briefly that dealt with the desoxypentose nuclease of yeast cells[1]. This investigation afforded a possibility of exploring the mechanisms by which an enzyme concerned with the disintegration of desoxypentose nucleic acid is controlled in the cell. Our starting point again was the question of the specificity of desoxypentose nucleic acids; but the results were entirely unexpected. Since we had available a number of nucleic acids from different sources, we wanted to study a pair of desoxypentose nucleic acids as distant from each other as possible, namely that of the ox and that of yeast, and to investigate the action on them of the two desoxypentose nucleases from the same cellular sources. The desoxyribonuclease of ox pancreas has been thoroughly investigated, as was mentioned before. Nothing was known, however, regarding the existence of a yeast desoxypentose nuclease.

It was found that fresh salt extracts of crushed cells contained such an enzyme in a largely inhibited state, due to the presence of a specific inhibitor protein. This inhibitor specifically inhibited the desoxypentose nuclease from yeast, but not that from other sources, such as pancreas. The yeast enzyme depolymerized the desoxyribose nucleic acids of yeast and of calf thymus, which differ chemically, as I have emphasized before, at about the same rate. In other words, the enzyme apparently exhibited inhibitor specificity, but not substrate specificity. It is very inviting to assume that such relations between specific inhibitor and enzyme, in some ways reminiscent of immunological reactions, are of more general biological significance. In any event, a better understanding of such systems will permit an insight into the delicate mechanisms through which the cell manages the economy of its life, through which it maintains its own continuity and protects itself against agents striving to transform it.

XI. *Concluding Remarks*

Generalizations in science are both necessary and hazardous; they carry a semblance of finality which conceals their essentially provisional character; they drive forward, as they retard; they add, but they also take away. Keeping in mind all these reservations, we arrive at the following conclusions. The desoxypentose nucleic acids from animal and microbial cells contain varying proportions of the same four nitrogenous constituents, namely, adenine, guanine, cytosine, thymine. Their composition appears to be characteristic of the species, but not of the tissue, from which

[1] E. VISCHER, S. ZAMENHOF, and E. CHARGAFF, J. Biol. Chem. *177*, 429 (1949).
[2] M. KUNITZ, J. Gen. Physiol. *24*, 15 (1940).
[3] M. KUNITZ, Science *108*, 19 (1948).
[4] M. MCCARTY, J. Gen. Physiol. *29*, 123 (1946).
[5] From S. ZAMENHOF and E. CHARGAFF, J. Biol. Chem. *178*, 531 (1949).
[6] S. ZAMENHOF and E. CHARGAFF, J. Biol. Chem. *178*, 531 (1949).
[1] S. ZAMENHOF and E. CHARGAFF, Science *108*, 628 (1948); J. Biol. Chem. *180*, 727 (1949).

they are derived. The presumption, therefore, is that there exists an enormous number of structurally different nucleic acids; a number, certainly much larger than the analytical methods available to us at present can reveal.

It cannot yet be decided, whether what we call the desoxypentose nucleic acid of a given species is one chemical individual, representative of the species as a whole, or whether it consists of a mixture of closely related substances, in which case the constancy of its composition merely is a statistical expression of the unchanged state of the cell. The latter may be the case if, as appears probable, the highly polymerized desoxypentose nucleic acids form an essential part of the hereditary processes; but it will be understood from what I said at the beginning that a decision as to the identity of natural high polymers often still is beyond the means at our disposal. This will be particularly true of substances that differ from each other only in the sequence, not in the proportion, of their constituents. The number of possible nucleic acids having the same analytical composition is truly enormous. For example, the number of combinations exhibiting the same molar proportions of individual purines and pyrimidines as the desoxyribonucleic acid of the ox is more than 10^{56}, if the nucleic acid is assumed to consist of only 100 nucleotides; if it consists of 2,500 nucleotides, which probably is much nearer the truth, then the number of possible "isomers" is not far from 10^{1500}.

Moreover, desoxypentose nucleic acids from different species differ in their chemical composition, as I have shown before; and I think there will be no objection to the statement that, as far as chemical possibilities go, they could very well serve as one of the agents, or possibly as the agent, concerned with the transmission of inherited properties. It would be gratifying if one could say—but this is for the moment no more than an unfounded speculation—that just as the desoxypentose nucleic acids of the nucleus are species-specific and concerned with the maintenance of the species, the pentose nucleic acids of the cytoplasm are organ-specific and involved in the important task of differentiation.

I should not want to close without thanking my colleagues who have taken part in the work discussed here; they are, in alphabetical order, Miss R. DONIGER, Mrs. C. GREEN, Dr. B. MAGASANIK, Dr. E. VISCHER, and Dr. S. ZAMENHOF.

Zusammenfassung

Die Betrachtung der Nukleinsäuren, sowohl der Desoxypentosen als der Pentosen enthaltenden Verbindungen, als organische Makromoleküle macht eine Auseinandersetzung mit den Problemen notwendig, welche sich auf die Bestimmung von Identität oder Verschiedenheit solcher aus verschiedenen Zellen isolierten hochpolymeren Substanzen beziehen. Dies führt zu einer kritischen Besprechung der sicherlich nicht haltbaren Tetranukleotidhypothese und zur Formulierung eines Arbeitsprogramms für die Aufklärung der Zusammensetzung individueller Nukleinsäuren.

Die mikrochromatographischen und spektrophotometrischen Methoden zur Trennung und quantitativen Bestimmung der stickstoffhaltigen Nukleinsäurebestandteile werden kurz geschildert. Sie ermöglichen die quantitative Analyse der Purine Adenin, Guanin, Hypoxanthin und Xanthin und der Pyrimidine Cytosin, Uracil und Thymin im Bereiche von 2 bis 40 γ. An die Beschreibung der Verfahren zur Isolierung der als Analysenmaterial dienenden hochpolymerisierten Nukleinsäurepräparate aus verschiedenen Zellen schließt sich eine Besprechung der Hydrolysen- und Analysenmethoden, die auf sehr geringe Nukleinsäuremengen (2 bis 3 mg) anwendbar sind.

Alle bis jetzt untersuchten Desoxypentosenukleinsäuren enthielten 2-Desoxyribose als Zucker und Adenin, Guanin, Cytosin und Thymin als Stickstoffkomponenten in für die betreffende Zelle konstanten, von der Tetranukleotidhypothese weit abweichenden Proportionen. Ihre Zusammensetzung ist spezifisch für die Spezies, die als Ausgangsmaterial dient, jedoch nicht für das Ausgangsgewebe. Weit auseinanderliegende Arten, wie z. B. Säugetiere gegenüber Mikroorganismen, enthalten völlig verschieden zusammengesetzte Desoxypentosenukleinsäuren. In manchen Fällen lassen sich jedoch auch bei näherliegenden Nukleinsäuren, z. B. denen des Ochsen und des Menschen, ins Gewicht fallende Verschiedenheiten aufzeigen. Die Untersuchung der Pentosenukleinsäuren, die auf einer quantitativen Bestimmung der sie zusammensetzenden Mononukleotide beruht, ist noch nicht so weit gediehen. Sie hat vorläufig gezeigt, daß sich die Verbindungen aus Säugetiergewebe von denen aus Hefe durch einen relativ sehr hohen Gehalt an Guanylsäure unterscheiden, und hat Anhaltspunkte dafür gegeben, daß die Pentosenukleinsäuren eher organ- als spezies-spezifisch sind.

Als Beispiele für den Beitrag, den die Untersuchung enzymatischer Reaktionsmechanismen zum Problem der chemischen Spezifizität der Nukleinsäuren leisten kann, werden schließlich Versuche mit der kristallisierten Desoxyribonuklease aus Pankreas und mit einer durch interessante Hemmungsstoffspezifizität ausgezeichneten Desoxypentosenuklease aus Hefe geschildert. Zum Abschluß werden einige der Probleme gestreift, die sich aus der hier nachgewiesenen Existenz vieler verschiedener Nukleinsäuren ergeben.