THE BIOCHEMISTRY OF THE NUCLEIC ACIDS

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WITH 4 PLATES AND
15 TEXT ILLUSTRATIONS

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PREFACE

IT should be made quite clear at the outset that this little book does not claim to be a monograph giving an exhaustive treatment of the biochemistry of nucleic acids. It is intended rather to provide an elementary outline of the main features of the nucleic acids and nucleoproteins for the benefit of students of biochemistry, of chemists who wish to know something about the biological aspects of the subject, and of biologists who wish to learn a little about the chemical aspects.

So much work is being done in the nucleic acid field at the present time, and progress is so rapid, that some of the material presented here will inevitably be superseded by more recent discoveries before the book is in print. This defect is inevitable and no apology need be made for it. More serious is the drawback that in a book of this size the author cannot hope to do justice to the work of all the many investigators whose contributions to the subject are significant and even fundamental. But each chapter is provided with a small though carefully selected list of references to reviews and important original papers from which those who wish to seek further may obtain additional authoritative information.

It is a pleasure to acknowledge with gratitude the help of those who have allowed me to reproduce figures and diagrams. In particular I should like to thank Professor W. T. Astbury and the Clarendon Press, Oxford, for Fig. 4.3; the Company of Biologists for Figs. 4.1 and 4.2; Professor T. Caspersson for Figs. 6.2 (Royal Microscopical Society), 11.1 and 11.2 (Springer-Verlag, Berlin), 14.1 (P. A. Norstedt & Söner, Stockholm) and 14.2 (The Company of Biologists); Dr. I. Gersh and the Wistar Institute of Anatomy and Biology, Philadelphia, for Fig. 6.3; The Long Island Biological Association for Fig. 9.1;

Professor S. Spiegelman and the American Association for the Advancement of Science, Washington, for Fig. 14.3; Professor B. Malmgren and Einar Munksgaard, Copenhagen, for Plate III and Fig. 15.1; Professor M. Stacey and the Royal Society of London for Plate IV; Dr. J. C. White and the Pathological Society of Great Britain for Plate II. I should also like to thank the Cambridge University Press for permission to reproduce Figs. 4.1, 4.2, 9.2 and 14.2.

In conclusion I should like to thank Dr. W. C. Hutchinson, Dr. H. S. D. Garven, Mr. I. Leslie and Mr. W. M. McIndoe for valuable suggestions and Miss Alison Reid for typing the manuscript.

J. N. D.

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CHAPTER I

INTRODUCTION

THE fundamental investigations which led to the discovery of the nucleic acids were made by Friedrich Miescher(1) (1844–95), who may be regarded as the founder of our knowledge of the chemistry of the cell nucleus. In early work carried out in 1868, in the laboratory of Hoppe-Seyler in Tübingen, he isolated the nuclei from pus cells obtained from discarded surgical bandages and showed that the nuclear material contained an unusual phosphorus compound named by him 'nuclein', which we now know to have been nucleoprotein. Miescher's investigations were continued in Basel, where most of his working life was spent. There he became interested in salmon sperm as a source of nuclear material, and in 1872 he showed that isolated sperm heads contained an acidic compound now recognized as nucleic acid, and a base to which the name 'protamine' was given. It was subsequently shown that nucleic acids were normal constituents of all cells and tissues which were examined, and Miescher's investigation of the nucleic acids were continued by Altmann, who in 1899 described a method for the preparation of protein-free nucleic acids from animal tissues and from yeast. The work was continued later by Kossel in Basel, Jones in Baltimore, Levene in New York, Hammarsten in Stockholm, Gulland in Nottingham, and many others.

One of the best animal sources of nucleic acid was found to be the thymus gland, and it is not surprising therefore that most work was concentrated on nucleic acid from this source. On hydrolysis it was found to yield the purine bases, adenine and guanine, the pyrimidine bases, cytosine and thymine, a sugar, which was eventually shown to be D(-)2-deoxyribose and phosphoric acid. On the other hand, the nucleic acid from yeast on hydrolysis yielded adenine,

guanine, cytosine, and uracil, a pentose sugar, which was eventually shown to be D(-)ribose, and phosphoric acid. It therefore differed from thymus nucleic acid in containing uracil in place of thymine and ribose in place of deoxyribose. Since most nucleic acids from animal sources appear to resemble thymus nucleic acid, and since the only other nucleic acid which had been prepared in reasonable quantities from a plant source (so-called triticonucleic acid from the wheat embryo) appeared to be very similar to yeast nucleic acid, the impression grew up that deoxypentosenucleic acid of the thymus type was characteristic of animal tissues, and pentosenucleic acid of the yeast type was characteristic of plant tissues. Thus Jones in 1921 stated categorically that 'we come to understand quite clearly that there are only two nucleic acids in nature, one obtainable from the nuclei of animal cells and the other from the nuclei of plant cells'.

It was not long before the validity of this conception was questioned. It had been known since early times that pentose derivatives were present in animal tissues. For example, the so-called β-nucleoprotein, which was originally prepared from mammalian pancreas by O. Hammarsten(2) in 1894, was known to contain a pentose sugar, and Jorpes(3) eventually prepared from this material a nucleic acid of the pentose type which he showed to resemble yeast nucleic acid in many ways and to be abundant in pancreatic tissue in which it might form as much as 10 per cent of the total weight. The presence of pentose nucleic acids in the mammary gland was also suggested by the work of Odenius(4) and of Mandel and Levene(5). Pentose nucleotide derivatives were also demonstrated in chick embryo pulp by Calvery(6) in spleen and the liver by Jones and Perkins(7) and by Thomas and Berariu(8) and in sea urchin eggs by Blanchard(9). It thus appeared probable that pentosenucleic acids were normal constituents of animal tissues as well as of plant cells, and Jones and Perkins(7) expressed the view that 'the distinction between plant and

animal nucleic acids will in future not be so definitely drawn'. This view has been amply confirmed, as we shall see later, by recent work, and it is now clear that pentosenucleic acids are abundant in animal cells.

Deoxypentosenucleic acid has also been demonstrated in plant tissues(10–13) and has been isolated from yeast(14), and modern work has shown that the biological distinction between the pentosenucleic acids and deoxypentosenucleic acids lies not in the source from which they are obtained but in their cytochemical distribution within the cell. As will be seen later, pentosenucleic acids are found chiefly in the cytoplasm while deoxypentosenucleic acids are confined to the nucleus.

In view of the history of the discovery of the nucleic acids it is not surprising that the nomenclature should be confused. It is doubtful indeed whether the term nucleic acid is a good one, and modern usage suggests that a more satisfactory substitute would be 'polynucleotide'.

As deoxypentosenucleic acid is usually prepared from the thymus gland of the calf, it is sometimes referred to as thymonucleic acid or animal nucleic acid, and more recently, on account of its presence in nuclear chromatin, the name 'chromonucleic' acid has been suggested. Since we do not know whether deoxypentosenucleic acid from all sources is the same, it is desirable to prefix the name of the source, and since we do not know for certain that deoxyribose is the sugar in this type of nucleic acid from all sources it is strictly correct to use the term deoxypentose. Thus it would be legitimate to speak of 'thymus deoxyribosepolynucleotide' or 'liver deoxypentose polynucleotide', but such names are clumsy and for most purposes the term *deoxyribonucleic acid* (DNA) is fairly acceptable, provided that we use it as a generic term without necessarily assuming that the material from different organs and different species is necessarily identical.

The nucleic acid originally obtained from yeast has been named 'zymonucleic acid' or 'phytonucleic acid', and more

recently the term 'plasmonucleic acid' has been suggested (15). As we shall see later, there is some evidence that the pentosenucleic acid from all sources is not identical and it would therefore be strictly accurate to use the terms 'yeast ribose polynucleotide' or 'spleen pentose polynucleotide'. Since the sugar has been identified as D(-)ribose only in the polynucleotides from yeast and from a limited number of other sources, it would in the meantime be logical to restrict the use of the term ribonucleic acid or ribopolynucleotide only to the material obtained from these sources. Nevertheless, for the sake of convenience and in conformity with common usage, the term *ribonucleic acid* (RNA) is frequently employed to indicate the pentose polynucleotide from any source, and the use of this term can be justified so long as its limitations are recognized and so long as we keep in mind that this also is a generic term and not the name of a single molecular species.

Several books and reviews on the nucleic acids have been published during the last few years and are listed in the References(16–31).

CHAPTER II

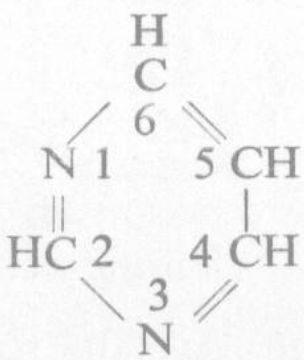
THE HYDROLYSIS PRODUCTS OF THE NUCLEIC ACIDS

2.1

Before any account is given of the structure of the nucleic acids proper it is desirable at this stage to discuss the structure of the component parts out of which the nucleic acid molecule is constructed. Complete hydrolysis of the nucleic acids yields pyrimidine and purine bases, a sugar component and phosphoric acid. Partial hydrolysis yields compounds known as nucleosides and nucleotides. Each of these component parts will be discussed in turn.

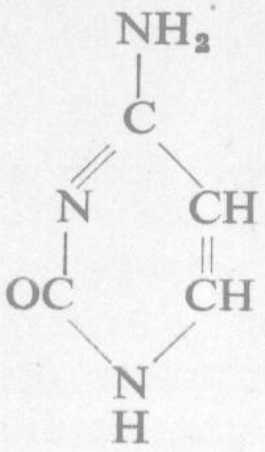
2.2 *Pyrimidine bases*

The pyrimidine bases are all derivatives of the parent compound pyrimidine, and the three derivatives found in

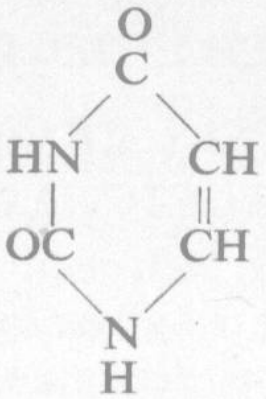


Pyrimidine

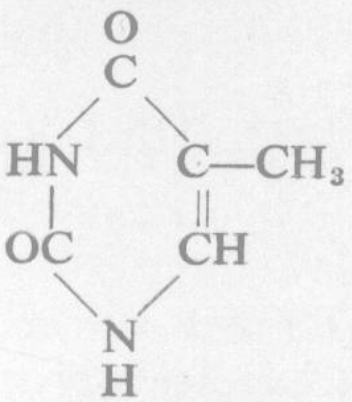
the nucleic acids are cytosine found in both types of nucleic acid, uracil found in the RNA type, and thymine found in the DNA type.



cytosine
2-hydroxy-6-amino-pyrimidine



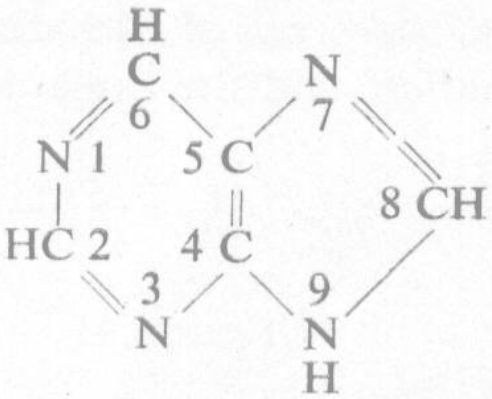
uracil
2 : 6-dihydroxy-pyrimidine



thymine
5-methyl-uracil

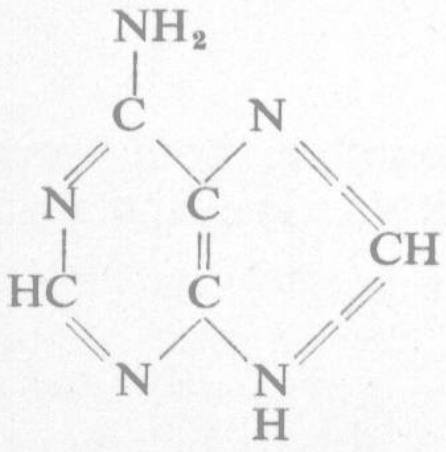
2.3 *Purine bases*

Both types of nucleic acids contain the same purine bases, adenine and guanine. They are derivatives of the parent compound purine which is formed by the fusion of a pyrimidine ring and an iminazole ring.

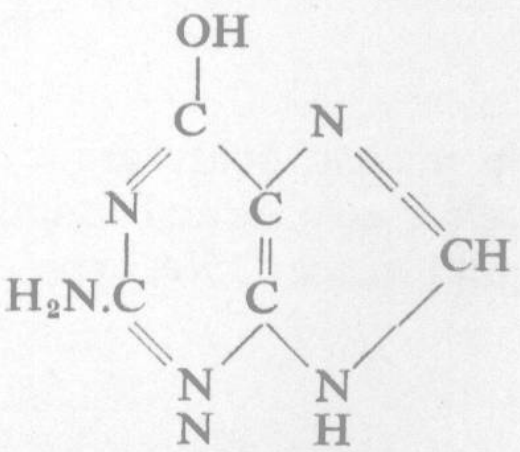


Purine

Adenine and guanine have the following structures:

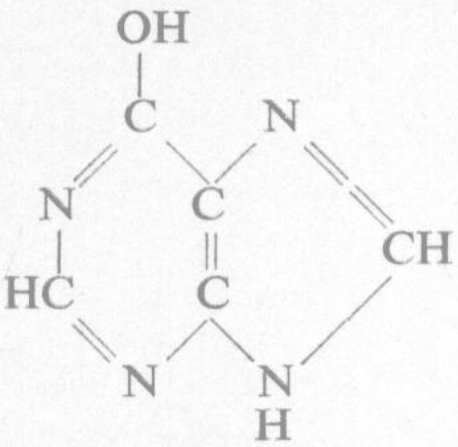


adenine
(6-aminopurine)

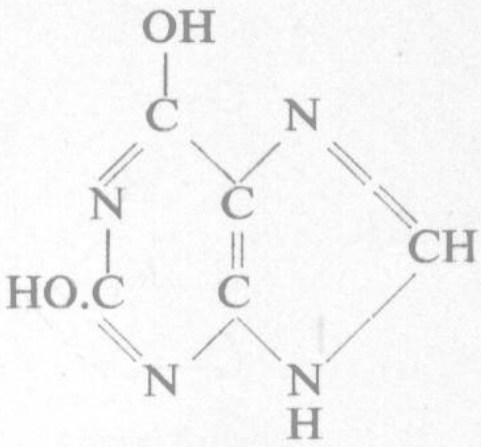


guanine
(2-amino-6-hydroxypurine)

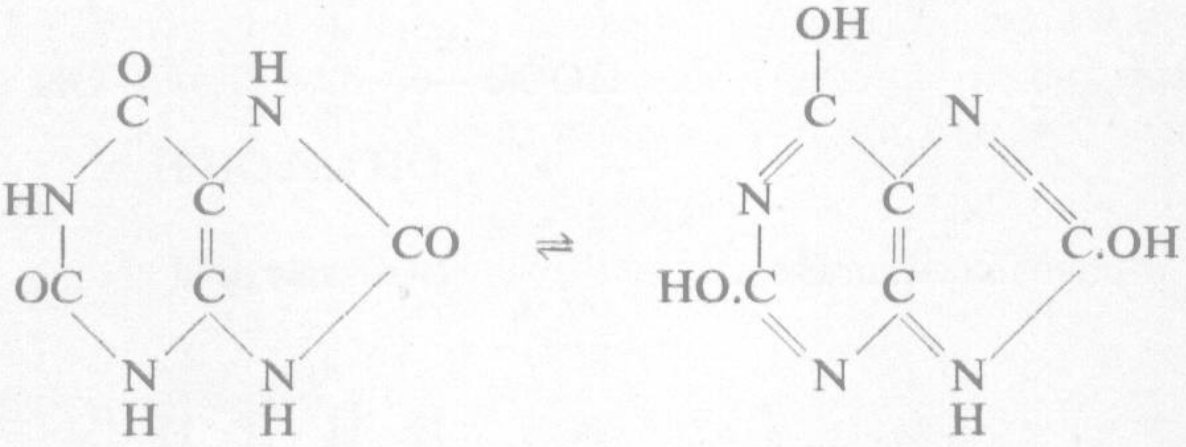
Other naturally occurring purine derivatives include hypoxanthine, xanthine, and uric acid.



hypoxanthine
(6-hydroxypurine)



xanthine
(2:6-dihydroxypurine)



keto form ⇌ enol form

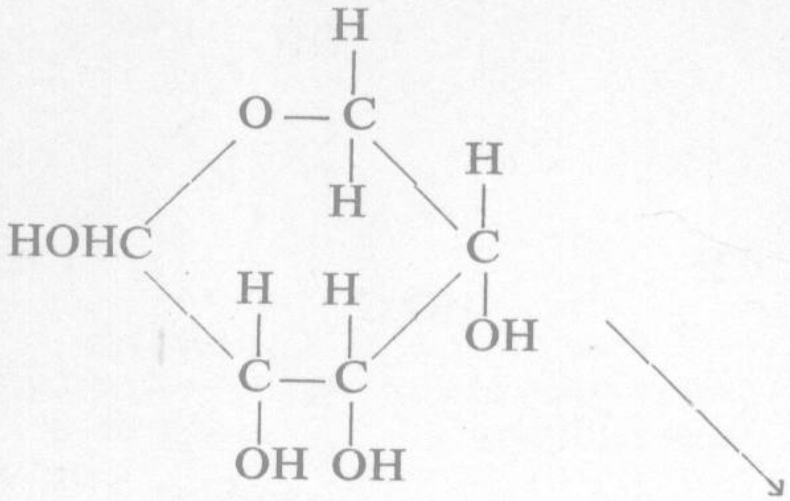
uric acid
(2:6:8-trihydroxypurine)

The chemistry of the pyrimidines and purines has been reviewed by Lythgoe(35).

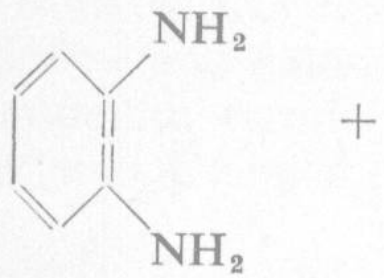
2.41 *Pentose sugars*

It has long been recognized that the nucleic acid originally prepared from yeast contained a pentose sugar which was identified as ribose by Levene(1) in 1909, using methods which were not absolutely conclusive. More recently Gulland(2) and his colleagues have proved without doubt that the pentose in yeast RNA is D(-)ribose by converting the aldonic acid from the sugar obtained on hydrolysis of

the purine nucleotides of yeast RNA to the corresponding benziminazole which is easily identified.

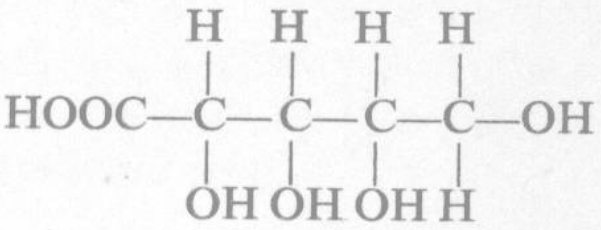


D-ribose

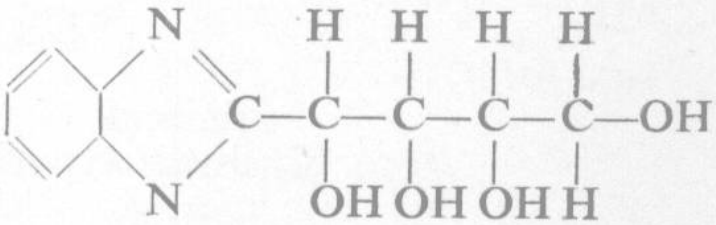


o-phenylenediamine

+

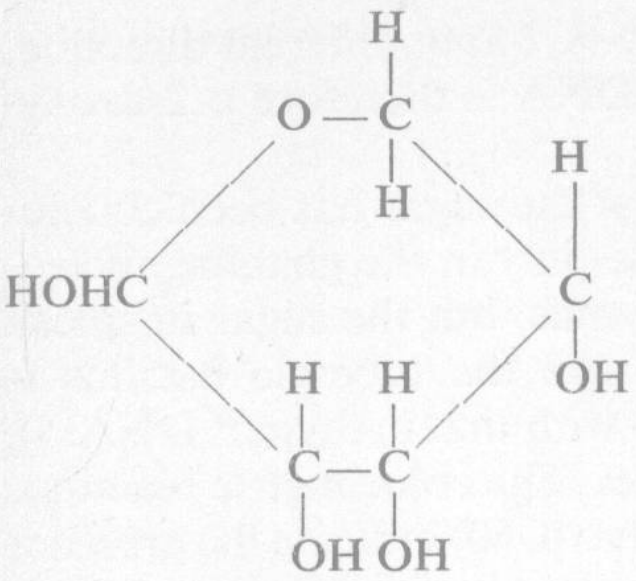


D-ribonic acid

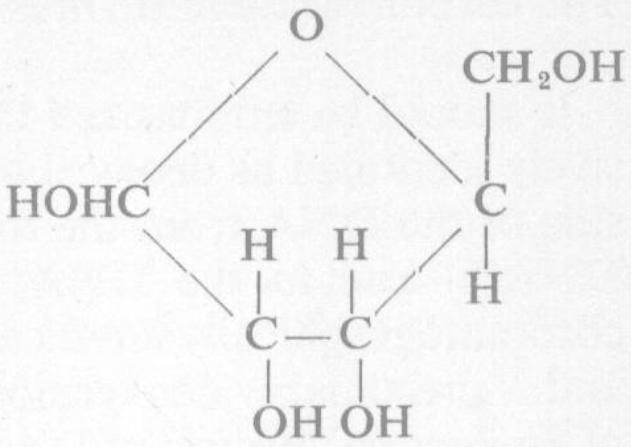


D-ribobenziminazole

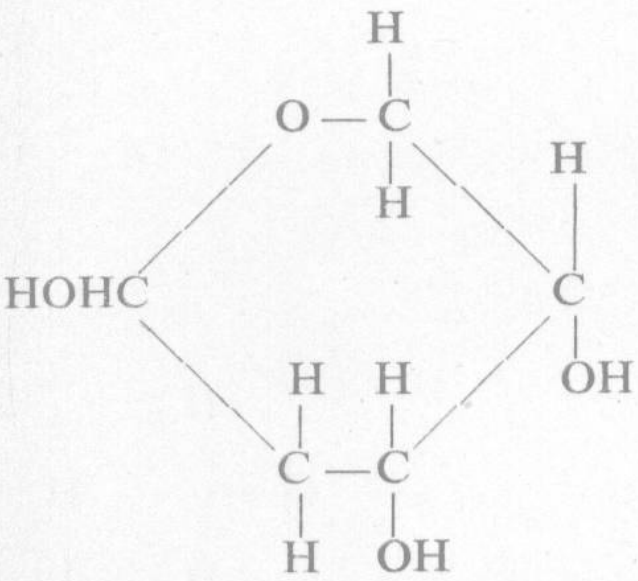
The sugar component in liver RNA has been proved to be ribose by identification as the *p*-bromophenylhydrazone(3). Since the pentose in pancreas RNA and in the RNA of the tubercle bacillus is chromatographically identical with that in yeast RNA, it also is ribose(4), and since the nucleotides from the pentose nucleic acid of tobacco mosaic virus are identical with their analogues from yeast RNA, the sugar in the virus nucleic acid is presumably also ribose(6).



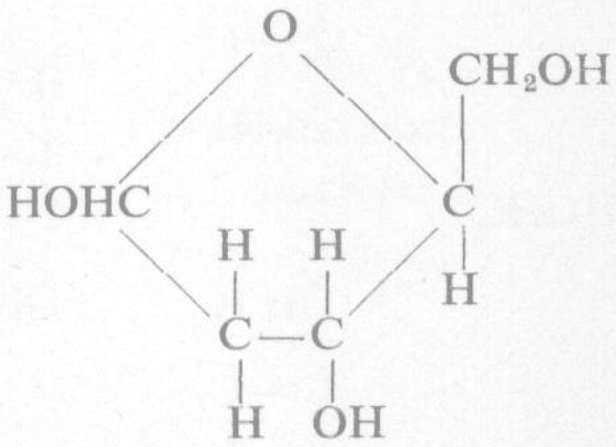
D-ribopyranose



D-ribofuranose



D-2-deoxyribopyranose



D-2-deoxyribofuranose

2.42 *Deoxypentose sugars*

Doubts surrounding the nature of the sugar present in thymus DNA were resolved when Levene and Mori(7) isolated the sugar from the guanine nucleoside of this nucleic acid and showed that it was a deoxypentose. Only two 2-deoxypentose sugars can exist, deoxyribose or ribodeose (arabinodeose) derived from ribose and arabinose, and deoxyxylose or xylodeose (lyxodeose) derived from xylose and lyxose.

Synthetic L-deoxyribose (and its benzylphenyl hydrazone) showed rotations of exactly the same value as the

deoxypentose from thymus DNA, but of different direction. The carbohydrate in thymus DNA is therefore D-2-deoxyribose.

It should be emphasized that the sugar has been conclusively identified as deoxyribose only in the guanine nucleoside of the DNA from the thymus, but the sugar in spleen DNA(8) and in the DNA from the tubercle bacillus is chromatographically identical with that in thymus DNA(5), and is presumably deoxyribose. The colorimetric reactions of Feulgen (p. 50) and of Dische (p. 60) indicate the presence of a deoxy sugar and are not specific for a deoxypentose, far less deoxyribose.