Reports and Working Notes on DNA

Publication/Creation

1951-1954

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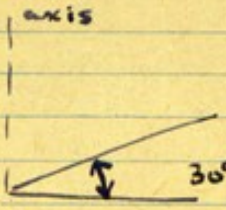
Brief Summary of main Conclusions & Result on D.N.A.

From. I.R. Spectra

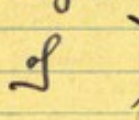
1. Purine & Pyrimidine rings preferentially oriented $\perp^r$ to direction of shear.

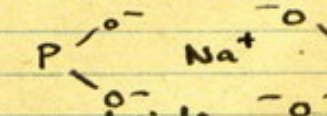


Results set limit ~ 30° from $\perp^r$ but probable that nearer to 20°.

Nature

Prog. in Biophysics

2. Absorption bands corresponding to symmetrical & antisymmetrical vibrations of  indicate equivalence of the P–O⁻ bonds. Dichroism indicates that POO plane is within 30° of $\perp^r$.

3. Failure of NH, NH_2, $CEOH$, of bases to exchange with D_2O at rm. temperature indicate that solution leaves the H-bonds intact. i.e. The solution involves rupture of  type of attraction. ergo phosphates outside. Also coiled chains or associated chains held by H-bonds & V.d.Waals. forces.

Thesis

4. Approximate calculations show that energy to be expected from:—

a) H bonds ~ 7 k.cal. per mole of nucleotide
b) Van der Waals (Bases) ~ 16 k.cal per .. .. ..
c) Sodium–Phosphate–Bonds — No exact calculations but estimated considerable say ~ 20–30 kal per mole of nucleotide.

Nos.

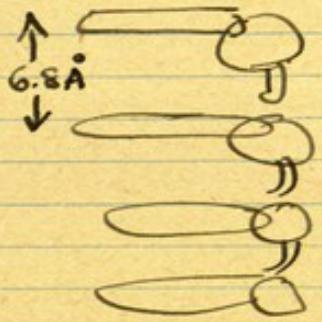
models.

models show:—

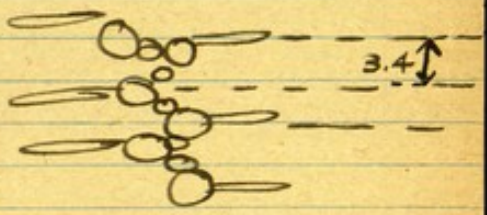
① Fully extended chain

[Not previously reported]

gives ⊥r Bases
6.8Å between bases.



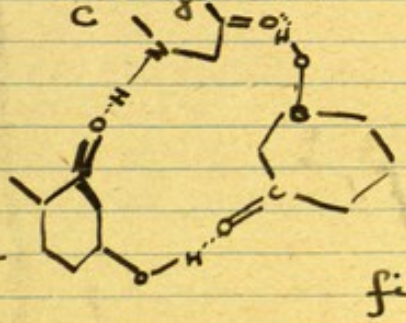
② Furberg-like modes



Photos of 1 & 2 available.

Formation of H-bonds.

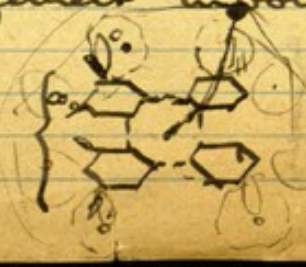
Suggested arrangement (wire model)



Ring formation all bases may be fitted.

next layer sits on top giving max. V. der Waals energy. 3 chain arrangement involves Twist
∴ HELIX (☠!)

Four chain arrangement.



can be built by putting sugars alternately each side in vertical array.

R E Franklin

PHYSICAL STUDIES OF NUCLEIC ACID

By Dr. M. H. F. WILKINS, R. G. GOSLING and W. E. SEEDS; Mrs. MARY J. FRASER and ROBERT D. B. FRASER

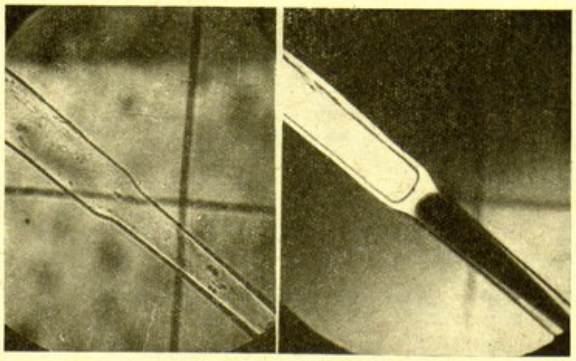
(Reprinted from Nature, Vol. 167, p. 759, May 12, 1951)

PHYSICAL STUDIES OF NUCLEIC ACID

Nucleic Acid: an Extensible Molecule ?

THE negative birefringence and ultra-violet dichroism of streaming solutions and fibres of sodium thymonucleate[1-3] has been taken as indicating a long molecule with purine and pyrimidine rings roughly at right-angles to the length. Astbury[4] has observed by X-ray diffraction a 3·4-A. spacing along the length of the fibres, and has suggested that the nucleotides are flat and lie 3·4 A. apart. We have studied in more detail the properties of sodium thymonucleate fibres and have confirmed the above general conception, but have observed an unusual stretching phenomenon in the fibres which suggests that the long molecules may be extended into a second form.

The main experimental facts may be summarized as follows. Fibres in air of 50 per cent humidity are partly crystalline with remarkably well-oriented crystallites. Birefringence, ultra-violet dichroism[3] and most of the infra-red dichroism[3] are all negative. The fibres have little elasticity, and, when a fibre of uniform diameter is stretched beyond the yield point, necks of approximately zero birefringence appear in the negative fibre. These necks have a uniform diameter about 20 per cent less than that of the negatively birefringent part of the fibre (see photograph). On further stretching, the shoulders at the ends of the necks move apart along the fibre. In addition to the increase in length of the necks due to this process, the necks also lengthen by stretching and become positively birefringent (0·02). Such material is non-crystalline, non-dichroic in the ultra-violet, and most of the infra-red dichroism is positive.



(*a*) (*b*)

Fibres of sodium thymonucleate. × 1,000

(*a*) Shoulder at end of neck; (*b*) same between crossed nicols, with compensation, showing thicker portion of fibre negatively birefringent and the thinner portion or neck positively birefringent

The fibre is roughly double its original length when the breaking point is reached. The positively birefringent fibre is stable at 50 per cent humidity but returns to the negative form, with complete disappearance of necks, when placed in a humid atmosphere, the length shrinking from about 1·5 to 1. Probably the reversible part of the stretching is in roughly this ratio, and some irreversible stretching occurs after the phase change of the necking has taken place. Similar phase changes may be produced by rolling or stretching sheets of thymonucleate and fibres of thymus nucleoprotein.

The fibres swell and shrink markedly when the humidity of the surrounding air is varied. In saturated water vapour the fibres are largely microcrystalline; but, on reducing the humidity to 50 per cent, a reversible change takes place, the greater part of the crystallinity disappears, length is reduced by approximately 30 per cent, cross-section is halved, and the birefringence per unit mass of nucleic acid and the ultra-violet dichroism are reduced. More complete dehydration is obtained by warming in air or *in vacuo*, when approximately 10 per cent shrinkage in diameter and length takes place, all crystallinity disappears, the infra-red dichroism is reduced, and the negative birefringence drops to 0·02, which is less than half that at 50 per cent humidity. The dried fibres are rapidly hydrated only if placed in an atmosphere of more than 50 per cent humidity.

There is little change in X-ray spacing along the length of the fibre on drying, and, in view of the great length of the molecules, which is probably at least 1000 A., it would appear likely that most of the increase of length of the fibre on hydration is associated with the change from the amorphous to the crystalline state, and is not due to water packing between crystallites. Presumably, on dehydration, the backbone of phosphate ester linkages in the molecule becomes crumpled and the purine and pyrimidine rings tilted; and, on hydration, the molecules pack regularly together and become extended in length with the purine and pyrimidine rings approximately at right-angles to the length. It is also unlikely that the reversible extension of the fibre on stretching is due to reversible slipping of molecules over one another, and it is difficult to avoid the conclusion that these molecules have been extended in length during the necking process. The optical observations show that the purine and pyrimidine rings have rotated during this process and lie on the average at about 45° to the length of the fibre.

When one considers the general form of the nucleic acid structure, a hypothesis which could explain these observations comes to mind. Nucleic acid consists broadly of large flat rings closely packed together[4,5], each nucleotide being joined to the next by a single chain of bonds. Such a system might be deformed very easily, and the rings might slide over one another from one equilibrium position to another (corresponding to the discontinuous process of necking) while keeping their separation roughly constant. If the general direction of the phosphate backbone of the molecule is kept straight during this process, extension of the backbone-length in the ratio of 1 to $\sqrt{2}$ would mean tilting the rings from 90° to 45° as observed. The extension could take place by rotations of the bonds in the backbone-chain relative to one another, the bond-angles being kept roughly constant.

Whatever the precise mechanism of the necking process, its study may well cast light on the structure of nucleic acid. So far the X-ray diffraction picture of the positive phase shows only diffuse rings, but in the case of infra-red dichroism the change of orientation of the units in the molecule gives much extra information[5].

We wish to thank Prof. R. Signer for sodium thymonucleate, Dr. K. A. Smith for nucleoprotein, and Prof. J. T. Randall and Dr. R. E. Franklin and colleagues for discussion. A fuller account of this work will be published later. We are indebted to the

Chemistry Department of King's College for the use of its X-ray equipment.

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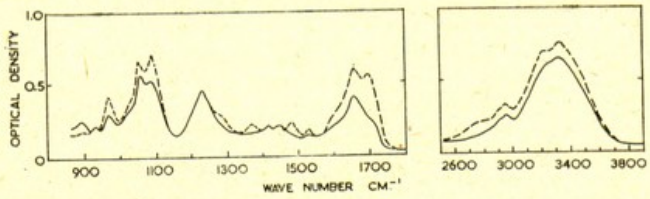
[1] Schmidt, W. J., "Die Doppelbrechung von Karyoplasma, Zytoplasma und Metaplasma" (Berlin, 1937).
[2] Signer, R., Caspersson, T., and Hammarsten, E., *Nature*, **141**, 122 (1938).
[3] Seeds, W. E., and Wilkins, M. H. F., Farad. Soc. Discuss., Cambridge (1950).
[4] Astbury, W. T., Symp. Soc. Exp. Biol., I. Nucleic Acid (Camb. Univ. Press, 1947).
[5] Fraser, M. J., and Fraser, R. D. B. (see following communication).
[6] Furberg, S., *Acta Chem. Scand.*, **4**, 751 (1950).

Evidence on the Structure of Deoxyribonucleic Acid from Measurements with Polarized Infra-Red Radiation

This communication is a preliminary account of a study of the nucleic acids by means of infra-red radiation; we shall discuss the extent to which our measurements support existing models of these polynucleotide structures.

On the basis of X-ray diffraction patterns from oriented sodium deoxyribonucleate fibres, Astbury[1-3] has proposed a model in which the nucleotides, which he supposed to be nearly planar, are piled directly on each other in stiff columns, with their planes roughly perpendicular to the axis of the molecule. Meridian arcs corresponding to axial repeat distances of 3·34 and 27 A. were observed in the X-ray diffraction patterns, and presumed to correspond to the separation of nucleotides, and a repeat of pattern every eight or sixteen nucleotides. The larger repeat was associated with the tetranucleotide constitution favoured at that time.

Since these ideas were put forward, little experimental support has been found for the tetranucleotide hypothesis, and recent X-ray studies of the ribose nucleosides by Furberg[4] have shown that in the crystal these nucleosides are not planar. The planes of the D-ribose and pyrimidine residues were found to be nearly normal. Furberg[4] supposes that this configuration is present in deoxyribose nucleic acid,



Infra-red spectrum of sodium deoxyribonucleate, obtained with polarized radiation. The full curve corresponds to the electric vector vibrating parallel to, and the broken curve perpendicular to, the direction of shear

and has proposed two modifications of Astbury's structure. In one case the backbone of sugar rings and phosphate groups forms a spiral enclosing the bases; in the other the backbone is a zigzag chain with the bases outermost. The infra-red dichroisms which we have observed in oriented films of sodium deoxyribonucleate can be interpreted in terms of a structure similar to that proposed by Furberg.

A detailed discussion of the chemical methods employed in preparing the material, and the assignment of the infra-red absorptions, will be given in a later publication, but the main features will be summarized here. The material was obtained from calf thymus by a modified form of the techniques used by Mirsky and Pollister[4], and Signer and Schwander[2]. Minced thymus gland was washed with citrate buffer (*p*H 6·8) containing a trace of thymol, and extracted with 0·14 *M* sodium chloride. The residue was extracted with 1 *M* sodium chloride, and the resulting nucleoprotein solution deproteinized by the Sevag[3] technique. The sodium chloride was removed by dialysing against distilled water, and the sodium deoxyribonucleate stored in the frozen-dried state until required.

Oriented samples were obtained by shearing viscous solutions on a microscope slide treated so as to have a non-polar surface. The films obtained by this method were extremely thin, and negatively birefringent with respect to the direction of shear. The infra-red measurements were made with a reflecting microscope, of numerical aperture 0·8, used in conjunction with a Grubb–Parsons infra-red spectrometer[5]. By this method small areas *c.* 0·5 mm. × 0·05 mm. were selected in which the film was of uniform thickness and showed high orientation.

The spectra obtained are indicated in the diagram, in which the optical density is plotted against wave-number with the electric vector vibrating parallel, and perpendicular, to the direction of shear. The main features of the spectra are the regions of intense

absorption around 3,300, 1,700 and 1,050 cm.$^{-1}$, all of which show a greater optical density with the electric vector vibrating perpendicular to the direction of shear (perpendicular dichroism).

The bands at 3,335 and 3,210 cm.$^{-1}$ are most probably N—H stretching vibrations of the amino- and imino-groups of the purine and pyrimidine residues, whereas the bands at 1,660 and 1,700 cm.$^{-1}$ are double-bond (C = O, C = N, C = C) vibrations of these ring systems. In both cases the transition moments associated with the vibrations will be in the plane of the ring. The perpendicular dichroism of these vibrations supports the approximate perpendicularity of the planar bases to the chain-axis. Further confirmation of this is obtained from the ultra-violet dichroism[10-12], and the fact that the optical birefringence is negative. The high water content of these films probably accounts for the different dichroic ratios of these bands, since water absorbs intensely near 3,450 and 1,640 cm.$^{-1}$.

The strong band at 1,087 cm.$^{-1}$ is believed to correspond to a C_1—O—C_4 vibration in the sugar residue, and the 1,052 cm.$^{-1}$ band to a C—O—P vibration[13]. The transition moment associated with the C_1—O—C_4 vibration is likely to be in the C_1—C_4 direction, so that the perpendicular dichroism observed is consistent with Furberg's model. Two P—O—C vibrations are to be expected, one corresponding to the nucleoside – phosphate ester linkage (P—O—C_3), the other to the internucleotide ester linkage (P—O—C_5). The relative intensities and directions of transition moment are not known ; but if it is assumed that the intensities are equal, and the transition moments are in the direction P—C_5 and P—C_3, the observed dichroism is of the correct character.

The valence vibration of the phosphoryl (P = O) group is known to occur around 1,250 cm.$^{-1}$ [13,14], and it seems likely that the main component of the band observed at 1,235 cm.$^{-1}$ is associated with this vibration. By analogy with the carbonyl group, lower frequencies are expected where hydrogen bonding occurs. The absence of dichroism indicates an effective transition moment inclined at 54½° to the chain-axis. The Furberg model requires a perpendicular dichroism for this vibration, but a consistent modification is obtained by a slight reorientation of the phosphate group. Alternatively, the partially resolved perpendicular band at *c.* 1,290 cm.$^{-1}$ could be assigned to this vibration, though this seems less probable.

Recent observations by Wilkins *et al.*[15] suggest that a reversible change occurs in the structure of oriented films and fibres when they are stretched or rolled. These observations are supported by infra-red measurements, which show that the dichroisms of the 3,335, 3,210, 1,700, 1,660, 1,087, 1,052 and 967 cm.$^{-1}$ bands are all reversed to parallel, whereas the dichroism of the 1,235 cm.$^{-1}$ band remains unchanged at a value of unity.

The results obtained so far do not contradict the original Astbury model, but we have preferred to interpret our results in terms of Furberg's modification, in view of his recent evidence on the stereochemistry of ribose nucleosides, and the steric distortions of the phosphate oxygens required in Astbury's model[2]. The assignment of the perpendicular band at 967 cm.$^{-1}$, which shows a marked reversal of dichroism on stretching, may throw new light on this question.

We wish to thank Prof. J. T. Randall for his continued advice and encouragement in this investigation, and Drs. M. H. F. Wilkins and W. C. Price for helpful discussions. One of us (R. D. B. F.) wishes to acknowledge a Medical Research Council Studentship.

Mary J. Fraser
Robert D. B. Fraser

Medical Research Council Biophysics Research Unit and Wheatstone Physics Laboratory, King's College, London, W.C.2.

Feb. 29.

[1] Astbury, W. T., *Nature*, **141**, 747 (1938).
[2] Astbury, W. T., Symp. Soc. Exp. Biol., I. Nucleic Acid (1947).
[3] Rudall, K. M., "Progress in Biophysics", **1**, 39 (1950).
[4] Furberg, S., *Acta Chem. Scand.*, **4**, 751 (1950).
[5] Furberg, S., Ph.D. thesis, University of London (1949).
[6] Mirsky, A. E., and Pollister, A. W., *J. Gen. Physiol.*, **30**, 117 (1946).
[7] Signer, R., and Schwander, H., *Helv. Chim. Acta*, **32**, 853 (1949).
[8] Sevag, M. G., Lackmann, D. B., and Smolens, J., *J. Biol. Chem.*, **124**, 425 (1938).
[9] Fraser, R. D. B., Farad. Soc. Discuss., Cambridge (1950).
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[11] Schmidt, W. J., "Die Doppelbrechung von Karyoplasma, Zytoplasma und Metaplasma" (Berlin, 1937).
[12] Signer, R., Caspersson, T., and Hammarsten, E., *Nature*, **141**, 122 (1938).
[13] Bellamy, L. J., and Beecher, J., *J. Chem. Soc.* (in the press).
[14] Gore, R. C., Farad. Soc. Discuss., Cambridge (1950).
[15] Wilkins, M. H. F., Gosling, R. G., and Seeds, W. E. (see preceding communication).

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Dr.Franklin

WHEATSTONE PHYSICS LABORATORY

Specific Optical Rotation of Nucleic Acid

One well-known type of optical rotation (e.g. quartz) arises from a helical arrangement of atoms in the crystal. I suppose it is technically too difficult to observe this in nucleic acid fibres, although it should be quite feasible in sheets. In fact it has already been observed in gelatin films by Robinson and Bott (Nature, 168, 325, 1951). An interesting point is that specific rotation in many substances is very high near an absorption band. Thus the optical rotation of a film of nucleic acid could be observed photographically (this is a standard technique) near λ2500 where the nucleotides absorb and the effect of moisture content and stress could also be studied. (The law is very similar to that of the Sellmeier dispersion formula).

The method has possibilities in solution as a means of correlation with light-scattering work. I doubt if it is theoretically possible to correlate the rotation with helical structure only since it can arise from asymmetric carbon atoms, but it might be a very useful method in showing changes of configuration arising from changes of pH etc.

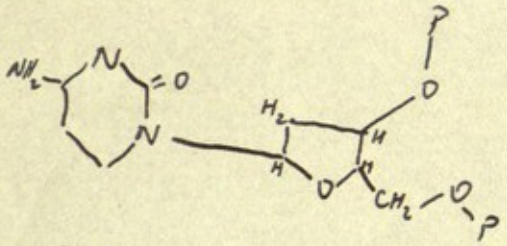
28.11.51

J.T.Randall

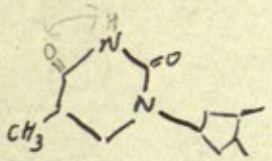
Chargaff's analysis

1.0 cytosine
1.5 thymine
1.6 adenine
1.3 guanine

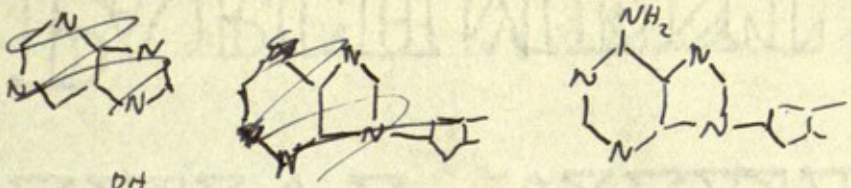
Cytosine



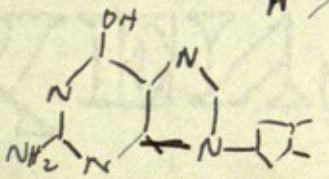
Thymine



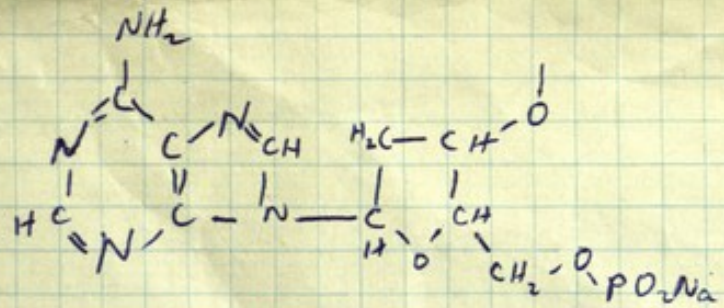
Adenine



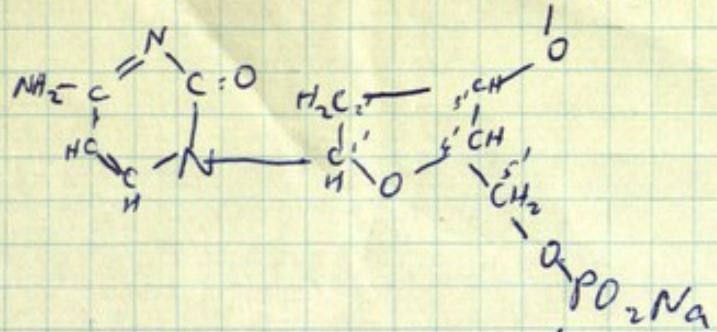
Guanine



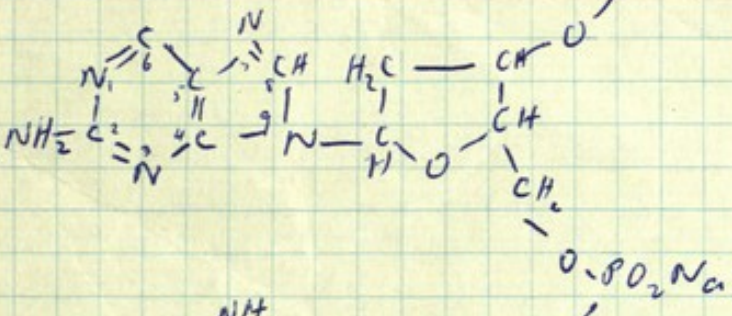
Adénine



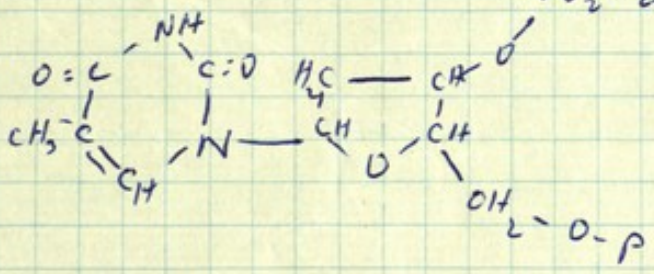
Cytosine



Guanine



Thymine



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July 25, 1973

27 JUL 1973

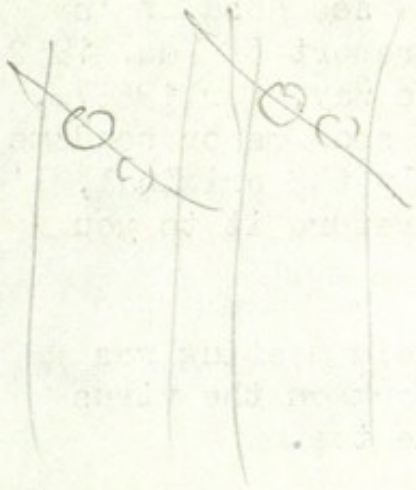
Dear Aaron:

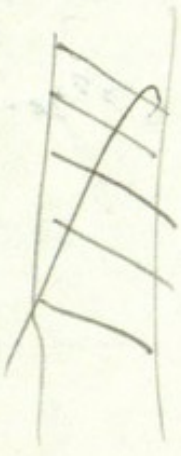
I'd very much like to see Rosalind's Turner-Newall fellowship report of Feb. 1952 (was there another, by the way, for 1953?). Is it something you could send me or send me a copy of? If you have only the original, I'd undertake to copy it and return it to you immediately.

I thought the Stockholm meeting was most exciting, and your work on the virus coat proteins was near the top.

With best regards,

David Sayre





ANNUAL REPORT

1st JANUARY, 1953 - 1st JANUARY, 1954

ROSALIND E. FRANKLIN,

Birkbeck College Crystallographic Laboratory,

21 Torrington Square, W.C.1.

The work carried out during the past year may be divided into three periods:

1. 1st January to 16th March. King's College.

During this period I continued to work in King's College on the structure desoxyribonucleic acid. Two papers entitled:

"Fibre Diagrams of Sodium Thymonucleate:
I. The Influence of Water Content
II. The Cylindrically Symmetrical Patterson Function."

were written (in collaboration with R.G. Gosling) and sent to Acta Crystallographica in March 1953. These have now been published, and copies are attached to this report.

Further work on the 3-dimensional Patterson function was carried out, but no quantitative results were obtained in this way.

Measurements on the X-ray fibre-digrams of Structure B (the less ordered form of sodium desoxyribonucleate, and that which we believe to exist more or less unmodified both in solution and in natural nucleo-protein) yielded a considerable amount of information. This is summarised in a note to Nature written in collabora-

-tion with R.G. Gosling (25th April, 1953) and entitled: "Molecular Configuration in Sodium Thymonucleate". A copy is enclosed with this report.

It is shown that the molecule of sodium thymonucleate in Structure B must consist of a two-strand helix, rather similar to that proposed by Watson and Crick (Nature, 25th April, 1953) but of smaller radius.

Since Structure B (~~NaDNA~~) has a 2-strand helical molecule, and since the change A $\rightleftarrows$ B is, in general, readily reversible, it follows that a 2-strand helical molecule must also exist in Structure A. Evidence for a 2-strand helix in structure A was obtained from a study of the cylindrically averaged Patterson function.

2. March 1953 - November 1953. Birkbeck College.

Owing to unexpected delays in obtaining the necessary apparatus for carrying out a programme of X-ray crystallographic research on viruses, a substantial part of this period was spent in continuing the interpretation of the X-ray diagrams of nucleic acid and their Patterson functions. At the same time, a literature survey was carried out of previous work on the molecular structure of viruses.

The evidence for a two-strand helical molecule of the Structure A form of DNA was presented in a note to Nature, 25th July, 1953, written in collaboration with R.G. Gosling. A copy is enclosed. The helix is of radius 9A and has 11 residues per turn. The evidence is based mainly on a study of the cylindrically symmetrical Patterson function of Structure A. It has also been

shown that the proposed structure accounts for many of the strongest features of the 3-dimensional Patterson function.

3. November - December 1953. Birkbeck College.

During this period X-ray diffraction studies of tobacco mosaic virus were started. For this purpose an Ehrenberg-Spear fine-focus X-ray tube is used, with nickel-filtered copper Kα radiation. The X-ray camera is the Phillips micro-camera modified to take a specimen-film distance of 30 mm. or 60 mm. as well as the usual distances of 10 mm. and 15 mm. It is filled with hydrogen during all exposures.

The virus solution was kindly given to this laboratory by Dr. R. Markham.

The research is a continuation of the earlier studies of Bernal and Fankuchen (1942) and of Watson (1953).

Highly detailed diffraction diagrams of orientated virus specimens (prepared by the method of Bernal and Fankuchen) containing varying amounts of water have already been obtained. While the greater part of the high-angle pattern is substantially independent of water content, the reflections corresponding to distances of about 20 A vary strikingly. This suggests that the water most closely associated with the virus may lie on either side of some structural component having at least one dimension of about 20 A.

A detailed study of the small differences in the intra-particle pattern for wet and dry viruses should make it possible to calculate the Patterson function of the difference, and hence to locate the water.

Further, intensity measurements of the equatorial reflections,

which are related to inter-particle, should make it possible to decide whether or not the ribonucleic acid forms a central core in the rod-like particle, as has been suggested by several authors. Preliminary measurements indicate the presence of a heavy core (presumably RNA) in the rod.

4. Miscellaneous.

(a) In April 1953 I was invited to the "Steinkohlentagung" at Aachen, Germany. There I read a paper on "The Mechanism of Crystallite Growth in Carbons" which is to be published (in German) in Brennstoff-Chemie in December 1953. Reprints are not yet available.

The new part this work consisted in a kinetic explanation of the sharpness of the separation of carbonaceous solids into two classes, the graphitising and non-graphitising, and an explanation of the apparent elongated shape of the crystallites in graphitising carbons.

(b) In June 1953 I read a short paper on "Le rôle de l'eau dans l'acide graphitique" to an international colloquium in Paris on "Water in Solids". In this paper a new type of structure for graphitic acid is proposed. A reprint is enclosed.

(5 reprints enclosed)

2ndAAugust 1973

Dr. D. Sayer,
Department of Zoology,
Laboratory of Molecular Biophysics,
South Parks Road,
Oxford
OX1 3PS

Dear David,

I enclose copies of the reports you asked for, I believe I have sent these on to Anne before, but in any case they may be difficult to find.

It was good to have your company to, and at, Stockholm.

Yours sincerely,

A. Klug

Encl.

R E Franklin

A PROPOSED STRUCTURE FOR THE NUCLEIC ACIDS

By

LINUS PAULING AND ROBERT B. COREY

Gates and Crellin Laboratories of Chemistry, California Institute of Technology

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A PROPOSED STRUCTURE FOR THE NUCLEIC ACIDS

By Linus Pauling and Robert B. Corey

Gates and Crellin Laboratories of Chemistry,* California Institute of Technology

Communicated December 31, 1952

The nucleic acids, as constituents of living organisms, are comparable in importance to the proteins. There is evidence that they are involved in the processes of cell division and growth, that they participate in the transmission of hereditary characters, and that they are important constituents of viruses. An understanding of the molecular structure of the nucleic acids should be of value in the effort to understand the fundamental phenomena of life.

We have now formulated a promising structure for the nucleic acids, by making use of the general principles of molecular structure and the available information about the nucleic acids themselves. The structure is not a vague one, but is precisely predicted; atomic coordinates for the principal atoms are given in table 1. This is the first precisely described structure for the nucleic acids that has been suggested by any investigator. The structure accounts for some of the features of the x-ray photographs; but detailed intensity calculations have not yet been made, and the structure cannot be considered to have been proved to be correct.

The Formulation of the Structure.—Only recently has reasonably complete information been gathered about the chemical nature of the nucleic acids. The nucleic acids are giant molecules, composed of complex units. Each unit consists of a phosphate ion, HPO_4^{--}, a sugar (ribose in the ribonucleic

acids, deoxyribose in the deoxyribonucleic acids), and a purine or pyrimidine side chain (adenine, guanine, thymine, cytosine, uracil, 5-methylcytosine). The purine or pyrimidine group is attached to carbon atom 1′ of the sugar, through the ring nitrogen atom 3 in the case of the pyrimidine nucleotides,[1] and the ring nitrogen atom 9 in the case of the purine nucleotides.[2] Good evidence has recently been obtained as to the nature of the linkage between the sugar and the phosphate, through the investigations of Todd and his collaborators;[3] it seems likely that the phosphate ester links involve carbon atoms 3′ and 5′ of the ribose or deoxyribose. New chemical evidence that the natural ribonucleosides have the β-D-ribofuranose configuration has also been reported by Todd and his collaborators,[4] and spectroscopic evidence indicating that the deoxyribonucleosides have the same configuration as the ribonucleosides has been obtained.[5] The β-D-ribofuranose configuration has been verified for cytidine by the determination of the structure of

TABLE 1

ATOMIC COORDINATES FOR NUCLEIC ACID

ATOM	ρ	ϕ	z	ATOM	ρ	ϕ	z
P	2.65 Å	0.0°	0.00 Å	O_I'	4.4 Å	45.4°	2.65 Å
O_I	2.00	28.3°	−0.67	O_2'	6.1	81.0°	2.1
O_{II}	2.00	−28.3°	0.67	N_3	6.7	52.8°	2.8
$O_{III} = O_3'$	3.72	13.5°	0.93	C_4	7.85	59.3°	2.8
$O_{IV} = O_5'$	3.72	−13.5°	−0.93	C_5	9.1	55.2°	2.8
C_1'	3.4	35.3°	0.7	C_6	9.35	46.9°	2.8
C_4'	3.2	51.6°	1.9	N_8	10.7	44.9°	2.8
C_3'	3.8	74.6°	1.55	N_1	8.45	39.9°	2.8
C_2'	5.3	70.3°	1.75	C_2	7.05	41.5°	2.8
C_5'	5.3	58.2°	2.8	O_2	6.35	32.4°	2.8

Identity distance along z axis = 27.2 Å.

Twenty-four atoms of each kind, with cylindrical coordinates (right-handed axes): $\rho, \phi + n \cdot 105.0°, n \cdot 3.40 + z$; $\rho, \phi + n \cdot 105.0° + 120°, n \cdot 3.40 + z$; $\rho, \phi + n \cdot 105.0° + 240°, n \cdot 3.40 + z$; $n = 0, 1, 2, 3, 4, 5, 6, 7$.

the crystal by x-ray diffraction; cytidine is the only nucleoside for which a complete x-ray structure determination has been reported.[6]

X-ray photographs have been made of sodium thymonucleate and other preparations of the nucleic acids by Astbury and Bell.[7, 8] It has recently been reported by Wilkins, Gosling, and Seeds[9] that highly oriented fibers of sodium thymonucleate have been prepared, which give sharper x-ray photographs than those of Astbury and Bell. Our own preparations have given photographs somewhat inferior to those of Astbury and Bell. In the present work we have made use of data from our own photographs and from reproductions of the photographs of Astbury and Bell, especially those published by Astbury.[10] Astbury has pointed out that some information about the nature of the nucleic acid structure can be obtained from the x-ray photographs, but it has not been found possible to derive the structure from x-ray data alone.

A configuration of polypeptide chains in many proteins is the α helix.[11] In this structure the amino-acid residues are equivalent (except for differences in the side chains); there is only one type of relation between a residue and neighboring residues, one operation which converts a residue into a following residue. Through the continued application of this operation, a rotation-translation, the α helix is built up. It seems not unlikely that a single general operation is also involved in the construction of nucleic acids, polynucleotides, from their asymmetric fundamental units, the nucleotide residues. The general operation involved would be a rotation-reflection, and its application would lead to a helical structure. We assume, accordingly, that the structure to be formulated is a helix. The giant molecule would thus be cylindrical, with approximately circular cross section.

Some evidence in support of this assumption is provided by the electron micrographs of preparations of sodium thymonucleate described by Williams.[12] The preparation seen in the shadowed electron micrograph is clearly fibrous in nature. The small fibrils or molecules seem to be circular in cross-section, and their diameter is apparently constant; there is no evidence that the molecules are ribbon-like. The diameter as estimated from the length of the shadow is 15 or 20 Å. Similar electron micrographs, leading to the estimated molecular diameter 15 ± 5 Å, have been obtained by Kahler and Lloyd.[13] Also, estimates of the diameter of the molecules of native thymonucleic acid in the range 18 to 20 Å have been made[14, 15] on the basis of sedimentation velocity in the ultracentrifuge and other physicochemical data. The molecular weights reported are in the range 1 million to 4 million.

The x-ray photographs of sodium thymonucleate show a series of equatorial reflections compatible with a hexagonal lattice. The principal equatorial reflection, corresponding to the form 10·0, has spacing 16.2 Å or larger, the larger values corresponding to a higher degree of hydration of the substance. The minimum value,[7] 16.2 Å, corresponds to the molecular diameter 18.7 Å. From the average residue weight of sodium thymonucleate, about 330, and the density, about 1.62 g. cm.$^{-3}$, we calculate that the volume per residue is 338 Å.^{3} The cross-sectional area per residue is 303 Å^2; hence the length per residue along the fiber axis is about 1.12 Å.

The x-ray photographs show a very strong meridional reflection, with spacing about 3.40 Å. This reflection corresponds to a distance along the fiber axis equal to three times the distance per residue. Accordingly, the reflection is to be attributed to a unit consisting of three residues.

If the molecule of nucleic acid were a single helix, the reflection at 3.4 Å. would have to be attributed to a regularity in the purine-pyrimidine sequence, or to some other structural feature causing the three nucleotides in the structural unit to be different from one another. It seems unlikely

that there is a structural unit composed of three non-equivalent nucleotides.

The alternative explanation of the x-ray data is that the cylindrical molecule is formed of three chains, which are coiled about one another. The structure that we propose is a three-chain structure, each chain being a helix with fundamental translation equal to 3.4 Å, and the three chains being related to one another (except for differences in the nitrogen bases) by the operations of a threefold axis.

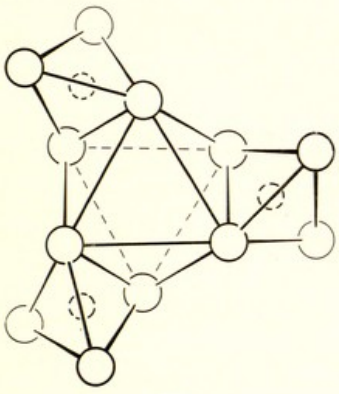
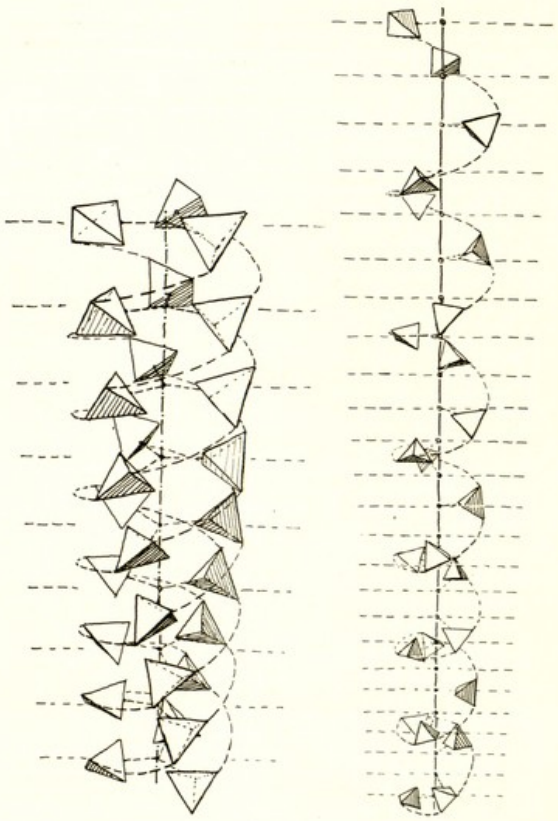


FIGURE 1

A group of three phosphate tetrahedra near the axis of the nucleic acid molecule. Oxygen atoms are indicated by full circles and phosphorus atoms by dashed circles.

The first question to be answered is that as to the nature of the core of the three-chain helical molecule—the part of the molecule closest to the axis. It is important for stability of the molecule that atoms be well packed together, and the problem of packing atoms together is a more difficult one to solve in the neighborhood of the axis than at a distance away from the axis, where there is a larger distance between an atom and the equivalent atom in the next unit. (An example of a helical structure which seems to satisfy all of the structural requirements except that of close packing of atoms in the region near the helical axis is the 5.2-residue helix of polypeptide chains. This structure seems not to be represented in proteins, whereas



the similar α helix, in which the atoms are packed in a satisfactorily close manner about the axis, is an important protein structure.) There are three possibilities as to the composition of the core: it may consist of the purine-pyrimidine groups, the sugar residues, or the phosphate groups. It is found by trial that, because of their varied nature, the purine-pyrimidine groups cannot be packed along the axis of the helix in such a way that suitable bonds can be formed between the sugar residues and the phosphate groups; this choice is accordingly eliminated. It is also unlikely that the sugar groups constitute the core of the molecule; the shape of the ribofuranose group and the deoxyribofuranose group is such that close packing of these groups along a helical axis is difficult, and no satisfactory way of packing them has been found. An example that shows the difficulty of achieving close packing is provided by the polysaccharide starch, which forms helixes with a hole along the axis, into which iodine molecules can fit. We conclude that the core of the molecule is probably formed of the phosphate groups.

A close-packed core of phosphoric acid residues, HPO_4^{--}, can easily be constructed. At each level along the fiber axis there are three phosphate groups. These are packed together in the way shown in figure 1. Six oxygen atoms, two from each tetrahedral phosphate group, form an octahedron, the trigonal axis of which is the axis of the three-chain helical molecule. A similar complex of three phosphate tetrahedra can be superimposed on this one, with translation by 3.4 Å along the fiber axis, and only a small change in azimuth. The neighborhood of the axis of the molecule is then filled with oxygen atoms, arranged in groups of three, which change their azimuthal orientation by about 60° from layer to layer, in such a way as to produce approximate closest packing of these atoms.

The height (between two opposite edges) of a phosphate tetrahedron is about 1.7 Å. If the same distance were preserved between the next oxygen layers, the basal-plane distance along the fiber axis would be 3.4 Å. This value is the spacing observed for the principal meridional reflection.

It is to be expected that the outer oxygen atoms of the complex of three phosphate groups would be attached to the ribofuranose or deoxyribofuranose residues, and that the hydrogen atom of the HPO_4^{--} residues

FIGURE 2

Figure 2 (*left*). A 24-residue 7-turn helix representing a single polynucleotide chain in the proposed structure for nucleic acid. The phosphate groups are represented by tetrahedra, and the ribofuranose groups by dashed arcs connecting them.

FIGURE 3

Figure 3 (*right*). One unit of the 3-chain nucleic acid structure. Eight nucleotide residues of each of the three chains are included within this unit. Each chain executes $3^1/_3$ turns in this unit.

would be attached to one of the two inner oxygen atoms, and presumably would be involved in hydrogen-bond formation with another of the inner oxygen atoms, of an adjoining phosphate group. The length of the O–H $\cdots$O bond should be close to that observed in potassium dihydrogen phosphate, 2.55 Å. The angle P—O—H should be approximately the tetrahedral angle. It is found that the spacing 3.4 Å is not compatible with this bond angle, if the hydrogen bonds are formed between one phosphate group

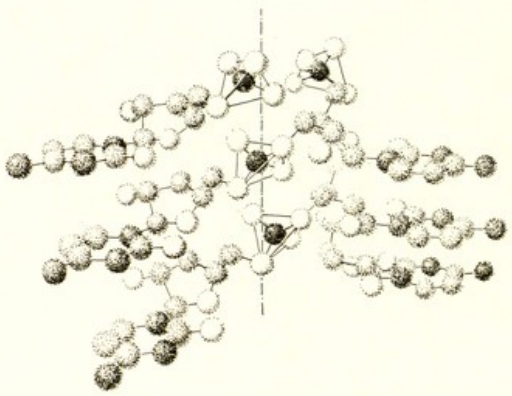


FIGURE 4

Perspective drawing of a portion of the nucleic acid structure, showing the phosphate tetrahedra near the axis of the molecule, the β-D-ribofuranose rings connecting the tetrahedra into chains, and the attached purine and pyrimidine rings (represented as purine rings in this drawing). The molecule is inverted with respect to the coordinates given in table 1.

and a group in the layer above or below it. Accordingly we assume that hydrogen bonds are formed between the oxygen atoms of the phosphate groups in the same basal plane, along outer edges of the octahedron in figure 1.

The maximum distance between the oxygen atoms 3′ and 5′ of a ribofuranose or deoxyribofuranose residue permitted by the accepted structural parameters (C—C = 1.54 Å, C—O = 2.43 Å, bond angles tetrahedral, with the minimum distortion required by the five-membered ring, one atom of

the five-membered ring 0.5 Å from the plane of the other four, as reported by Furberg[6] for cytidine) is 4.95 Å. It is found that it is very difficult to assign atomic positions in such a way that the residues can form a bridge between an outer oxygen atom of one phosphate group and an outer oxygen atom of a phosphate group in the layer above, without bringing some atoms into closer contact than is normal. The atomic parameters given in Table

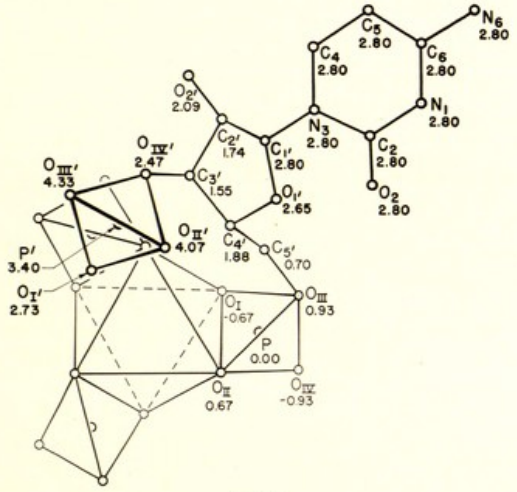


FIGURE 5

A plan of the nucleic acid structure, showing four of the phosphate groups, one ribofuranose group, and one pyrimidine group.

1 represent the best solution of this problem that we have found; these parameters, however, probably are capable of further refinement. The structure is an extraordinarily tight one, with little opportunity for change in position of the atoms.

The phosphate groups are unsymmetrical: the P—O distance is 1.45 Å for the two inner oxygen atoms, and 1.60 Å for the two outer oxygen atoms

which are involved in ester linkages. This distortion of the phosphate group from the regular tetrahedral configuration is not supported by direct experimental evidence; unfortunately no precise structure determinations have been made of any phosphate di-esters. The distortion, which corresponds to a larger amount of double bond character for the inner oxygen atoms than for the oxygen atoms involved in the ester linkages, is a reason-

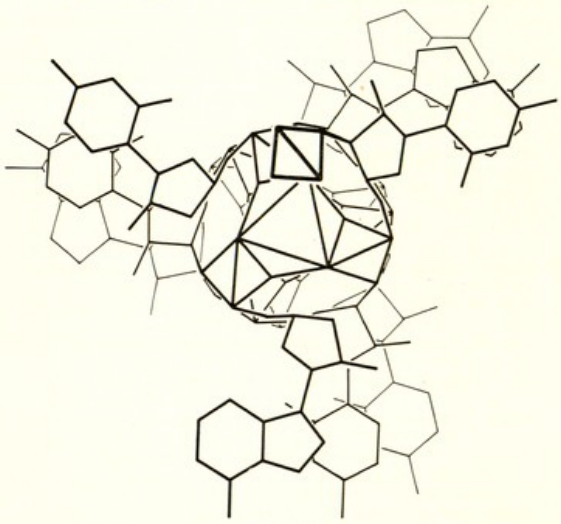


FIGURE 6

Plan of the nucleic acid structure, showing several nucleotide residues.

able one, and the assumed distances are those indicated by the observed values for somewhat similar substances, especially the ring compound S_3O_9, in which each sulfur atom is surrounded by a tetrahedron of four oxygen atoms, two of which are shared with adjacent tetrahedra, and two unshared. The O—O distances within the phosphate tetrahedron are 2.32 Å (between the two inner oxygen atoms), 2.46 Å, 2.55 Å, and 2.60 Å. The

hydrogen-bond distance is 2.50 Å, and each phosphate tetrahedron has two O—O contacts at 2.50 Å, with tetrahedra in the layer above. The group of three phosphate tetrahedra in each layer is obtained from that in the layer below by translation upward by 3.40 Å, and rotation in the direction corresponding to a left-handed screw by the azimuthal angle 15°. Thus there are strings of phosphate tetrahedra that are nearly superimposed, and execute a slow twist to the left. These strings are not connected together into a single polynucleotide chain, however. The sugar residues connect each phosphate group with the phosphate group in the layer above that is obtained from it by the translation by 3.40 Å and rotation through the azimuthal angle 105°, in the direction corresponding to a right-handed screw, as shown in figure 2. This gives rise to a helical chain, with pitch 11.65 Å, and with 3.43 residues per turn of the helix. The chain has an identity distance or approximate identity distance of 81.5 Å, corresponding to 24 nucleotide residues in seven turns, as shown in figure 3. The three chains of the molecule interpenetrate in such a way that the pitch of the triple helix is 3.88 Å, and the identity distance or approximate identity distance is 27.2 Å, corresponding to eight layers (see also Figs. 4, 5, and 6).

The structure requires that the sugar residues have the β-furanose configuration; steric hindrance would prevent the introduction of purine or pyrimidine groups in the positions corresponding to the α configuration. The planes of the purine and pyrimidine residues may be perpendicular or nearly perpendicular to the axis of the molecule. This causes these groups to be superimposed in layers that execute a slow left-handed turn about the molecule, the distance between the planes of successive groups being 3.4 Å. The orientation of the groups is accordingly that required by the observed strong negative birefringence of the nucleic acid fibers. The assignment of the sense of the helical molecules corresponding to the right-handed screw is required by the nature of the structure (the packing of the atoms near the axis, and the absolute configuration of the sugar, as given by the recent experimental determination[16] that absolute configurations are correctly given by the Fischer convention).

The structure bears some resemblance to the structures that have been suggested earlier, and described in a general way, without atomic coordinates. Astbury and Bell[9] suggested that the nucleic acid molecule consists of a column of nucleotide residues, with the purine and pyrimidine groups arranged directly above one another, in planes 3.4 Å apart. Astbury[10] considered the possibility that the nucleotides are arranged in a spiral around the long axis of the molecule, and rejected it, on the grounds that it does not lead to a sufficiently close packing of the groups, as is required by the high density of the substance. He pointed out that it is unlikely that adjacent molecules could interleave their purine and pyrimidine residues in such a way as to lead to the high density. Our structure solves this problem by

the device of intertwining three helical polynucleotide chains, in such a way that there are three nearly vertical purine-pyrimidine columns, consisting of purine and pyrimidine residues from the three chains in alternation. Furberg[17] suggested two single helical configurations, each resembling in a general way one of our helical polynucleotide chains, but his structures involve orientations of phosphate tetrahedra and the ribofuranose rings that are quite different from ours, and it is doubtful that three chains with either of the configurations indicated in his drawing could be intertwined.

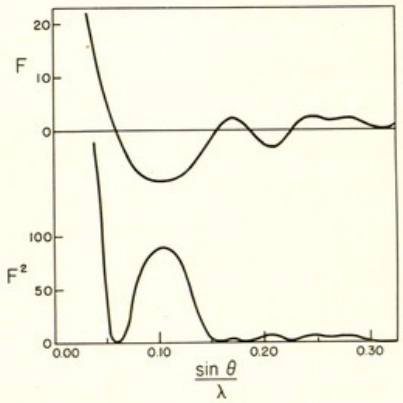


FIGURE 7

The calculated x-ray form factor F and its square F^2 for equatorial reflections of nucleic acid.

The proposed structure accounts moderately well for the principal features of the x-ray patterns of sodium thymonucleate and other nucleic acid derivatives. The spacing 3.40 Å between successive layers of three nucleotides along the molecular axis is required to within about 0.10 Å by the structural parameters of the nucleotides. The prediction that the helixes have 24 nucleotide residues per turn, corresponding to identity distance 8×3.4 Å in the direction of the fiber axis, is in good agreement with the fact that the x-ray diagrams can be reasonably well indexed by placing the 3.4 Å meridional reflection on the eighth layer line. The formula of Cochran,

Crick, and Vand[18] for the form factor for helical structures requires that the orders of Bessel functions for the successive layer lines from 0 to 8 be 0, 3, 6, 9, 12, 9, 6, 3, and 0. The layer-line intensities agree satisfactorily with this prediction, in the region from layer line 4 to layer line 8. There is an unexplained blackening near the meridian for layer lines 2 to 4, which, however, differs in nature for sodium thymonucleate and clupein thymonucleate, and which probably is to be attributed to material between the polynucleotide chains.

The distribution of intensity along the equator can be accounted for satisfactorily. In figure 7 there are shown the calculated form factor in the

TABLE 2

CALCULATED AND OBSERVED EQUATORIAL X-RAY REFLECTIONS FOR SODIUM THYMONUCLEATE. HEXAGONAL UNIT WITH a_0 = 22.1 Å

hkl	$d_{calc.}$	F_1	F_2	pF_2^2	$I_{obs.}$[a]	$d_{obs.}$[a]
10·0	19.1 Å	55	47	6600	m	18.1 Å
11·0	11.0	9.6	21	1350	m	11.2
20·0	9.5	4.7	−1.0	3		
21·0	7.22	−3.4	−8.9	480	w	7.16
30·0	6.37	−7.7	3.1	29		
22·0	5.52	−9.2	1.3	5		
31·0	5.30	−9.4	−14.6	1280	m	5.30
40·0	4.78	−9.3	−14.4	620		
32·0	4.38	−9.1	−14.1	1200	m	19[b]
41·0	4.17	−8.8	1.0	6		
50·0	3.83	−6.1	−10.8	350		
33·0	3.68	−5.1	4.2	53		
42·0	3.61	−4.3	−8.9	480	vw	3.57
51·0	3.43	−2.6	−7.1	300		

The symbol p in column 5 is the frequency factor for the form.

[a] The observed intensity values and interplanar distances are those reported by Astbury and Bell.

[b] The reflection covers the angular range corresponding to interplanar distances 4.0 to 4.4 Å, and may arise in part from overlapping from the adjacent layer lines.

equatorial direction, and the square of the form factor. It is seen that the form factor vanishes at a spacing of about 8 Å, and has a maximum in the region near 5 Å. Calculated intensities, given in table 2, are obtained by making a correction for interstitial material, at the coordinates 1/3 2/3 and 2/3 1/3, the amount of this material being taken as corresponding in scattering power to 1.5 oxygen atoms per nucleotide residue. There is reasonably satisfactory agreement with the experimental values; on the other hand, similar agreement might be given by any cylindrical molecule with approximately the same diameter. A comparison of observed and calculated radial distribution functions would provide a more reliable test of the structure; this comparison has not yet been carried out.

It is interesting to note that the purine and pyrimidine groups, on the periphery of the molecule, occupy positions such that their hydrogen-bond forming groups are directed radially. This would permit the nucleic acid molecule to interact vigorously with other molecules. Moreover, there is enough room in the region of each nitrogen base to permit the arbitrary choice of any one of the alternative groups; steric hindrance would not interfere with the arbitrary ordering of the residues. The proposed structure accordingly permits the maximum number of nucleic acids to be constructed, providing the possibility of high specificity. As Astbury has pointed out, the 3.4-Å x-ray reflection, indicating a similar distance along the axis of the molecule, is approximately the length per residue in a nearly extended polypeptide chain, and accordingly the nucleic acids are, with respect to this dimension, well suited to the ordering of amino-acid residues in a protein. The positions of the amino-acid residues might well be at the centers of the parallelograms of which the corners are occupied by four nitrogen bases. The 256 different kinds of parallelograms (neglecting the possibility of two different orientations of each nitrogen base) would permit considerable power of selection for each position.

(Added in proof.) Support of the assumed phosphorus-oxygen distances in the phosphate di-ester group is provided by the results of the determination of the structure of ammonium tetrametaphosphate.[19, 20] In this crystal there are P_4O_{12} complexes, consisting of four tetrahedra each of which shares two oxygen atoms with other tetrahedra. The phosphorus-oxygen distance is 1.46 Å for the oxygen atoms that are not shared, and 1.62 Å for those that are shared. These values are to be compared with the values that we have assumed, 1.45 Å for the inner oxygen atoms (which are not shared), and 1.60 Å for the outer ones, which have bonds to carbon atoms.

This investigation was aided by grants from The National Foundation for Infantile Paralysis and The Rockefeller Foundation.

* Contribution No. 1766.

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[12] Williams, R. C., *Biochimica et Biophysica Acta*, **9**, 237 (1952).
[13] Kahler, H., and Lloyd, B. J., Jr., *Biochim. et Biophys. Acta*, in press.
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[15] Kahler, H., *J. Phys. Colloid Chem.*, **52**, 207 (1948).
[16] Bijvoet, J. M., Peerdeman, A. F., and van Bommel, A. J., *Ibid.*, **168**, 271 (1951).
[17] Furberg, S., *Acta Chemica Scand.*, **6**, 634 (1952).
[18] Cochran, W., Crick, F. H. C., and Vand, V., *Acta Cryst.*, **5**, 581 (1952).
[19] Romers, C., Ketelaar, J. A. A., and MacGillavry, C. H., *Nature* **164**, 960 (1949).
[20] Romers, C., dissertation, Amsterdam. 1948.

Proposed structure for nucleic acids

Pauling & Corey Proc. Nat Acad Sci 39 84 (1953)

Used Astbury's published X-ray data & their own

- excluded equatorial reflections incompatible with hexag. lattice – not true

16 A is not an equatorial reflection

(this is given as min. value for '100')

consecutive of this 19A at of density for 1.6 to 1.5

will lead to 2 residues / 3.4 A instead of 3

Not clear why structure which is so empty in its outer parts must give by X-rays the outside diameter

(Helix has pitch 11.65A and identity distance 81.5A)

but longer-line period is 34 A!

3-chain helix has identity period 27.2 A = 8×3.4

but this is characteristic of structure A which has no 3.4A reflection!

M. Wt. of Sheep of DNA

ME Richmond, R Vinograd, B. Doty, JACS 74 3203 (1952)

DNA prepared by Sigiura method has η_{sp} 7.7 in 0.2 M NaCl (cf Schneider & Sigiura who found 6.7 n)

At pH 6.5, 3-dimensional structure only slightly more asymmetric than random coil. Max. dimension ~ 6500 Å

Lowering of pH to 2.6 by dialysis of the 0.2 M NaCl soln

→ rod 3000 Å long, with M. Wt. unchanged

Removing NaCl at pH 6.5 → 60% increase in max. dimension

If it were a linear polynucleotide coil, and expect removal of NaCl to → of expansion ∴ change. By analogy w polymethacrylic acid (Ott & Doty ~~JACS~~ J Phys Chem 56 43 (1952)) would expect 10-fold incr max. dimension - 100-fold incr. red. sp. η. Red. sp. η incr only 55 @ 16 mg/100 cc → 85 @ 1 mg/100 cc

These expts are considered to indicate a slightly branched or cross-linked polymer

R E Franklin

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SOME THEORETICAL AND EXPERIMENTAL STUDIES OF X-RAY AND LIGHT SCATTERING BY COLLOIDAL AND MACROMOLECULAR SYSTEMS

SOME THEORETICAL AND EXPERIMENTAL STUDIES OF X-RAY AND LIGHT SCATTERING BY COLLOIDAL AND MACROMOLECULAR SYSTEMS

By D. P. Riley and G. Oster

Received 9th April, 1951

The principal results are given of a general theory of X-ray and light scattering by colloidal and macromolecular systems. Particular attention is devoted to the derivation of molecular size and shape in concentrated solutions and an outline is presented of an independent X-ray method for this purpose. The method is applied to solutions of bovine serum albumin and human haemoglobin. An investigation of the structure of concentrated aqueous systems of thymonucleic acid is reported and the derivation of micellar size in oil-water disperse systems discussed briefly.

The size, shape and relative positions of colloidally dispersed particles (e.g. macromolecules or micelles) may be determined in principle by either X-ray or light-scattering methods. The theory of the angular dependence of the intensity of scattering for these two types of radiation is identical if, in the case of light scattering, the particles have an index of refraction close to that of the medium. A further identity exists between the kinetic theory of gases and the thermodynamic theory of solutions of compact macromolecules, the equation of state, in the latter case, being given by the osmotic pressure as a function of concentration and temperature.

Perhaps the macromolecule most extensively studied by these methods is that of tobacco mosaic virus. It has been examined in concentrated

solutions by means of X-rays [1] and in dilute solution,[2] concentrated solution,[3] concentrated solution [3, 4] and the crystalline state [4, 5] by means of visible light. The crystalline condition of this substance of very large molecular weight shows with visible light many of the diffraction phenomena given by simple ionic crystals with X-rays.

In this contribution to the Discussion we shall first present, in summarized form, the results of some theoretical deliberations on the subject. These have been chosen partly because they are amenable to exact mathematical treatment and are therefore, in some degree, an idealized abstraction of possible reality. The structural models considered possess either spherical or cylindrical symmetry and serve as a necessary basis for comparison with experiments involving approximately spherical or cylindrical molecules or micelles. Scattering from both dilute and concentrated systems is treated.

The outline of an independent X-ray method for the determination of molecular size and shape in paracrystalline solutions is next given and illustrated by examples. The oil-water micellar systems of Schulman and his co-workers are briefly mentioned as examples of less-ordered structures.

Spherically Symmetric Systems.—The normalized intensity of scattering (i.e. intensity per particle) for a system of particles the centres of which are spherically symmetrically disposed, on an average, is given by the Zernicke and Prins relation [6]

$$F^2\left[1 - \nu\int_0^\infty 4\pi r^2(1 - (gr))\,\frac{\sin kr}{kr}\,\mathrm{d}r\right], \quad . \quad . \quad . \quad (1)$$

where ν is the number of scattering particles per unit volume and F is the scattering factor for isolated particles discussed below. The probability of finding centres of particles at a distance r from the centre of any reference particle is given by the radial distribution function $g(r)$. Further $k = (4\pi \sin\theta)/\lambda$, where λ is the wavelength of the radiation in the medium and 2θ is the scattering angle. Eqn. (1) assumes, as Debye and Menke [7] have pointed out, that the irradiated volume is large compared with the wavelength of the incident radiation.

F^2 is the normalized intensity of scattering from an ideal (i.e. very dilute) system of particles. It is the square of the amplitude F of the scattered radiation from a single isolated particle, where F is given in the case of spherically symmetric particles by [8]

$$\frac{\int_0^\infty 4\pi r^2 G(r)\,\frac{\sin kr}{kr}\,\mathrm{d}r}{\int_0^\infty 4\pi r^2 G(r)\mathrm{d}r} \quad . \quad . \quad . \quad . \quad . \quad (2)$$

$G(r)\mathrm{d}r$ is the probability density of scattering material within a spherical shell limited by radii r and $r + \mathrm{d}r$ measured from the centre of the particle as origin. In order to make the theory apply to any wavelength and any particle size, we shall write all expressions for F in terms of the dimensionless parameter kR, where R is the radius of the spherical particle. We have calculated [9] explicit relations for F for the following cases: (*a*) solid spheres,[10] (*b*) spherical shells of finite thickness, (*c*) infinitesimally

[1] Bernal and Fankuchen, *J. Gen. Physiol.*, 1941, **25**, 111.
[2] Oster, Doty and Zimm, *J. Amer. Chem. Soc.*, 1947, **69**, 1193.
[3] Oster, *Rec. trav. chim.*, 1949, **68**, 1123.
[4] Oster, *J. Gen. Physiol.*, 1950, **33**, 445.
[5] Wilkins, Stokes, Seeds and Oster, *Nature*, 1950, **166**, 127.
[6] Zernicke and Prins, *Z. Physik*, 1927, **41**, 184.
[7] Debye and Menke, *Ergebn. Tech. Rontgenk.*, 1931, **2**, 1.
[8] Debye, *Physik. Z.*, 1930, **31**, 419.
[9] Oster and Riley, *Acta Cryst.*, 1952, **5**, 1.
[10] Rayleigh, *Proc. Roy. Soc. A*, 1914, **90**, 219.

thin spherical shells, (*d*) periodic concentric shells. A particularly instructive example is that of a spherical particle made up of a number of concentric shells periodically disposed. It presents analogies with the crystalline case in that the sharpness of the inter-shell diffraction maximum increases with increasing number of shells (cf. "line-broadening" from small crystallites) and, at the same time, the position of this maximum approaches that required by Bragg's law as applied to the inter-shell spacing. Bragg's law is thus of more general applicability than might at first be realized.

Several authors have calculated F^2 values, averaged over all orientations, for particles of various shapes (for bibliography see ref. (11) and (12)). Numerically computed values are also available for ellipsoids of revolution with various axial ratios.[13] As Guinier [14] has stated, F^2 can be represented quite generally by a power series in k with coefficients given by the spatial moments of the particle. The first two terms in the series are given by the expression $1 - q(kR)^2$, where $2R$ is here the largest dimension of the particle and q is simply related to the moment of gyration of the particle. Guinier approximates the power series by a Gaussian error curve.

In Table I are given values of q for various shapes and for various types of spheres. We have also included values for clusters of spheres [9] such as might be encountered in colloidal systems.

TABLE I

Values of q

Spherical shell of outer radius R and inner radius cR ($c < 1$)	$\frac{1}{5}\left[c^2 + \frac{c+1}{c^2+c+1}\right]$
Solid sphere	1/5
Infinitesimally thin spherical shell	1/3
Two spheres of radius R in contact	0·53
Five spheres of radius R in contact (centred tetrahedron)	1·28
Thirteen spheres of radius R in contact (close packing about central sphere)	1·50
Thin circular disc of radius R	1/6
Elongated right circular cylinder of length $2R$ and axial ratio $1/m$	$1 + \frac{2}{9}m^2/9$
Ellipsoid of revolution with major axis $2R$ and axial ratio $1/m$	$2 + m^2/15$

For single particles, the value of q is greatest (i.e. greatest decrease of intensity with angle for a given value of R) for a solid sphere ($c = 0$) and least for an infinitely long and thin isotropic rod (circular cylinder of infinite axial ratio). That is, q increases with the number of pairs of scattering centres making up the particle.

In the cases for which the two-term expression for the intensity of scattering is valid (i.e. $\lambda/2R$ large compared to $2\pi\sqrt{q}\sin\theta$), we obtain the following simple expressions which are useful in light scattering studies:

$$\frac{I_\phi}{I_{180°-\phi}} = 1 + q\left(\frac{4\pi R}{\lambda}\right)^2 \cos\phi, \quad . \quad . \quad . \quad (4a)$$

$$I_{90°} = 1 - \frac{q}{2}\left(\frac{4\pi R}{\lambda}\right)^2, \quad . \quad . \quad . \quad . \quad (4b)$$

[11] Kratky and Porod, *J. Colloid Sci.*, 1949, **4**, 35.
[12] Oster, *Chem. Rev.*, 1948, **43**, 319.
[13] Roess and Schull, *J. Appl. Physics*, 1947, **18**, 308.
[14] Guinier, *Ann. Physique*, 1939, **12**, 161.

where $\phi = 2\theta$, the scattering angle. Eqn. (4*a*) has been called the dissymmetry of light scattering.[15] Eqn. (4*b*) is the factor due to the dissymmetry of light scattering by which the intensity of right-angle scattering must be corrected in order to obtain the correct molecular weight of the particle (see, for example, ref. (2)). To this degree of approximation it is also the ratio of the intensity integrated over all angles to that expected for point " Rayleigh scatterers " of the same molecular weight.[15, 16]

The two-term approximation is not, of course, valid for larger values of kR and has limited usefulness if the largest dimension $2R$ is unknown. One can only distinguish between various shapes by determining the scattering at relatively large values of kR where the curves for various shaped particles differ markedly.[3]

Eqn. (1) assumes that the distribution of the centres of the particles is on an average spherically symmetric, but for concentrated solutions of non-spherical particles this will obviously not be the case since correlation in orientation of the particles will set in.[17, 3] The procedure for the determination of the number of particles at distance r from any other particle, namely, $4\pi r^2 g(r)$, is straightforward, however, for spherical particles. One determines F^2 by observing the scattering as a function of angle for a very dilute colloidal solution. Then one determines the intensity as a function of angle at a concentration ν (eqn. (1)). By performing a Fourier inversion of eqn. (1), just as is done for X-ray scattering of liquids,[7] one obtains $4\pi r^2 g(r)$. Now the radial distribution is related to the osmotic pressure P and the inter-particle force $f(r)$ by the expression [18, 19] (an extension of Keesom's formula for the derivation of van der Waals' equation [20])

$$P = \nu kT\left[1 + \frac{\nu}{6kT}\int_0^\infty 4\pi r^2 g(r) f(r) r \, dr\right], \quad . \quad . \quad (5)$$

where k is Boltzmann's constant and T is the absolute temperature. Hence, by determining $4\pi r^2 g(r)$ at various concentrations one can in principle evaluate the average interparticle force $f(r)$.

We have discussed elsewhere [3] the relation of the low-angle scattering to the equation of state in ideal and various non-ideal systems. In deriving the scattering curves for spheres in non-crystalline concentrated systems (volume concentration of 70 %), we have preferred to use experimental values for $E(k)$, the term in square brackets in eqn. (1). This we obtained from Dr. R. E. Jenning's as yet unpublished work on liquid mercury at 22·7° C. Curves were thus obtained for the scattering in a system having the same relative configurations as in liquid mercury and in which the particles are (*a*) solid spheres, (*b*) hydrated or repelling solid spheres, (*c*) infinitesimally thin shells. All three cases give definite maxima, that of the second being the sharpest. A practical conclusion can be drawn from this work, namely, that for solid spheres, the centre-to-centre distance s of separation between neighbouring spheres in liquid-type configurations is given approximately by the apparent Bragg spacing d of the diffraction maximum. With shells, the correction factor is appreciable :

Solid spheres in liquid configurations	$s = 1{\cdot}05d$	(6*a*)
Hydrated or repelling solid spheres as above	$s = 1{\cdot}10d$	(6*b*)
Thin shells in liquid configurations	$s = 1{\cdot}33d$	(6*c*)

Eqn. (1) is of general applicability and is not confined to liquid-type structures. It applies equally to assemblies of crystallites in random

[15] Debye, *J. Physic. Chem.*, 1947, **51**, 18.
[16] Doty and Steiner, *J. Chem. Physics*, 1950, **18**, 1211.
[17] Onsager, *Ann. N.Y. Acad. Sci.*, 1949, **51**, 627.
[18] Yvon, *Act. Sci. Ind.*, No. 203 (1935).
[19] Kirkwood, *J. Chem. Physics*, 1935, **3**, 300.
[20] Keesom, *Comm. Phys. Lab., Leiden*, 1912, Suppl. 24*B*, 32.

orientation which would give a number of Debye-Scherrer rings. The appropriate Fourier inversion of the scattered intensity curve will, in all cases, lead to the radial distribution function of the structure, $4\pi r^2 g(r)$. If, however, it can be inferred from other considerations that the structures involved are crystalline, or nearly so, in two or three dimensions and are of a simple type, then the interparticle distance s can be deduced from an elementary geometrical argument. This approach was used in an earlier publication and seems to be justified in certain instances, as will be discussed in a later section. The relations between the apparent Bragg spacing d of the principal near-in band and the interparticle distance s is given by

$$s = \sqrt{(3/2)}d = 1{\cdot}22d, \quad . \quad . \quad . \quad . \quad (7)$$

if the spherical molecules fill space uniformly in " expanded " structures of the closest-packing type.

Cylindrically Symmetric Systems.[21]—The normalized scattered intensity for cylindrical rods of infinite length and in perfect parallel orientation is given by the expression

$$F^2\left[1 - \nu\int_0^\infty 2\pi r(1 - g(r))J_0(kr)dr\right], \quad . \quad . \quad . \quad (8)$$

where ν is the number of rods per unit area measured in the plane perpendicular to the long axis of the rod, r is the radial distance in this plane measured from the centre of any particle, and $J_0(x)$ is the zero order Bessel function. F is the scattering factor for an isolated rod (F^2 is the normalized intensity). Eqn. (8) is derived in a simple manner by applying the method of Zernicke and Prins to the case of two dimensions. For an infinitely long rod of radially symmetric cross-section, F is given by

$$\frac{\int_0^\infty 2\pi r G(r) J_0(kr)\, dr}{\int_0^\infty 2\pi r G(r)\, dr}. \quad . \quad . \quad . \quad . \quad . \quad (9)$$

In eqn. (8) and (9), $g(r)$ and $G(r)$ are radial distribution functions in two dimensions defined analogously to those in the three-dimensional case. The two equations apply equally (as regards relative intensity) to systems in which the overall symmetry is cylindrical (e.g. fibres) as to macroscopically isotropic systems in which the cylindrically symmetrical regions are randomly oriented relative to each other (e.g. micellar solutions). For a solid rod of circular cross-section with radius R, eqn. (9) gives

$$F(kR) = 2\frac{J_1(kR)}{kR},$$

where $J_1(x)$ is the first-order Bessel function, i.e. the amplitude of scattering for a circular hole or circular plate of radius R under Fraunhöfer conditions of observation. The value of the parameter q for this case is 1/4. Guinier [14] has applied this expression to oriented ramie fibres. A more detailed treatment, however, would require account to be taken of the relative positions of the fibres as expressed in eqn. (8). F values for other cylindrically symmetric particles (e.g. shells)have also been derived.

Eqn. (8) can be generalized to take into account relative orientations of the rods as well as relative positions. The mathematics, however, become extremely cumbersome except for some very special distributions. In practice, in order to explain the observed scattering from a given system of fibres, it might be simpler to construct macroscopic models of some suspected arrays and observe the scattering of the model under Fraunhöfer conditions. If, for example, a bundle of Nylon threads were arranged in a certain manner and imbedded in a medium of nearly, but

[21] Oster and Riley, *Acta Cryst.* (in press).

not exactly, the same index of refraction, the scattering by such a system could be projected on a screen, the light source being a monochromatic and parallel beam of epidiascope intensity.

If the system consists of n long rods in perfect parallel orientation and located at fixed positions in space, the normalized intensity of scattering is given by

$$\frac{1}{n^2}F^2\sum_i\sum_j J_0(hl_{ij}), \quad . \quad . \quad . \quad . \quad . \quad (10)$$

where l_{ij} is the distance between the ith and the jth rod measured in the equatorial plane. This is the 2-dimensional analogy of Debye's well-known expression for the scattering by rigid point assemblies and applies, as regards relative intensity, to both fibres and micellar solutions which are macroscopically isotropic. James [22] has treated the specific case of fibrous crystallites and the general method of averaging which we have used is his. It is of interest in this connection to consider the diffraction effects produced by micelles of finite lateral extent. It has been shown [21] by application of eqn. (10) that even small micelles consisting of only seven rod-like molecules in a centred hexagonal arrangement will give a reasonably sharp maximum if there is an appreciable gap between the molecules. The position of this band nearly corresponds to that of the Bragg spacing of the 10 planes in a 2-dimensional hexagonal lattice of infinite extent. The practical conclusion is that the intermolecular distance s can be obtained from the apparent Bragg spacing d of the observed diffraction band by using the 2-dimensional crystallographic relation

$$s = (2/\sqrt{3})d_{10} = 1{\cdot}15d. \quad . \quad . \quad . \quad . \quad (11)$$

The greater the lateral extent of the micelles, the better the approximation.

Size and Shape of Protein Molecules and other Charged Macromolecules from X-ray Scattering by Solutions.—The proteins which have already been studied (by us) in concentrated solutions are human haemoglobin [23, 24] egg albumin [25, 24] and bovine serum albumin. In addition, concentrated aqueous systems of thymonucleic acid have been examined. There is also the earlier work of Bernal and Fankuchen [1] on tobacco mosaic virus solutions, the particles of which are oriented in one direction by flowing the solutions in capillaries during the observations. It would appear from these observations that strong solutions of such charged macromolecules possess structures of almost crystalline regularity in two or three dimensions. This fact gives us the possibility of an independent technique for determining not only the size but also one or more linear dimensions of the molecule. A convenient feature with such solutions is that the intermolecular forces can be varied by the simple expedient of altering the pH or the salt concentration of the medium.

All these systems give one or more reasonably well-defined low-angle X-ray diffraction bands at high concentrations. The essential feature of the method is to observe the way in which these bands move with change of concentration, while always keeping the concentration high. The apparent spacing d of a band is derived from the angular position 2θ of the maximum by the straightforward application of Bragg's law, $d = \lambda/2 \sin\theta$. The values of d at different stages of dilution are plotted against the concentration (vol./vol.) on a double logarithmic scale. If the system has long-range order, the linearity or otherwise of the plot is evidently a measure of the degree of crystallinity in the solutions while the slope of the plot, if linear, indicates whether the lattice expansion on dilution is in two or three dimensions. Extrapolation to the value of the

[22] James, *The Optical Principles of the Diffraction of X-rays* (London, 1948), Chap. X, § 4.
[23] Riley and Herbert, *Biochim. Biophys. Acta*, 1950, **4**, 374.
[24] Riley, *Brit. Sci. News*, 1950, **3**, 7.

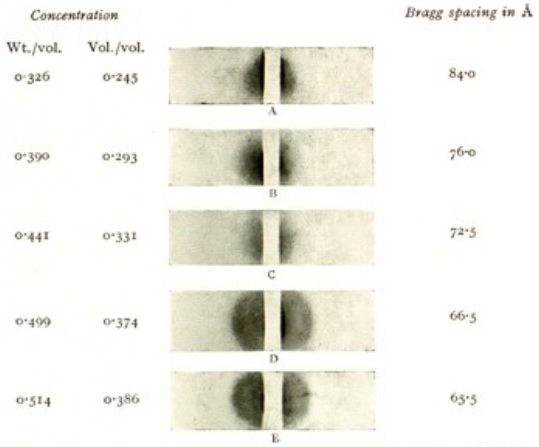

PLATE I.—Low-angle X-ray diffraction photographs given by solutions of bovine serum albumin of increasing high concentration. Cu$K\alpha$ radiation (Ni filter) at 30 cm. in vacuum camera.

[*To face page* 113.

volume concentration for close-packed cylinders or spheres gives a figure for the spacing in a hypothetical dry system of the same structure-type but in which the molecules are in contact. Multiplication by the appropriate geometrical factor (eqn. (7), (11) above) leads to a value for the diameter of the unhydrated molecule.

In the 3-dimensional case, the molecule is almost certainly spherical in shape, or nearly so, but this may be confirmed by comparison with the radius of gyration as determined by the Guinier method of low-angle scattering by dilute solutions (but *only* if there is no molecular association or aggregation in such solutions, which is not always true). In the non-spherical case (2-dimensional expansion), the permissible simple shapes for the molecule can be determined from a similar comparison. If the molecular volume (from the molecular weight and partial specific volume) is also known, a single most probable shape should be deducible with its actual linear dimensions. The method has been applied to the systems next discussed, that of nucleic acid being more complex than the others.

BOVINE SERUM ALBUMIN.—Five solutions of concentrations between 33 % and 51 % (wt./vol.) were examined and gave pronounced low-angle diffraction effects. Plate I shows the series of comparable photographs (apart from exposure time) obtained. The concentrations and apparent Bragg spacings of the principal band are given to the nearest 0·5 Å against each photograph. This diffraction maximum is most marked in the specimen of highest concentration but is just perceptible in the most dilute case. On dilution, the maximum moves inwards and the corresponding spacing therefore increases. At concentrations below 33 % no diffraction band is observed.

On the original photograph of the specimen of highest concentration, a very weak and diffuse band of spacing *ca.* 37 Å is visible. The ratio of the spacing of the main band, 65·5 Å, to that of the secondary band is nearly $\sqrt{3}$, which is the ratio of the 10 and 11 spacings in a 2-dimensional hexagonal lattice. Another band of higher spacing can be distinguished for the three most concentrated specimens. Its spacing is about 155 Å and does not appear to change with concentration.

If these values of d of the main band and the volume concentrations are plotted on a double logarithmic scale (Fig. 1), the points lie reasonably closely on a straight line of slope $-\frac{1}{2}$. This implies that the dilution-expansion measured by d is two-dimensional. By extrapolation to a volume concentration 0·91 (long cylinders in closest-packing), a value $d = 42{\cdot}5$ Å is found. If the molecules are assumed to be cylinders aggregated in a 2-dimensional hexagonal array in the diametral plane, the molecular diameter is therefore given from eqn. (11) as $\sigma = 49$ Å. The height of the cylinder corresponding to a molecular weight of 68,000 is 45 Å. The radius of gyration of a cylinder of these dimensions is 22 Å which is not in close agreement with the value of 26·6 Å given by X-ray scattering measurements [25] on dilute solutions.

Solutions of serum albumin exhibit no [26] or only very slight [27] flow-birefringence. The molecule cannot therefore be very anisometric in form. The fact that there are no low-angle diffraction bands at low concentrations of the type observed with nucleic acid (see later) also indicates that the particles are not highly elongated. We therefore conclude that the dimensions 150 Å long by 38 Å wide [28] which had been previously deduced from a combination of physical measurements are probably in error. Molecular association to form trimers in solution might explain the discrepancy, and the spacing of 155 Å mentioned earlier may be related to the height of the trimer. Nor is it probable that the form of the molecule is exactly spherical for, in that case, the observed value of 26·6 Å

[25] Ritland, Kaesberg and Beeman, *J. Chem. Physics*, 1950, **18**, 1237.
[26] Sadron, Bernot and Mosimann, *J. Chim. Phys.*, 1939, **36**, 78.
[27] Edsall and Foster, *J. Amer. Chem. Soc.*, 1948, **70**, 1860.
[28] Oncley, Scatchard and Brown, *J. Physic. Chem.*, 1947, **51**, 184.

for the radius of gyration would correspond to a molecular diameter of 69 Å and to a molecular weight of 139,000, which is twice too large.

HUMAN HAEMOGLOBIN.—The data reported in an earlier publication [23] have been re-examined and the hypothesis that the molecule of this protein is nearly spherical in shape has been confirmed. When the spacings given there are plotted against concentration on a double logarithmic scale (Fig. 1), the points lie on a straight line of slope $-\frac{1}{3}$ approximately, which suggests that the dilution-expansion is 3-dimensional. Extrapolation to a volume concentration 0·74 (closest-packing of spheres) gives $d = 45$ Å. (More weight is given to the better data for the two most concentrated solutions in this extrapolation.) Assuming that the spherical molecules are arranged with nearly crystallographic regularity, this spacing when substituted into eqn. (7), leads to a diameter for the unhydrated molecule of 55 Å. The diameter of a spherical molecule of molecular weight 66,700 and partial specific volume 0·75 is 54 Å. The

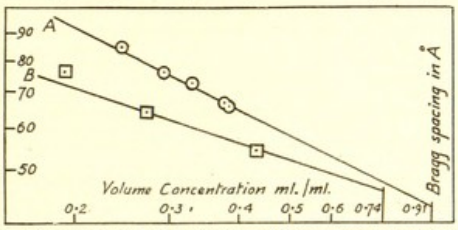

FIG. 1.—Double logarithmic plots of apparent Bragg spacings against volume concentrations for solutions of
A. bovine serum albumin (slope $-\frac{1}{3}$),
B. human haemoglobin (slope $-\frac{1}{3}$).

volume concentration of the saturated solution (0·579 g./ml.) is 0·434 whereas the calculated concentration on the basis of an "expanded" close-packed arrangement of spheres is 0·419. There would not, therefore appear to be separate micelles in these very strong solutions. The molecules take up mean positions equidistant from each other throughout the whole volume and are presumably kept at these points by the effect of the additive repulsive forces between the charged molecular particles. The structure is the 3-dimensional analogy of the 2-dimensional regularity in solutions of tobacco mosaic virus. The observed spacing of 54·5 Å in the saturated solution leads to an intermolecular distance s of 66·5 Å. As the molecular diameter σ is 55 Å, the gap between neighbours $s - \sigma$ is 12 Å and corresponds to four layers of water molecules. A saturated solution therefore contains just enough water to form a bimolecular layer of water of hydration around each protein molecule.

Scattering Studies of Thymonucleic Acid.—It was demonstrated in 1938 [29] by combined flow birefringence and viscosity measurements that nucleic acid from thymus gland is a highly elongated molecule with negative birefringence. Recently Signer and Schwander [30] have prepared thymonucleic acid (desoxyribonucleic acid, Na salt) of a very high degree of polymerization. This material has been extensively studied by means of flow birefringence [31] and viscosity [32] by workers in the Centre

[29] Signer, Caspersson and Hamersten, *Nature*, 1938, **141**, 122.
[30] Signer and Schwander, *Helv. chim. Acta.*, 1949, **32**, 853.
[31] Schwander, *J. Chim. Phys.*, 1950, **47**, 718; Schwander and Cerf, *Helv. chim. Acta*, 1949, **32**, 2356.
[32] Vallet and Schwander, *Helv. chim. Acta.*, 1949, **32**, 2508.

d'Études de Physique Macromoleculaire, Strasbourg, and the conclusion reached is that the particles, even in the presence of 10 % NaCl, are fibres about 8000 Å long. Prof. R. Signer of the University of Bern has kindly presented us with some of this material, the light scattering and X-ray scattering results of which we report below.

The light-scattering results are somewhat anomalous at first sight. In Table II are given the dissymmetries $I_\phi/I_{180°-\phi}$ measured [33] with green light (wavelength in the medium 4090 Å) compared with that calculated for some ideal models. These results, which are extrapolations to infinite dilution, are not appreciably different in the presence of NaCl. The large dissymmetry over that expected for very long rods can be explained for the most part if one takes into account the strong negative birefringence of the particles, i.e. optically the fibres behave as if they had a great thickness. A quantitative theory of this phenomenon has been recently developed.[34] Other workers [35] have reported widely varying results from sample to sample. Care must be taken, however, to ensure that the sample is free from denatured protein, a small amount of which could considerably augment the dissymmetry. The protein might perhaps be removed by trypsin which had been carefully purified to remove traces of desoxyribonuclease.

TABLE II

Dissymmetry	Scattering Angle				
	45°	50°	60°	70°	80°
Observed	6·00	4·73	3·21	2·12	1·38
Thin rod, infinitely long. .	2·41	2·15	1·73	1·43	1·19
Random coil, infinitely large .	5·82	4·60	3·00	2·06	1·77
Sphere with diameter of one-half of wavelength . .	5·30	4·64	3·24	2·26	1·51

The structural nature of nucleic acid preparations is markedly dependent on the quantity of water present and on the way in which the sample is prepared. If insufficient water is present, or again if there is too much, the structure is disordered. There is, however, a wide range of concentrations over which the gels have an ordered structure, including a narrow range where the material appears to be really crystalline. Over a wide range of concentrations, from about 0·4 to 0·02 vol./vol. the nucleic acid gels possess a partly-ordered structure of the "liquid-crystalline" type.

The X-ray scattering effects [36] from all the samples examined can be grouped into eight ranges. First of all, there are the 3 Å, 4 Å and 5 Å ranges which are given by all samples and must correspond to intramolecular periodicities. The intramolecular structure remains substantially unaltered on this scale under all the conditions studied. There is also a similar, but less definite, 8 Å region. The crystalline and liquid-crystalline specimens show, in addition, pronounced diffraction effects in the higher spacing ranges which have been the subject of special study. Two groups of well-defined lines are given by the crystalline specimens: a triplet of spacings 10·6 Å, 11·3 Å and 11·8 Å and a doublet of spacings 14·9 Å and 16·5 Å. Under conditions of poor resolution, the two sets of lines appear as two bands of spacings 10·9 Å and 15·7 Å. On addition of water, these

[33] Oster, *J. Chim. Phys.*, 1950, **47**, 717. (Abstr.).
[34] Horn, Benoit and Oster, *Compt. rend.*, 1951, **232**, 810.
[35] Smith and Sheffer, *Can. J. Res. B*, 1950, **28**, 96.
[36] Riley and Oster, *J. Chim. Phys.*, 1950, **47**, 715 (abstr.), and *Biochim. Biophys. Acta*, 1951 (in press).

bands weaken and finally disappear while two further bands of higher spacings make an appearance.

These two bands are characteristic of the liquid-crystalline state. Their spacings increase as the systems are diluted, the inner band shifting more rapidly than the outer. The outer band, of lower spacing, is usually the sharper and more intense and its spacing can vary between the wide limits of 19 Å and 95 Å. For a specimen of given concentration, the spacing of this band is effectively independent of the source of the nucleic acid employed. The spacing, definition and intensity of the inner-high-spacing band are, on the contrary, greatly affected by the source of the nucleic acid material as well as by the concentration of the gel and the way in which it was prepared. The spacings lie within the range 50 Å to 200 Å.

The fact that both sets of spacings are inversely proportional to the square root of the concentration (as shown by the slope of $-\frac{1}{2}$ of the lines which result from a double-logarithmic plot) indicates that the expansion on dilution is 2-dimensional in each case. Detailed consideration of the data leads to the following structural scheme for the strong solutions. The nucleic acid molecule is very long and narrow and is effectively rigid. Pictured as a cylinder, it has a diameter of about 16 Å. Its length is about 8000 Å in Signer's samples and much smaller, about 500 Å in the commercial preparations. These long rod-like molecules aggregate into roughly cylindrical bundles or micelles which are themselves regularly packed together. The evidence is that the highly purified and non-degraded Signer's sample gives highly organized micellar systems if the specimens are prepared without mechanical agitation. On stirring or on keeping for long periods at room temperature, this structural perfection is broken down and the systems resemble those made with other samples of nucleic acid. The structure of the more ordered systems has been studied in some detail. It appears that not only are the molecules grouped into identical micelles (the centred hexagonal 7-molecule micelles discussed earlier), but the micelles are themselves arranged in a distinctive hexagonal 2-dimensional pattern of an open honeycomb kind. On dilution, the whole structure expands uniformly in 2-dimensions by interpolation of water between the molecules and, in addition, tends to become more disordered. This is a markedly different state of affairs from that existing in gels of tobacco mosaic virus, also a long rod-like molecule. In this case the molecules simply fill space uniformly.

Oil-Water Disperse Systems.—Schulman and collaborators [37] have shown that dispersions of sub-microscopic oil droplets in water, or vice-versa, may be made by the use of suitable stabilizing agents. The diameters of the spherical micelles can be arrived at in three ways: (*a*) by calculation from the chemical model,[38] (*b*) from X-ray studies,[38, 39] (*c*) from light-scattering measurements.[40] Comparison of results shows that the best values for the distances s separating centres of neighbouring micelles is given by placing $\kappa = 1$ in the relation $s = \kappa d$, where d is the apparent Bragg spacing of the low-angle X-ray diffraction band. In the original paper, κ was taken to be 1·23. It would thus appear that the micelles resemble solid spheres more than thin shells as regards their X-ray scattering properties, and that the spheres are arranged in liquid-type configurations (see eqn. (6*a*), (6*b*), (6*c*)). The scattering from assemblies of long cylindrical micelles of similar chemical type can be calculated from the general theory given earlier.

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21 *Albemarle Street,*
*London, W.*1.

[37] Schulman and McRoberts, *Trans. Faraday Soc.*, 1946, **42B**, 165.
[38] Schulman and Riley, *J. Colloid Sci.*, 1948, **3**, 383.
[39] Schulman, McRoberts and Riley, *J. Physiol.*, 1948, **107**, 49P.
[40] Schulman and Friend, *J. Colloid Sci.*, 1949, **4**, 497.

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213. *Deoxypentose Nucleic Acids. Part II. Electrometric Titration of the Acidic and the Basic Groups of the Deoxypentose Nucleic Acid of Calf Thymus.*

By J. Masson Gulland, D. O. Jordan, and H. F. W. Taylor.

The acidic and the basic groups of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus and of barium thymate derived therefrom have been titrated electrometrically, employing hydrogen and glass electrodes. This polynucleotide is found to possess three amino-, two purine–pyrimidine enolic hydroxyl, four primary phosphoryl, and not more, and probably less, than 0·25 secondary phosphoryl dissociations for every four atoms of phosphorus. These data are consistent with a chain structure for this acid in which branching, if it occurs, is infrequent as compared with yeast ribonucleic acid. The main internucleotide bond is an ester linkage. For every four atoms of phosphorus it is found that there are 1·0 guanine, 1·0 thymine, 1·0 to 1·2 cytosine, and 1·0 to 0·8 adenine radicals.

The initial dissociation curves of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus obtained by titrating from pH 6·9 with acid and alkali are abnormal, being displaced from the back-titration curves. This discrepancy between the forward- and back-titration curves persists in high concentrations of neutral salt. It is concluded that the purine–pyrimidine hydroxyl groups and some of the amino-groups are blocked, most probably by a hydrogen bond between these groups. The significance of this linkage in the macromolecular structure of the tetrasodium salt of the deoxypentose nucleic acid is discussed.

The conflicting results obtained previously from investigations of the acid–base properties of thymus deoxypentose nucleic acid (Steudel, *Z. physiol. Chem.*, 1912, **77**, 497; Feulgen, *ibid.*, 1919, **104**, 189; Levene and Simms, *J. Biol. Chem.*, 1925, **65**, 519; 1926, **70**, 327; Makino, *Z. physiol. Chem.*, 1935, **232**, 229; 1935, **236**, 201; Bredereck, Köthnig, and Lehmann, *Ber.*, 1938, **71**, 2613; Bredereck and Köthnig, *ibid.*, 1939, **72**, 121; Ahlström, Euler, Fischer, Hahn, and Högberg, *Arkiv Kemi, Min. Geol.*, 1945, **20**, *A*, 1) may be ascribed to the different degrees of degradation of the samples studied (Schmidt, Pickels, and Levene, *J. Biol. Chem.*, 1939, **127**, 251; Cohen, *ibid.*, 1942, **146**, 471; Tennent and Vilbrandt, *J. Amer. Chem. Soc.*, 1943, **65**, 424; Gulland, Barker, and Jordan, *Ann. Rev. Biochem.*, 1945, **17**, 175). In all probability the least degraded specimens which have hitherto been examined were those prepared by the method of Bang (Hofmeister's " Beiträge chem. Physiol. Path.", 1903, **4**, 331) and studied conductimetrically by Hammarsten (*Biochem. Z.*, 1924, **144**, 383) and electrometrically by Jorpes (*Biochem. J.*, 1934, **28**, 2102) and Stenhagen and Teorell (*Trans. Faraday Soc.*, 1939, **35**, 743). They were found to possess four acid-dissociating groups per hypothetical tetranucleotide, having the very approximate pK_a' values of 2·4, 3·7, 4·3, and 5·2. The Hammarsten–Bang method of isolation, however, gave a product which on analysis was found to be very deficient in nitrogen and phosphorus (N, 11·97; P, 7·09%; Hammarsten, *loc. cit.*) when compared with the theoretical for the tetrasodium salt (N, 15·85; P, 9·37%). Furthermore, Hammarsten studied the free acid obtained from the sodium salt by the action of hydrochloric acid; as will be shown in this paper this treatment causes an irreversible change in that the free acid isolated from solutions more acid than pH 3·5 does not show the same acid–base properties as the sodium salt isolated at pH 7·0.

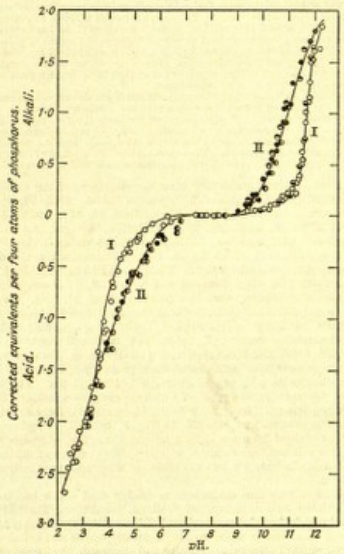
The sodium salt of calf thymus deoxypentose nucleic acid which has been studied in this investigation was isolated by a mild method (Gulland, Jordan, and Threlfall, Part I, this vol., p. 1129), throughout which the solution employed did not vary significantly from pH 7·0.* The solid was fibrous and dissolved in water to give a faintly opalescent solution, having a pH of 6·90, which exhibited marked structural viscosity and streaming birefringence (Creeth, Gulland, and Jordan, Part III, this vol., p. 1141). A second specimen, prepared by the Hammarsten–Bang procedure and supplied by Professor Caspersson through Professor Astbury in 1939, has also been studied. It was found to contain a small amount of protein which was removed by the method of Sevag, Lackman, and Smolens (*J. Biol. Chem.*, 1938, **124**, 425), and is believed to be identical with that studied with ultracentrifuge and viscosity methods by Signer, Caspersson, and Hammarsten (*Nature*, 1938, **141**, 122) and with *X*-ray methods by Astbury and Bell (*ibid.*, p. 747).

* It is necessary to correct a point in the paper of Tennent and Vilbrandt (*J. Amer. Chem. Soc.*, 1943, **65**, 424). The sample of " Thymonucleic acid TNA2, prepared by Gulland ", referred to by these authors, was a purchased commercial sample, and the " barium thymate BTl, prepared by Gulland " was made from it by the usual method. These were given to Professor Astbury in 1939 with a warning that their purity and homogeneity was open to doubt; they were not intended for the type of investigation to which they have been put by Tennent and Vilbrandt, and in our view results obtained with them are of no value in connection with nucleic acid structure.

Results of the Present Investigation.—All electrometric titration curves have been corrected at the extremes of pH for the titration of the water by the method of Jordan and Taylor (*J.*, 1946, 994).

(i) *Titration of the sodium salt of thymus deoxypentose nucleic acid.* The titration curve of this sample is shown in Fig. 1, curve I. It will be seen that on the addition of acid or alkali to

FIG. 1.



The dissociation curves of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus :

I. *Titration with acid or alkali from pH 6·9,* ○, ◒. *The smooth curve drawn through these points is calculated for 1 equiv. each of* pK_a' *values 2·6, 3·5, 5·2, 10·4 and 11·4.*

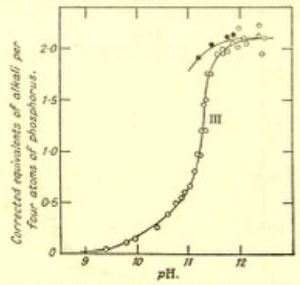
II. *Back titration with acid from pH 12·0,* ●, ◓ *and with alkali from pH 2·5,* ◐.

Points marked ◒ *and* ◓ *obtained with "Alki" glass electrode (Cambridge Instrument Co., Ltd.) ; all other points obtained with hydrogen electrode.*

the solution in water no groups are titrated at first between pH 5·0 and 11·0, but that outside these limits there occurs a rapid liberation of groups titrating in the ranges pH 2·0 to 6·0 and pH 9·0 to 12·0. On back titration either with acid from pH 12·0 or with alkali from pH 2·5, a curve (II) is obtained which is different from that representing the initial titration, and it is significant that the same curve (II) is obtained whether the back titration is with alkali from pH 2·5 or with acid from pH 12·0. This complete identity of the back-titration curves suggests that acid and alkali have an identical effect in liberating both sets of groups.

The back-titration curve exhibits a well-defined point of inflection in the neutral region, and shows incipient points of inflection in the regions of pH 12·0 and pH 2·0, corresponding respectively to approximately 2·0 equivalents of alkali and 3·0 equivalents of acid for each four atoms of phosphorus. There is some difficulty in interpreting electrometric titration

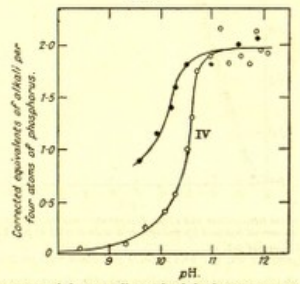
FIG. 2.



The dissociation curves of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus in M*-potassium chloride.*

Titration with alkali from pH 6·9, ○ *; back titration with acid from pH 12·5,* ●.

FIG. 3.



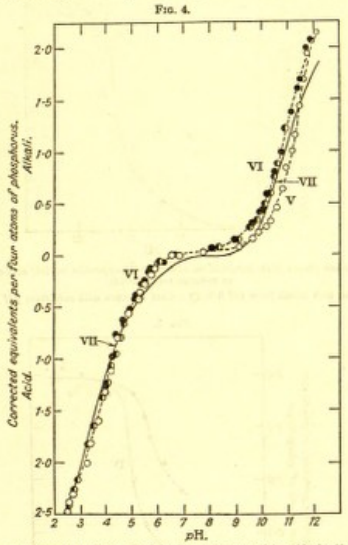
*The dissociation curves of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus in 2·6*M*-guanidine sulphate.*

Titration with alkali from pH 6·9, ○ *; back titration with acid from pH 12·0,* ●.

data above pH 11·0 and below pH 3·0 owing to the dependence of the water correction on the ionic strength (Jordan and Taylor, *loc. cit.*), which, for a polybasic substance such as nucleic acid, cannot be estimated with certainty. In obtaining the data given in Fig. 1, the assumption has been made that the acidic and basic groups contribute independently to the ionic strength. This approximation is justified by the fact that no appreciable proportion of the phosphorus

atoms carry more than one dissociating group (see below). It appeared desirable, however, to have additional confirmation of the number of groups titrating in the alkaline region, and titration of the sodium salt of thymus deoxypentose nucleic acid was therefore carried out in M-potassium chloride. The presence of the potassium chloride has the effect of masking all other contributions to the ionic strength (Cohn, Green, and Blanchard, *J. Amer. Chem. Soc.*, 1937, **59**, 509), and the water correction is obtained from a titration of M-potassium chloride. The results of this titration, which are given in Fig. 2, curve III, show conclusively that the

FIG. 4.



The dissociation curves of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus after alkaline treatment followed by precipitation with alcohol at pH 7 :

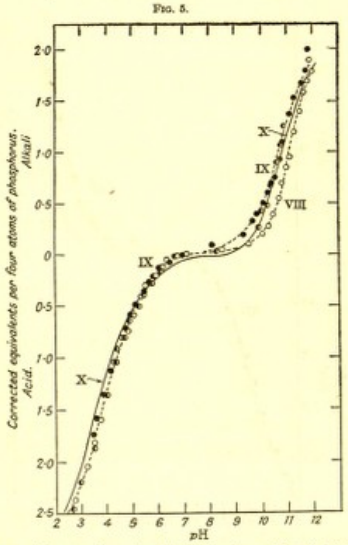
V. *Titration with alkali from pH* 6·7, ○.
VI. *Titration with acid from pH* 6·7, ○ ; *back titration with alkali from pH* 2·5, ◐ ; *back titration from pH* 12·0, ●.
VII (*full curve*). *Mean titration curve of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus from Fig.* 1.

number of groups dissociating in the range pH 8·0 to 12·0 is 2·05. Owing to the ease with which precipitation of the deoxypentose nucleic acid occurs in the presence of M-potassium chloride when acid is added, a complete back titration below pH 11·2 was not practicable; the data which have been obtained, however, are sufficient to indicate that the discrepancy between the forward- and back-titration curves described above persists in the presence of M-potassium chloride. Very similar results were obtained by titration in 2·53M-guanidine sulphate ($[C(NH_2)_3]_2SO_4$; Fig. 3, curve IV). Owing, however, to the existence of an unsteady liquid-

junction potential between this solution and the saturated potassium chloride bridge, the pH values were not very reproducible, especially in strongly alkaline solutions.

(ii) *Titration of the sodium salt of thymus deoxypentose nucleic acid precipitated with alcohol at pH* 7·0 *after treatment with alkali at pH* 12·0, *or with acid at pH* 3·0. The titration curves of an alkali-treated sample are shown in Fig. 4, curves V and VI; similar results were obtained with two other samples. The results of titration on the alkaline side of neutrality resemble those obtained with the original substance, in that a shift in the dissociation curve is observed

FIG. 5.



The dissociation curves of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus after acid treatment followed by precipitation with alcohol at pH 7 :

VIII. *Titration with alkali from pH* 7·1, ○.
IX. *Titration with acid from pH* 7·1, ○ ; *back titration from pH* 2·3, ◐ ; *back titration from pH* 12·0, ●.
X (*full curve*). *Mean titration curve of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus from Fig.* 1.

on back titration, although to a somewhat smaller extent. No such effect, on the other hand, was observed on the acid side. The back-titration curves from pH 12·0 or pH 2·5 are identical, and are very similar to the curve obtained with the original substance.

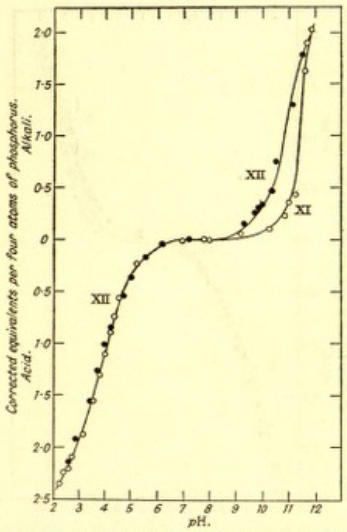
The results obtained with an acid-treated specimen (Fig. 5, curves VIII and IX) are almost identical ith those described above for the alkali-treated material.

(iii) *Titration of the sodium salt of thymus deoxypentose nucleic acid supplied by Professor Caspersson.* The titration curves of this sample are shown in Fig. 6, curves XI and XII, and

are intermediate between those of the alkali- and acid-treated materials prepared by us and those of our original material. Viscosity studies (Creeth, Gulland, and Jordan, *loc. cit.*) support the view that, compared with the acid prepared by us, the sample of Caspersson exhibits different, probably less, hydrogen bonding (see below) and lower viscosity.

(iv) *Titration of the barium salt of thymic acid.* On treating thymus deoxypentose nucleic acid with dilute sulphuric acid at 80°, quantitative removal of guanine and adenine takes place (Feulgen, *Z. physiol. Chem.*, 1918, **101**, 296; Feulgen and Landmann, *ibid.*, **102**, 262; Bredereck and Müller, *Ber.*, 1939, **72**, 115); the resulting thymic acid is isolated as its barium salt. The empirical formula weight of this substance is defined, for convenience, as the amount containing 4 g.-atoms of phosphorus, and the values obtained from the phosphorus contents of the two preparations studied were 1310 and 1315. The electrometric titration curve calculated on the basis of these formula weights is shown in Fig. 7, curve XIII, well-marked points of inflection being observed at pH 2·5, 8·0 and 11·5 after the neutralisation of 1·85 and 1·0 equivalents respectively. A titration has also been carried out in the presence of formaldehyde in order to ascertain what proportion of the more acidic dissociation represents that of an amino-group (curve XIV).

FIG. 6.



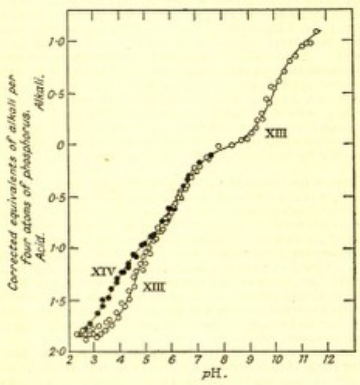
The dissociation curves of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus supplied by Professor Caspersson:

XI. *Titration with alkali from pH* 7·8, ○.
XII. *Titration with acid from pH* 7·8, ○; *back titration from pH* 11·8, ●.

Discussion.—(i) *The nature of the acidic and the basic groups of deoxypentose nucleic acid.* The problem of the initial structure of deoxypentose nucleic acid before alkali or acid treatment will be considered in section (ii) of this discussion, and in this section the back-titration curve (Fig. 1, curve II) only will be treated.

Owing to the fact that the deoxyribonucleotides have not yet been isolated in quantities sufficient to permit an investigation by electrometric methods, it is necessary to refer to dissociation-constant data of the ribonucleotides in order to interpret this curve. This procedure is to some extent justified by the fact that the pK_a' values of the amino-groups of adenylic

FIG. 7.



The dissociation curves of the barium salt of thymic acid:

XIII. *Titrations with acid and alkali from approximately pH* 7, ○. *The smooth curve drawn through these points is calculated for* 1·1 *equivs. of* pK_a' 4·6, 0·75 *equiv. of* pK_a' 6·8, *and* 1·0 *equiv. of* pK_a' 10·0.
XIV. *Titration in* 1·875% *formaldehyde solution,* ●.
(*The zero of equivalents is fixed arbitrarily at pH* 8·4, *the titrations being carried out on two samples of slightly different barium content.*)

and cytidylic acids do not differ very greatly from those of adenine and cytosine respectively, as shown by the following data:

Adenine	4·15	Adenylic acid	3·70 [1]
Cytosine	4·60 [1]	Cytidylic acid	4·24 [1]

[1] Data of Levene and Simms (*loc. cit.*).

It is not expected, therefore, that replacement of *d*-ribose by deoxypentose would give rise to any considerable change in the pK_a' values.

There has been some confusion in the literature between the pK_a' values assigned to the amino- and the primary phosphoryl dissociations of nucleic acids. Levene and Simms (*loc. cit.*) considered the groups dissociating in the range pH 2·0 to 6·0 to be the amino-groups, whereas Hammarsten (*loc. cit.*) and Fletcher, Gulland, and Jordan (*J.*, 1944, 33) considered them to be the primary phosphoryl dissociations. Consideration of the pK_a' values for the

nucleosides and sugar phosphates (given below) in the light of the modern theory of zwitterions points conclusively to the former view being correct.*

Adenosine ..... 3·45 (Levene and Simms, *loc. cit.*).
Sugar phosphates... pK_{a_1}', 0·8 to 1·1; pK_{a_2}', 6·0 to 6·5 (Kumler and Eiler, *J. Amer. Chem. Soc.*, 1943, **65**, 2355).

Adenylic acid ..... pK_{a_1}', 0·89; pK_{a_2}', 3·70; pK_{a_3}', 6·01 (Levene and Simms, *loc. cit.*).

The pK_{a_2}' value of adenylic acid is clearly that of a primary phosphoryl dissociation, the pK_{a_3}' an amino-dissociation, and the pK_{a_1}' a secondary phosphoryl dissociation. At the isoelectric point, therefore, nucleic acids will exist almost entirely in the zwitterionic form.

The dissociations which will be titrated in the range pH 2·5 to 8·0 are therefore those of the amino- and the secondary phosphoryl groups. Examination of the lower portion of the back-titration curve (Fig. 1, curve II) shows that groups of both types are present, although the amount of secondary phosphoryl dissociation for every four atoms of phosphorus is relatively very small (see below). The curve is in approximate agreement with a theoretical curve constructed for 1·0 equivalent each, for every four atoms of phosphorus, of the amino-dissociations of guanylic, adenylic, and cytidylic acids, the pK_a' values of which are 2·3, 3·7, and 4·24 respectively (Levene and Simms, *loc. cit.*; Fletcher, Gulland, and Jordan, *loc. cit.*). It appears, however, that the pK_a' value of the amino-group of cytidylic acid (*viz.*, 4·24) which has been assumed for the amino-group of cytosine deoxypentose nucleotide is low, and much better agreement with the experimental curve is obtained by employing the pK_a' values of 2·5, 3·5, and 5·2 for constructing the theoretical curve; the curve shown in Fig. 1 is calculated on this basis.

The titration curve in the range pH 5·5 to 7·5 indicates the presence of a small amount of a group having a pK_a' value of 6·0 to 6·5, which is considered to be a secondary phosphoryl group. The determination of the exact quantity of this dissociation is dependent upon a precise knowledge of its pK_a' value and of the amounts of the amino-dissociations and their pK_a' values, but using 6·5 for the pK_a' value of the secondary phosphoryl dissociation, which is that observed for thymic acid, and assuming that there is no overlap of the amino- and the secondary phosphoryl dissociations above pH 6·5 (*i.e.*, no amino-dissociation greater than pK_a' 4·5), the amount of secondary phosphoryl dissociation is 0·25 equivalent for every four atoms of phosphorus. This represents the maximum amount of this group which can be present. Since, however, the pK_a' value of the weakest amino-group is of the order of 5·2, overlapping of the dissociations above pH 6·5 must occur, and thus the amount of the secondary phosphoryl dissociation present will be less than the maximum value.

The analysis of the sodium salt of deoxypentose nucleic acid shows that there are four sodium atoms for every four atoms of phosphorus, and in view of the fact that the amount of secondary phosphoryl dissociation is small, the four atoms of sodium must be combined largely or entirely with four primary phosphoryl dissociations. The deoxypentose nucleic acid of calf thymus differs in this respect markedly from the ribonucleic acids of yeast (Fletcher, Gulland, and Jordan, *loc. cit.*) and of *Calliphora erythrocephala* (Khouvine and Grégoire, *Bull. Soc. Chim. biol.*, 1944, **16**, 421), both of which show 1·0 equivalent of secondary phosphoryl dissociation (on correction for the phosphorus analysis) for every four atoms of phosphorus, and it cannot therefore possess the branched chain structure postulated for yeast ribonucleic acid by Fletcher, Gulland, and Jordan (*loc. cit.*). The data recorded are, however, consistent with the view that the thymus deoxypentose nucleic acid of calf thymus has a long, unbranched chain structure (Signer, Caspersson, and Hammersten, *loc. cit.*; Astbury and Bell, *loc. cit.*).

The groups titrating in the range pH 8·0 to 12·0 are considered to be the purine–pyrimidine hydroxyl groups of thymine and guanine deoxypentosides, and the upper part of the back-titration curve is in agreement with a theoretical curve constructed for 1·0 equivalent each of pK_a' values 10·4 and 11·4. The corresponding pK_a' value for thymine is 9·94 (Levene, Bass, and Simms, *J. Biol. Chem.*, 1926, **70**, 229; and confirmed by us) and for guanylic acid, 9·36 (Levene and Simms, *loc. cit.*), which, although of the same order of magnitude as the upper dissociations of deoxypentose nucleic acid, are nevertheless appreciably lower. The same effect is seen to a lesser extent in yeast ribonucleic acid which shows 2·0 dissociations of pK_a' 10·2 as compared with 9·36 for guanylic acid and 9·43 for uridylic acid (Levene and Simms, *loc. cit.*; Fletcher, Gulland, and Jordan, *loc. cit.*). The reason for this discrepancy is not clear,

* Acceptance of this view does not alter the main conclusions of Fletcher, Gulland, and Jordan about the structure of yeast ribonucleic acid, except that the triply-bound phosphoryl group is not necessarily that of uridylic acid.

especially in view of the better agreement which exists between the pK_a' values for the amino-dissociations of the ribonucleotides and of a yeast ribonucleic acid. It may, however, be related to the degree of polymerisation of the nucleic acid since it is not observed in thymic acid, which is considered to have a low molecular weight (see below).

The data given in Fig. 2, curve III, show that, other than the purine–pyrimidine hydroxyl groups, there are no dissociating groups titrating with alkali which have a pK_a' value in water less than at least 13·5. This confirms the absence of free sugar hydroxyl groups in this sample, since the pK_a' values of the primary hydroxyl dissociations of many sugars are in the region of 12·5 (Hirsch and Schlags, *Z. physikal. Chem.*, 1929, **141**, *A*, 387; Stearn, *J. Physical Chem.*, 1931, **35**, 2226; Urban and Shaffer, *J. Biol. Chem.*, 1932, **94**, 697; Urban and Williams, *ibid.*, 1933, **100**, 237), and suggests that no other sugar than a deoxypentose is present in any appreciable quantity in this sample of deoxypentose nucleic acid of thymus. Furthermore, taken in conjunction with the presence of one primary phosphoryl dissociation for every atom of phosphorus, the guanine, adenine, and cytosine dissociations and the guanine and thymine hydroxyl dissociations, this fact supports the view that the internucleotide bond is an ester linkage between the phosphoryl groups and the two hydroxyl groups of the sugar which are not involved in the glycosidic ring structure. Other types of linking may, however, exist in the nucleic acid, but the sensitivity of the titration method precludes the occurrence of such other linkages to a greater extent than one for every ten to twenty nucleotides.

The titration of thymic acid, taken in conjunction with the preceding data for deoxypentose nucleic acid, supplies information concerning the proportions of the four bases guanine, adenine, cytosine, and thymine present in this sample of nucleic acid. The titration curve for thymic acid (Fig. 7) shows 1·0 dissociation per four atoms of phosphorus in the pH range 8·0 to 12·0; this group can only be the enolic hydroxyl group of thymine. The 2·0 dissociations therefore observed in this pH range for deoxypentose nucleic acid (see above) must indicate the presence of one molecule each of thymine and guanine for every four atoms of phosphorus. It is not possible to determine with certainty the relative proportions of cytosine and adenine from the titration data of the sodium salt of deoxypentose nucleic acid, although the total amount of these groups is approximately 2·0. Thymic acid, however, possesses for every four atoms of phosphorus 1·0 to 1·2 dissociations of pK_a' 4·5 which must be the amino-dissociation of cytosine, and thus it is probable that the ratio of cytosine to adenine in the deoxypentose nucleic acid is as 1·0—1·2 is to 1·0—0·8.

The presence in barium thymate of 0·75 equivalent of a secondary phosphoryl dissociation for every four atoms of phosphorus suggests, on the basis of the straight-chain structure for deoxypentose nucleic acid, that the average number of nucleotides per molecule of thymic acid is approximately 5, and thus that the average molecular weight of the free acid is of the order of 1200.

(ii) *The macromolecular structure of deoxypentose nucleic acid.* As is shown in Fig. 1, the amino- and the enolic hydroxyl groups of thymus deoxypentose nucleic acid are partly or completely blocked until the material has been treated with acid or alkali; an irreversible change then takes place with the accompanying liberation of titratable groups. The release of the groups on treatment with alkali takes place sharply in the neighbourhood of pH 11·5, but less sharply in the range pH 3·5 to 4·5 on treatment with acid. In both cases equilibrium is established almost instantaneously, and the liberation of groups is accompanied by a marked fall in the viscosity and a disappearance of streaming birefringence (Hammarsten, *loc. cit.*; Vilbrandt and Tennent, *J. Amer. Chem. Soc.*, 1943, **65**, 1806; Creeth, Gulland, and Jordan, *loc. cit.*).

The decrease in viscosity brought about by the addition of acid or alkali was considered by Vilbrandt and Tennent (*loc. cit.*) to be caused by a depolymerisation which was slowly reversed when the solution was returned to neutrality. Our results show that such a depolymerisation cannot involve the rupture of the internucleotide ester linkages since no increase of secondary phosphoryl dissociation is observed in the back-titration curve. The complete identity of the back-titration curves from pH 2·5 and pH 12·0 strongly suggests that acid and alkali have the same effect in liberating the amino- and the hydroxyl groups. Two possibilities may be considered to explain this behaviour. It could be caused by easily hydrolysed radicals, hitherto unidentified in the breakdown products of the nucleic acid, which either substitute in the amino- and the hydroxyl groups separately or form a bridge between them. There are, however, certain limitations in the type of radical which could be involved; first, it could not contain groups which are titrated in the pH range examined in this investigation, since the back-titration curve shows no liberation of such groups and is moreover almost identical with the titration curve of the acid or alkali treated samples (Figs. 4 and 5), and secondly, the stability

to acid and alkali of the links involving the amino- and the hydroxyl groups would have to be very similar. In our opinion these restrictions make the preceding explanation of the observed behaviour improbable, and a simpler and preferable explanation is that in which the amino- and the hydroxyl groups are linked by hydrogen bonds. Bonding of this type has frequently been suggested as being important in protein structure, and our observations on the behaviour of deoxypentose nucleic acid resemble in some respects those recorded for egg albumen (Cannan, Kibrick, and Palmer, *Ann. N.Y. Acad. Sci.*, **1941**, **41**, 243; Crammer and Neuberger, *Biochem. J.*, **1943**, **37**, 302), which have been interpreted by postulating a hydrogen bond between a phenolic hydroxyl group and a carboxylate ion. Although it is undesirable at the present stage to speculate too far as to the macromolecular structure of deoxypentose nucleic acid, a hydrogen bond between an amino-group and either the –NH– group or the enolic –C(OH)– group of an adjacent guanine or thymine radical could explain satisfactorily our experimental results. The large number of such bonds which are possible, the maximum number being two for every four atoms of phosphorus, would give a degree of stability to the untreated deoxypentose nucleic acid, and in order to degrade the nucleic acid it might be necessary to break many of the hydrogen bonds simultaneously. Such a process would lead to an abnormal titration curve of the type shown in Fig. 1. It is not possible on the basis of these data to decide whether the hydrogen bonds unite nucleotides in the same, or in different chains; this aspect is considered in the light of viscosity and streaming birefringence by Creeth, Gulland, and Jordan (*loc. cit.*).

The increase in viscosity observed at approximately pH 7 when a solution of the nucleic acid was adjusted to that value after treatment with acid or alkali (Vilbrandt and Tennent, *loc. cit.*; Creeth, Gulland, and Jordan, *loc. cit.*) does not involve the blocking of the amino- and the enolic hydroxyl groups, since the titration curves obtained were identical with the back-titration curve shown in Fig. 1 whether the solution was titrated immediately or was allowed to remain at approximately pH 7 for 96 hours in the absence of atmospheric oxygen. During this period the viscosity had risen to a value of the same order as that observed with the original acid (Creeth, Gulland, and Jordan, *loc. cit.*).

A different behaviour was observed in samples which had been precipitated by alcohol at pH 7 after acid or alkaline treatment. Some blocking of the enolic hydroxyl groups occurred (Figs. 4 and 5), but to a much smaller extent than that found with the original material, and the properties of the precipitated material appeared to be independent of the viscosity changes, since very similar titration curves were obtained whether the product was precipitated immediately or after 96 hours. Precipitation would thus seem to be the important factor. There is no evidence from titration data that precipitation effects blocking of the amino-groups.

Greenstein and Jenrette (*J. Nat. Cancer. Inst.*, **1940**, **1**, 77; *Cold Spring Harbor Symp. Quant. Biol.*, **1941**, **9**, 236) have postulated on the evidence of viscosity measurements that reversible depolymerisation of thymus deoxypentose nucleic acid takes place on the addition of neutral salts. Titration of the sodium salt of thymus deoxypentose nucleic acid in M-potassium chloride (Fig. 2) and in 2·53 molar guanidine sulphate (Fig. 3) showed that the changes in viscosity which occur bear no relation to the irreversible change which takes place on treatment with acid or alkali, since the discrepancy between the forward- and back-titration curves was still present in these salt solutions. The lowering of the viscosity by the addition of salt must therefore involve a different type of physico-chemical change to that occurring on treatment with acid or alkali.

Experimental.

Apparatus.—The electrometric titrations were carried out according to the method described by Fletcher, Gulland, and Jordan (*loc. cit.*), and the titration curves were corrected for the titration of water at the extremes of pH by the method of Jordan and Taylor (*loc. cit.*). The solutions for titration contained 100 to 200 mg. of the samples in 20 ml. of water.

Preparation of Alkali-treated Samples of the Sodium Salt of Thymus Deoxypentose Nucleic Acid.—To a solution of sodium salt of deoxypentose nucleic acid (2 g.) in water (100 ml.), 0·5*N*-sodium hydroxide (25 ml.) was added with mechanical stirring. The solution then had a reaction of pH 12·30. 0·5*N*-Hydrochloric acid (24 ml.) diluted with water (51 ml.) was added slowly with rapid stirring to avoid precipitation. The pH was finally adjusted to 7·0. The solution was added immediately or at the time required to ethyl alcohol (750 ml.). The white granular precipitate (1·6 g.) was collected, washed with alcohol, and dried in a vacuum over phosphoric oxide.

Preparation of Acid-treated Samples of the Sodium Salt of Thymus Deoxypentose Nucleic Acid.—To a solution of the sodium salt of deoxypentose nucleic acid (2 g.) in water (150 ml.), 0·08*N*-hydrochloric acid (50 ml.) was added slowly with constant stirring, the final reaction being pH 3·1. 0·195*N*-Sodium hydroxide (24 ml.) was added slowly with stirring, and the reaction adjusted to pH 7·1 with dilute hydrochloric acid. The solution was added immediately or later as required to ethyl alcohol (750 ml.).

The white granular precipitate (1·6 g.) was collected, washed with alcohol, and dried in a vacuum over phosphoric oxide.

Preparation of Barium Thymate.—A solution of the sodium salt of thymus deoxypentose nucleic acid (15 g.) in water (400 ml.) was warmed to 80° and mixed with 10% sulphuric acid (30 ml.) at the same temperature. A white solid formed and redissolved in 5 minutes. The solution was maintained at 80° for 30 minutes. Silver sulphate (5 g.) was added and the suspension shaken for 1 hour, cooled in water and then to 0°, and mixed with a solution of barium acetate (20 g.) and barium chloride (5 g.) in water (30 ml.). Next day the supernatant was decanted, and the solid collected by centrifuge and washed repeatedly with 90% alcohol and then with 100% alcohol and ether. The product (4·6 g.) was dried in a vacuum over phosphoric oxide.

Analyses.—All samples were dried at 110° in a vacuum over phosphoric oxide. Details of the analytical methods are given in Part I (*loc. cit.*). Sodium salt of deoxypentose nucleic acid of calf thymus prepared by Professor Caspersson and deproteinized by us (see text), found: C, 35·5; H, 4·14; N, 15·4; P, 9·4; Na, 7·2 (colorimetric)%. Alkali-treated sodium salt of deoxypentose nucleic acid of calf thymus (respective values for three preparations), found: C, 35·3, 35·7, 36·1; H, 3·62, 3·83, 3·79; N, 15·4, 15·6, 15·5; P, 9·30, 9·63, 9·58; Na, —, 6·42, 6·28 (gravimetric) %. Acid-treated sodium salt of deoxypentose nucleic acid of calf thymus, found: C, 35·9; H, 3·76; N, 15·8; P, 9·41; Na, 6·33 (gravimetric) %. Calc. for a large polynucleotide consisting of the tetrasodium salts of tetranucleotides containing on average 1 mol. each of guanine, adenine, cytosine, and thymine deoxypentose nucleotides, *i.e.* $(C_{39}H_{45}O_{24}N_{15}P_4Na_4)_n$, the additional HONa of the terminal groups being ignored: C, 35·4; H, 3·4; N, 15·9; P, 9·4; Na, 6·95%. Calc. for a large polynucleotide consisting of the tetrasodium salts of tetranucleotides containing on average 1 mol. each of guanine and thymine, and 1·2 mols. of cytosine and 0·8 mol. of adenine deoxypentose nucleotides, *i.e.* $(C_{37.6}H_{43}O_{24}N_{14.4}P_4Na_4)_n$, the additional HONa of the terminal groups being ignored: C, 35·4; H, 3·4; N, 15·5; P, 9·4; Na, 6·98%.

Barium thymate (respective values for two preparations), found: C, 27·1, —; H, 3·46, —; N, 5·44, 5·53; P, 9·47, 9·44; Ba, 23·3, 23·1%. Calc. for a molecule containing four atoms of phosphorus, 1·2 mols. of cytosine, 1·0 mol. of thymine, 2·22 atoms of barium and a terminal OH group to every five atoms of phosphorus, *i.e.*, $C_{39.4}H_{49.0}O_{24.9}N_{15.4}P_4Ba_{2.22}$: C, 29·9; H, 3·10; N, 5·96; P, 9·44; Ba, 23·2%. Calc. for a molecule containing four atoms of phosphorus, 1·0 mol. each of cytosine, and thymine, 2·22 atoms of barium, and a terminal OH group to every five atoms of phosphorus, *i.e.*, $C_{36}H_{40.1}O_{24.9}N_{14}P_4Ba_{2.22}$: C, 26·8; H, 3·08; N, 5·37; P, 9·56; Ba, 23·4%.

It is a pleasure to record our thanks to Imperial Chemical Industries Ltd. for the loan of apparatus; to Professor W. T. Astbury, F.R.S., for the gift of the sample prepared by Professor Caspersson; to Mr. J. M. Creeth, B.Sc., for preparing two of the alkali-treated samples, for preliminary work on the acid treated material, and for some of the sodium analyses; to Mr. C. J. Threlfall, B.Sc., for preparing the samples of barium thymate; and to Mr. J. E. Still, B.Sc., and Mr. D. S. R. Cameron for the microanalyses.

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MOLECULAR CHARACTERISTICS OF SODIUM DESOXYRIBONUCLEATE

by

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It has been the purpose of many studies[1-10] on sodium desoxyribonucleate (DNA) to determine molecular parameters in order to define the molecular shape and size. Consideration of this work suggests that a large sample to sample and technique to technique variation is responsible for the enormous range ($0.5 - 20 \cdot 10^6$) of molecular weight values.

We have prepared a high molecular weight sample by the MIRSKY-POLLISTER[11], SEVAG, LACKMAN AND SMOLENS method[12] as modified by GULLAND *et al.*[13] and studied its properties by light scattering, electron microscopy and viscometry. In addition, the viscometric behavior of a second sample prepared by a modified HAMMARSTEN method by GREENSTEIN AND HOYER[14] was studied. This investigation is still incomplete but the measurements thus far obtained are interesting enough to warrant publication at this time. A more detailed account will be submitted upon completion of this investigation.

LIGHT-SCATTERING STUDIES

The intrinsic asymmetries of approximately fifty solutions of the Gulland preparation of varying DNA concentration ($0.40 - 7.0 \cdot 10^{-4}$ g/ml) and salt concentration

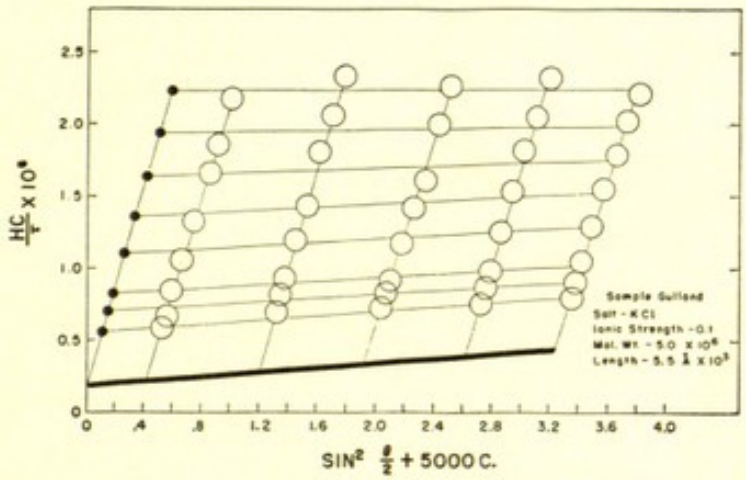

Fig. 1. Typical Zimm plot. The lowest angle of observation of each of the five concentrations, over the range of $1.0 - 7.0 \cdot 10^{-4}$ g/ml, is 40°, the highest point is 100°.

* National Institutes of Health, Public Health Service, Federal Security Agency.

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(0.0–2.5 *M*) fell between 2.8 and 3.6. The double extrapolation method of ZIMM[15] was found to be applicable to the data. A typical Zimm plot at one ionic strength is shown in Fig. 1. The molecular configuration was intermediate between that of a rod and a coil as shown in Fig. 2. The long thread-like molecules contracted in length from $6.8 \cdot 10^3$ A to $4.5 \cdot 10^3$ A when the ionic strength of the added electrolyte was increased from 0 to $2.0 \cdot 10^{-2}$.

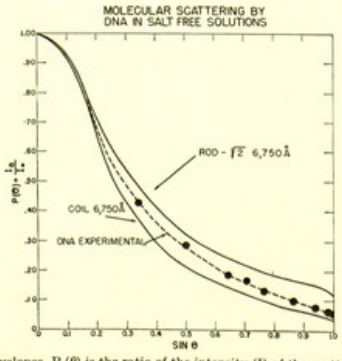

Fig. 2. Scattering envelopes. P (θ) is the ratio of the intensity (I) of the scattered light at any angle θ to the intensity of the scattered light at 0°.

ELECTRON MICROSCOPY STUDIES

In previous electron microscope studies[16, 16a] of DNA the diameters measured and reported bore no resemblance to the sizes obtained by other methods and undoubtedly were measurements of multiple filaments. In this investigation a great many electron-micrographs of both of the above-mentioned preparations shadow cast under a variety of conditions with platinum, chromium, and uranium were made. We have succeeded by the use of high resolving power methods and by the proper interpretation of shadow lengths in obtaining measurements on the finest fibers and found them to be in the range of 10–20 A. This value of the molecular diameter is in fair agreement with the values obtained by light-scattering and viscometric methods. Contrary to earlier indications[9, 17, 18] the molecule appears to be not a stiff rod but one which is best described as a slightly kinked and slightly flexible rod. One of its most striking properties is its tendency to spiral, twist and intertwine with neighboring molecules.

VISCOMETRY STUDIES

The viscometric properties of both samples have been studied and found to be quite comparable. The study of the Gulland preparation is still incomplete. It was found possible to correct the relative viscosities obtained with a Bingham viscometer to zero

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shear rate by two methods which gave essentially identical results. In the first method the pressure was plotted versus shear rate and the limiting slope at zero shear rate was used. In the second method the reciprocal of the product of pressure and time was plotted against pressure and the limiting viscosity was obtained from the extrapolated value of PT[19]. A plot of the specific viscosity versus concentration is shown in Fig. 3. The intrinsic viscosity 5060 ml/g lead to an axial ratio of 330 using SIMHA's equation for a rod[20].

Fair agreement was found in molecular parameters as estimated by the above three methods. The molecular diameter was found to be in the range of 15–24 A, the axial ratio between 330 and 400 and the molecular weight between four and five million ($4.51 - 0.53 \cdot 10^6$).

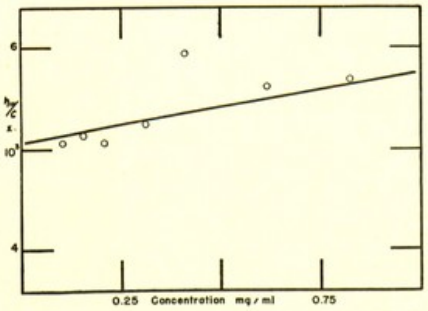

Fig. 3. Intrinsic viscosity determined by extrapolation of the plot of specific viscosity divided by concentrations (mg/ml) *vs.* concentration.

SUMMARY

Studies by three different physical methods on a sample of Na desoxyribonucleate are described.

1. Light-scattering studies indicate that the molecular length decreased from 6800 A for solvent ionic strength zero, to 4500 A for ionic strength 0.02. The molecular weight was found to be $4.5 \cdot 10^6$.

2. Viscosities extrapolated to zero shear rate indicate an axial ratio of 330.

3. Electron microscopic photographs reveal filamentous particles whose diameters are in agreement with values obtained by indirect methods.

RÉSUMÉ

Nous avons étudié un échantillon de désoxynucléate de sodium par trois méthodes physiques différentes.

1. Des expériences avec de la lumière dispersée indiquent que la longueur moléculaire diminue de 6800 A à 4500 A lorsque la force ionique du solvent passe de 0 à 0.2. La valeur trouvée pour le poids moléculaire est de $4.5 \cdot 10^6$.

2. La valeur de la viscosité extrapolée pour une vitesse de cisaillement zéro indique un rapport axial de 330.

3. Des photographies prises au microscope électronique révèlent des particules filamenteuses dont la valeur du diamètre est en accord avec les valeurs trouvées par des méthodes indirectes.

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ZUSAMMENFASSUNG

Untersuchungen einer Probe Natriumdesoxyribonucleat mit drei verschiedenen physikalischen Methoden werden beschrieben.

1. Untersuchungen im Streulicht zeigen, dass die Molekülläge von 6800 A in Lösungen mit der Ionenstärke 0 abnimmt auf 4500 A bei Ionenstärke 0.02. Das Molekulargewicht wurde zu $4.5 \cdot 10^6$ gefunden.

2. Die für Schergeschwindigkeit Null extrapolierte Viskosität zeigt ein Achsenverhältnis von 330 an.

3. Aufnahmen mit dem Elektronenmikroskop zeigen faserige Teilchen, deren Durchmesser in Übereinstimmung sind mit den durch indirekte Methoden erhaltenen Werten.

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Molecular characteristics of Nc DNA

JW Rowen, M Eden, H Keller, Biochim Biophys Acta 10 89 (1903)

Light scattering → length 6800 Å for zero ionic strength → 4500 Å for 0.02
M.Wt. 4.5×10^6

Viscosity ~~exp~~ extrap. → zero shear axial ratio 330

EM shows fibre 10-20 Å diameter
"slightly kinked + flexible rod" with "tendency to spiral twist
↳ interwine w neighboring molecules

Electron microscopy of sodium desoxyribonucleate

H. Kahler & B.O. Lloyd (National Cancer Institute, Bethesda, Md, USA) Biochim Biophys Acta 10 355 (1953)

Diff proj° all → 15 ± 5 Å diameter of fibres of Na DNA (thymus) (measured by shadow length)

Calf thymus nucleoprotein → diameter ≥ 5 Å

(but uncertainty: "poss dissoc" or drying)

Form, intermediate betw. rod & random coil

- as indicated by light-scattering

[v good photos]

Light-scattering studies of NaDNA

U. W. Arnes, Biochim. Biophys. Acta, 10, 391, (1953)

Results agree w others

but also studied effect of enzymatic degradation

- found ~ 25%, not de-polymerized
- this fraction isolated & found to behave differently both in light-scattering & in infra-red
- light scattering gives it as rigid rod.

AN X-RAY DIFFRACTION INVESTIGATION OF AQUEOUS SYSTEMS OF DESOXYRIBONUCLEIC ACID (Na SALT)

by

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Nucleic acid is an essential constituent of living animal cells. In its desoxy variety, it constitutes the major part of the nucleus, in association or combination with protein. The exact nature of the interaction between protein and nucleic acid is not known. Chemical analyses show[1] that the nucleus contains about 14% by weight of desoxyribonucleic acid and in local regions the concentration may be higher. According to MIRSKY AND RIS[2], 37% by weight of chromatin threads consists of nucleic acid.

The aim of this work has not been to achieve a detailed analysis of the nucleic acid molecule itself, but to study molecular arrangement in aqueous systems at relatively high concentrations. It is felt that knowledge of molecular interaction in environments similar to those which exist in the living cell may be more important to an understanding of gross cytological phenomena than even a complete picture of the molecule taken in isolation. For this type of investigation, the method of X-ray diffraction analysis is uniquely suited. It has the virtue of being indifferent to the nature of the specimen, which can be a solid, a concentrated or dilute solution, or living tissue, and of not affecting the material in the course of experiment. Linear dimensions in the 1 A. to 100 A. range can be accurately measured and still higher values are accessible by the low-angle scattering technique. In this paper, we shall examine the X-ray scattering given by aqueous systems of the sodium salt of desoxyribonucleic acid in concentrations varying from about 0.03 to 1.8 g/ml (2% to 100% by volume). Certain aspects of the problem have already been briefly described by us elsewhere[3].

THE MATERIAL

Three samples of the sodium salt of desoxyribose nucleic acid (from calf thymus) having different molecular weights were studied. (1) Sample prepared by Prof. R. SIGNER AND Dr H. SCHWANDER[4] who kindly presented it to us. This material is of very high purity and contains less than 0.3% protein. (2) Sample prepared by Drs J. A. V. BUTLER AND K. A. SMITH[5] who kindly presented it to us. (3) Commercial sample purchased from the British Drug Houses Ltd. Samples (1) and (2) were prepared by modifications of the Hammarsten technique (salt extraction) and sample (3) was prepared by the Feulgen-Levene technique (alkali extraction). By a comparison of the ultra-violet

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Miss Franklin

Reprinted from

Biochimica et Biophysica Acta

7 (1951)

Riley + Oster

X-ray investigation of aqueous system of DNA

(from manuscript) - Biochim et Biophys Acta 1952

Used concentrations 0·3 → 1·8 gm/c.c. DNA (2% → 100% by vol)

And specimens Signer, Butler + BDH

M. Wts by light scattering + viscosities 3·26, 1·34, 0·51 $\times 10^6$

and axial ratios 175, 125, 19 respectively

see Oster J Chim Phys 47 (1950) 717

Partial sp. vol. of dry DNA assumed to be 0·55 throughout

[and presumably of water assumed to be 1·00]

Sedimentation + diffusion outrage. → ∞ dil gives particle dia. 18Å

i.e. single molecules apparently operative

cf Kaller J Phys Coll Chem 52 676 (1948)

Ceil + Oster JCS 1382 (1948)

EM shows fibres of dia 50-60Å (Nixon + Oster, unpublished)

Specimens - Pyrex tubes, weighed, + re-weighed after adding water + sealing. Left several days to equilibrate, till X-ray spacings showed no further change

(except Signer DNA, + changed further over period of months)

absorption of solutions of these samples with the absorption for samples dried over P_2O_5 and then dissolved in water (optical density of dehydrated nucleic acid at 260 mμ equals 0.200 for a solution of concentration $1.0 \cdot 10^{-5}$ g/ml) it was found that the air-dry samples, as received, contained (1) 30%, (2) 30% and (3) 10% water by weight respectively. By light scattering and viscosity studies, the molecular weights (in millions) of the samples have been found to equal (1) 3.26, (2) 1.34 and (3) 0.51[6], and the axial ratios 175, 125 and 19 respectively. The partial specific volume of nucleic acid is taken as 0.55, the mean of the observed values[7]. In all the work considered here the nucleic acids are in the form of their sodium salts.

From sedimentation and diffusion measurements extrapolated to infinite dilution[7,8], the diameter of the fundamental particle, assuming it to be an elongated ellipsoid obeying the laws of macroscopic hydrodynamics, is calculated to be 18.4 A. The rate of sedimentation for elongated particles is determined by their cross-sectional area. In the very dilute solutions required by this method, the molecules are apparently hydrodynamically independent and the dimensions thereby derived are those of the individual molecule. Electron microscope examination (with palladium shadowing) of dried solutions of SIGNER's sample shows fibres with diameters of about 50 to 60 A.[9] These apparently correspond to bundles of the fundamental molecular fibres, as will be seen from the later discussion.

THE SPECIMENS

Specimens of known concentration were prepared from the SIGNER and from the BUTLER samples* by the following method. Bundles of air-dry fibres were introduced into weighed thin-walled Pyrex glass tubes (average diameter 1.2 mm and wall thickness 0.04 mm). The tubes were then re-weighed and distilled water introduced with a microsyringe; a final weighing after careful sealing-off completed the process. Specimens prepared in this way, without stirring, were left to stand for several days to attain equilibrium. This was indicated by the fact that the spacings of the X-ray diffraction bands did not change on further keeping and were the same for different parts of the specimen. On keeping for several months, however, the SIGNER specimens do show changes which are discussed later. A solution of the SIGNER sample of known concentration was also prepared by rapid stirring in a small vessel before being sucked up into the specimen tube with the aid of a vacuum pump. This specimen also showed differences from those prepared without mechanical agitation. Solutions of the commercial sample are not highly viscous and were prepared separately before being sucked into the tubes.

Work on the crystalline-micelle transition was done with the aid of a small vacuum-tight cell with thin mica windows. This cell was also used for much of the wide-angle work and for a number of exploratory experiments where the concentrations were not measured in advance.

X-RAY PHOTOGRAPHY

All X-ray photographs were taken with CuKα radiation ($\lambda = 1.542$ A.) obtained from the large rotating-anode X-ray generator in the Davy-Faraday Laboratory. Con-

* We shall, for brevity, refer to these two samples by the names of the first mentioned authors in the references previously given[4,5].

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siderable modifications have been made to this unit since it was first described by MÜLLER AND CLAY[10], in order to make it less troublesome to operate for prolonged periods. As the X-ray effects examined consist of relatively well-defined bands, it was not considered necessary to use crystal-reflected radiation and sufficiently monochromatic X-rays were obtained by filtering through 0.025 mm of nickel.

The camera used was essentially an optical bench mounted in a long vacuum chamber so that specimen-to-film distances of up to 110 cm could be used if needed. The beam was defined by three slits, one on the tube-window and the other two in the vacuum chamber. The latter two were precisely adjustable by micrometer screw-threads and were faced with pure tungsten. The third slit, following the usual practice, merely suppressed secondary scattering from the second slit. Little difficulty was found in obtaining by this means fine well-collimated beams of height *ca* 3 mm at the specimen.

The low-angle photographs were taken on a flat film at various distances between 15 cm and 50 cm. The wide-angle photographs were mainly taken in a cylindrical camera of radius 5 cm although some were taken at longer distances on flat film to increase the resolution. The exposure varied considerably, the wide-angle photographs requiring from 15 to 30 minutes while the low-angle exposures were of about 2 hours duration with a power input of 15 KW, the long exposures being made necessary by the great distance between the X-ray tube focus and the film. In order to eliminate air-scattering, all low-angle photographs were taken in a vacuum; this procedure was also used for most of the wide-angle work.

GENERAL CLASSIFICATION OF THE SYSTEMS

The structural nature of nucleic acid preparations is markedly dependent on the quantity of water present and on the way in which the sample is prepared. If insufficient water is present, or again if there is too much, the structure is disordered. There is, however, a wide range of concentrations over which the gels have an ordered structure, including a narrow range where the material appears to be really crystalline.

Four principal regions of concentrations may be distinguished and will be described in turn.

a. *Dry region*

This region starts at the anhydrous material ($c = 1.0$)* and includes the air-dry fibres as received ($c = 0.57$ or 1.04 g/ml). The anhydrous material, prepared by drying out a wet gel in a vacuum chamber containing P_2O_5, is hard, white and opaque.

b. *Crystalline region*

Crystalline samples, in which the concentration is in the neighbourhood of 0.44 (weight concentration, 80%) can be prepared in the following way. A drop of distilled water is added to a small amount of the air-dry fibrous substance (*ex* SIGNER). The material rapidly imbibes water and loses its opacity and shape. A homogeneous solution is obtained by stirring with a needle and this is left to dry gradually for about 24 hours. The crystalline substance which results is a hard mass with a white waxy appearance. It is opaque in bulk but shows under low magnification strong domain birefringence in

* All concentrations c, unless stated otherwise, are given in terms of the volume of anhydrous material per unit total volume.

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thin sections, the domains being elongated in shape and negatively birefringent. Once obtained, the material can be wetted and dried between narrow limits without losing its essential crystalline nature.

c. *Micelle region*

Over a wide range of volume concentrations, from about 0.4 to 0.02 (73% to 4% wt/vol), the nucleic acid gels possess a partly-ordered structure of the "liquid-crystalline" type. The striking feature of this region is that the molecules aggregate into micelles; the distance apart of molecules within micelles, as of the micelles themselves, increases markedly with dilution. These characteristics will be fully discussed in a later section. The appearance of the samples varies from that of a moist semi-opaque fibrous mass to that of a typical transparent liquid gel of high viscosity. The more concentrated transparent samples exhibit pronounced domain birefringence under static conditions, the domains being in some cases as large as 0.1 mm in length, as well as strong streaming birefringence.

d. *Dilute region*

At concentrations less than about 0.02, the systems are transparent non-birefringent solutions of relatively low viscosity. There is no evidence of marked molecular ordering or aggregation in this region.

GENERAL DESCRIPTION OF THE X-RAY SCATTERING

Previous X-ray work on nucleic acid preparations by ASTBURY AND BELL[11] and ASTBURY[12], has been of a fundamentally different type from ours and has been aimed at elucidating the intramolecular structure. Fibre photographs obtained by these workers of stretched films in air showed marked preferred orientation. A list of spacings is not given but the presence of a strong meridian are of spacing 3.34 A. is emphasised: the true identity spacing along the fibre axis is stated to be at least 27 A. In his latest paper, ASTBURY[12] states that the true repeat distance along the fibre axis corresponds to the thickness of 8 or 16 nucleotides. The principal side-spacing reported has a value of 16.2 A. and higher spacings up to about 26 A. are also mentioned. It may not be possible to compare these data with our findings in detail as the material used was not identical and the specimens were prepared in different ways. We shall return to ASTBURY's work in the discussion at the end of the paper.

As will be seen by reference to Table I, the X-ray scattering effects observed by us from the samples examined can be grouped into eight ranges*. First of all, there are the 3 A., 4 A. and 5 A. ranges which are given under all conditions and must correspond to intramolecular periodicities. (They were not looked for in the most dilute systems owing to the confusing effect of scattering by the bulk of water present). In the anhydrous specimen, they take the form of very diffuse bands, as they do in the case of a wet gel. The air-dry fibres (BUTLER, as received) show a significant difference in that the 3 A. band becomes a sharp line of spacing 3.25 A., although the rest of the pattern remains diffuse. The crystalline specimens show only sharp lines, several in each region, and the diffuse bands in the disordered structures correspond to a blurred-out

* This statement refers specifically to the SIGNER AND BUTLER samples. Specimens made from the commercial material were examined for high spacings only.

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TABLE I

SUMMARY OF X-RAY RESULTS FOR NUCLEIC ACID UNDER VARIOUS CONDITIONS, SHOWING THE RELATION OF THE PRINCIPAL DIFFRACTION REGIONS TO THE DIFFERENT STRUCTURE TYPES

Specimen	Anhydrous	Air-dry fibres as received	Crystalline		Moist Crystalline	Wet gel	Wet gel
Volume Concentration	1.0	0.57	0.44		0.40	0.15	*ca* 0.15
Spacing Range							
3 A	3.3_1 *s vb*	3.25 *s sb*	2.97 *w* 3.15 *m* 3.34 *w* 3.50 *w*	a)	a)	(a)	3.3_2 *s b*
4 A	4.0_4 *s vb*	4.1_4 *vw vb*	3.88 *m* 4.04 *s* 4.20 *m*	(a)	(a)	(a)	4.1_3 *vw b*
5 A	5.3_0 *mw b*	5.46 *vw vb*	4.53 *w* 4.66 *w* 5.23 *mw* 5.88 *w*	(a)			5.7_3 *w b*
8 A	(b)	(b)	6.54 *w* 7.22 *w* 7.97 *w*	6.88 *w* 7.42 *w* 8.12 *w*	6.67 *w* 7.33 *vw* 8.14 *w*		8.5_6 *w b*
10 A			10.4 *s* 11.2 *m*	10.6 *s* 11.3 *s* 11.8 *m* (triplet)	10.8 *mw* 11.7 *m sb*		
15 A			15.7 *mw sb*	14.9 *mw* 16.5 *m* (doublet)	16.5 *w*	13.2 *vw vb*	13.0 *m b*
20 A	(c)			19.1 *w sb*	20.8 *vs sb*	21.2 *vw b* 35.8 *vs b*	(d)
Micelle Band 40 A	(a)	(a)	(a)		44.9 *vw b*	78.5 *ms b*	(a)
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)

vs = very strong; *s* = strong; *ms* = medium strong; *m* = medium; *mw* = medium weak; *vw* = very weak; *vb* = very broad; *b* = broad; *sb* = slightly broadened line.

a. This spacing range not recorded
b. Weak unlocalized scattering

Spacings are in A., concentrations in ml/ml.
The intensities given are relative intensities for any one specimen and are not directly comparable with those for another.

c. Intense diffuse central scattering
d. Intense low-angle scattering unresolved owing to small camera radius.

N.B. The columns are numbered at the base of the table.

version of the richer crystalline pattern. There is not necessarily an exact equivalence in spacing between the strongest crystalline lines and the diffuse bands but they are all obviously related to an intramolecular structure which remains substantially unaltered on this scale under all the conditions studied. The 8 A. region is also given at all concentrations but is much less definite and intense. It, too, is probably largely intramolecular in nature.

Scattering is not observed in the remaining four spacing-ranges (10 A., 15 A., 20 A., and 40 A. upwards) in the case of material from the dry region, if exception be made for the usual diffuse central (low-angle) blackening given by disordered colloidal substances. This also applies to dilute solutions, which give diffuse central scattering only.

Specimens in the crystalline and micelle region, however, show pronounced diffraction effects in the higher spacing ranges which have been the subject of special study. Broadly, the lines in the 10 A. and 15 A. ranges are characteristic of a crystalline specimen, while the bands in the 20 A. and 40 A. ranges correspond to micelle formation. Several series of photographs were taken to establish the nature of the transition, a given slightly moist specimen being repeatedly examined as it slowly dried.

A typical wet gel in the micelle region shows two bands at low angles whose spacings are markedly dependent on the concentration of nucleic acid in the specimen. The exact way in which these bands move with concentration will be the subject of the next section. Such a system may, if it is not too dilute, also show diffuse bands in the 3 A., 4 A., 5 A. and 8 A. ranges and, in addition, one at about 13 A. At the high concentration end of the region, lines typical of the crystalline state appear, first very faint and rather diffuse, then more and more definite as crystallisation sets in. Correspondingly, the micelle bands become fainter until they disappear, the band of higher spacing vanishing considerably ahead of the 20 A. band. The crystalline lines, once apparent, do not change appreciably in spacing or relative intensity. They consist of a triplet of lines of spacings 10.6 A., 11.3 A. and 11.8 A. and a doublet whose components are at 14.9 A. and 16.5 A., Under conditions of poor resolution, the two sets of lines appear as two bands of spacings 10.9 A. and 15.7 A. The latter composite band can be still further broadened at concentrations where the 20 A. micelle band is just appearing. Nevertheless, there is clear evidence from several photographs that the micelle band is a separate phenomenon and is not, as a rapid inspection of one or two patterns might misleadingly indicate, the 16 A. crystalline band shifted inwards. The transition from the crystalline state to a micelle system is reversible and a given specimen can be repeatedly changed, back and forth, from one to the other by adjusting the water-content.

The micelle band has not been observed with a spacing of less than 19.1 A. When its spacing has this value, the band is weak whereas the crystalline line-pattern is well developed. We may therefore take this figure as the lower limit of movement of the band. If the concentration of nucleic acid is then slightly increased by allowing specimen to dry a little, the micelle band disappears completely and the crystalline pattern only is seen.

THE X-RAY PHOTOGRAPHS

We have reproduced in Fig. 1, 2, and 3 a selection of X-ray photographs given by nucleic acid under various conditions.

Fig. 1 reproduces four wide-angle photographs taken in order to show the lower

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spacing regions in specimens of different moistness. Photographs Ia, b, c were taken in a cylindrical camera of 5 cm radius, while Id was taken on flat film at 5.5 cm distance.

Ia. A photograph of the crystalline material, showing a wealth of well-defined lines reminiscent of the patterns given by normal crystalline substances in powder form. The spacings and relative intensitites are listed in Table I, column 4. The substance was enclosed in the small air-tight cell described earlier.

Ib. A similar photograph of the same crystalline specimen in a slightly more moist state. Note the blurring of the lines which otherwise remain practically unchanged. The specimen was enclosed in a cell, as in Ia.

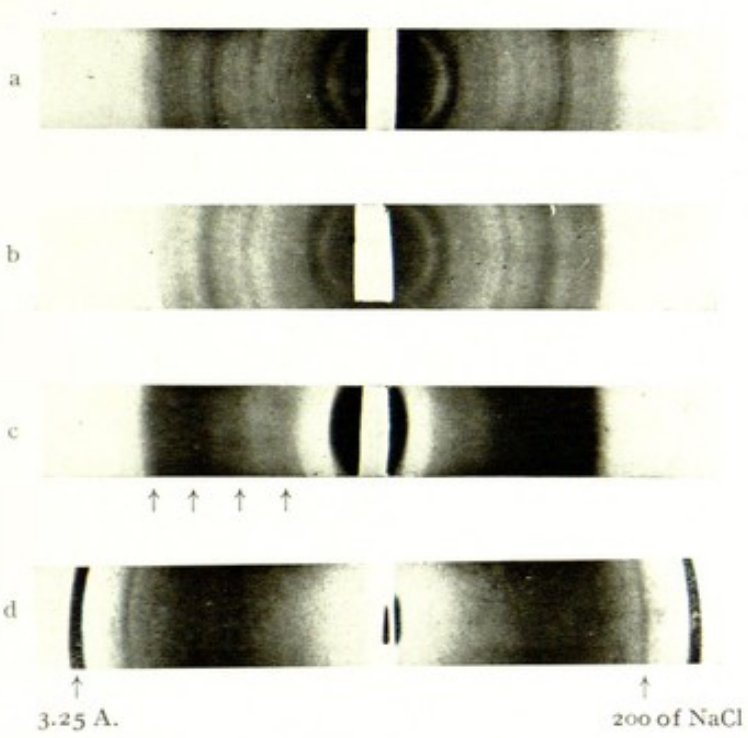

Fig. 1. Wide-angle photographs of various specimens of nucleic acid (Cu Kα radiation, Ni filter).
a. Crystalline specimen in cylindrical camera of 5 cm radius
b. Moistened crystalline specimen in same camera
c. Anhydrous (P_2O_5 dried) specimen in same camera
d. Bundle of air-dry fibres, as received, at 5.5 cm on flat film.

Ic. The pattern given by anhydrous material obtained by prolonged drying of a gel in a vacuum over P_2O_5. Details of the photograph are given in Table I, column 2. The photograph was taken in a vacuum camera with the specimen unsurrounded by any enclosure.

Id. A photograph of a bundle of air-dry fibres (BUTLER AND SMITH, as received). Details are given in Table I, column 3. The dominant feature is a sharp line of spacing 3.25 A. and the 200 line of NaCl present in the sample is also shown.

Fig. 2. Shows a series of photographs taken to illustrate the gradual transition from the crystalline state to the micelle region. All photographs were taken on flat film in a vacuum camera, 2a at 20 cm, the rest at 15 cm.

2a is a photograph of a crystalline specimen corresponding to the central higher-

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spacing region of Fig. 1a. The triplet and doublet, the spacings of which are given in Table I, column 5, are clearly resolved (T and D). There is no evidence of other crystalline lines of high spacing but the micelle band M_1 is just starting to be perceptible at its lowest observed spacing of 19 A.

2b shows how, on addition of a tiny amount of water, M_1 becomes more pronounced and slightly increases its spacing (Table I, column 6). The crystalline lines are still present.

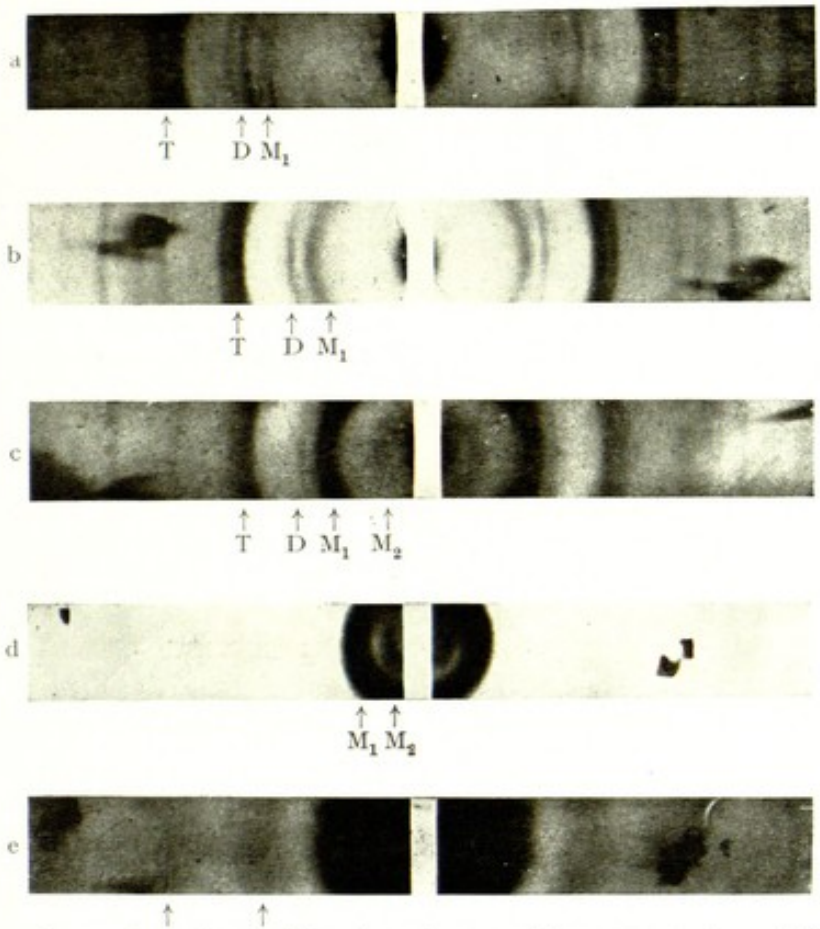

Fig. 2. Photographs to show the transition from the crystalline state to the micelle region. Cu$K\alpha$ radiation, Ni filter, in a vacuum camera. (a) at 20 cm on flat film (b) (c) (d) and (e) at 15 cm on flat film. (d) and (e) are under- and over-exposed prints of the same photograph. Micelle bands are shown by M_1 and M_2, crystalline doublets and triplets are labelled D and T. Arrows in (e) indicate diffuse low-spacing scattering regions. Concentration diminishing from (a) to (d) with consequent onset of micelle formation. Large spots are from mica-windows of specimen-cell.

2c shows the effect of further addition of water. M_1 is now the dominant feature of the pattern and the inner micelle band M_2 is just becoming visible. The crystalline pattern is weaker and less distinct.

2d and e are photographs of a transparent wet gel prepared from the above specimen by addition of water and stirring. 2d is a light print which shows clearly the two micelle bands, now at much higher spacings (Table I, column 7). 2e is a heavy print of the same photograph made in order to show how the perfection of the crystalline pattern has been lost and replaced by the broad scattering regions indicated by arrows.

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Fig. 3 reproduces low-angle photographs given by the three different samples of nucleic acid investigated. All were taken in a vacuum camera, 3a at 30 cm, the other two at 25 cm, with the specimens enclosed in thin-walled glass tubes.

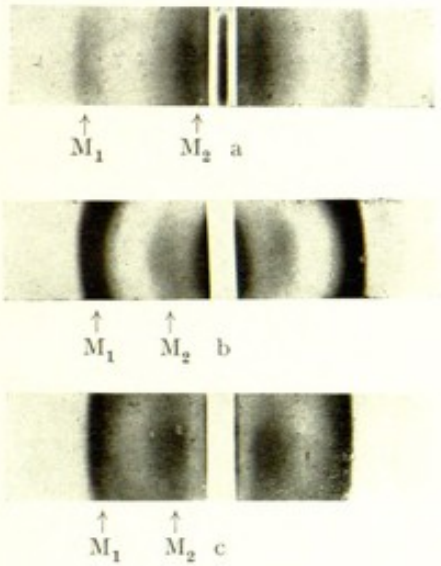

Fig. 3. Low-angle photographs showing differences in micelle bands given by three samples of nucleic acid.
a. SIGNER AND SCHWANDER, fresh and unstirred. Volume concentration 0.182 (33% wt/vol); b. BUTLER AND SMITH. Volume concentration 0.250 (45% wt/vol); c. Commercial (BDH). Volume concentration 0.274 (50% wt/vol) CuKα radiation, Ni filter, in vacuum camera. (a) at 30 cm (b) and (c) at 25 cm.

3a is a photograph of an unstirred SIGNER specimen of volume concentration 0.182. The inner band M_2 is markedly more intense than M_1.

3b is a similar photograph of a BUTLER specimen of volume concentration 0.250. The print has been over-exposed to bring up the weak and broad M_2 band. In the original, the outer band M_2 is sharper than appears here.

3c is a similar photograph of a commercial specimen of volume concentration 0.257. The inner band is markedly weaker and more diffuse than the outer.

THE MICELLE REGION

All three samples of nucleic acid give pronounced low-angle diffraction effects if the concentration is between certain limits and, although there are differences in detail, the general nature of the scattering is the same in each case. It consists of two well-separated bands of which the outermost, of lower spacing, is usually the sharper and more intense. The spacings of both bands increase as the systems are diluted but at different rates, the inner band shifting more rapidly than the outer. The sharpness and intensity of the bands also change with concentration.

As will be seen from Table II and Fig. 3, the SIGNER and SCHWANDER material gives photographs which are distinctly different from those given by the other two samples. The inner band M_2 is similar in definition and intensity to the outer band M_1 and, in the three more dilute specimens, is actually the stronger of the two. The commercial sample resembles that of BUTLER AND SMITH in that the outer band M_1 is always stronger and usually sharper than the inner, M_2. In all cases, dilution tends to broaden both M_1 and M_2. In the most dilute systems M_2 is no longer observed, and the region between M_1 and the central beam is filled in with a nearly uniform background. The more concentrated specimens prepared from the BUTLER material, and from the commercial sample, give an outer band M_1 which is unsymmetrical. Although quite sharp, it possesses a visible tail on the higher-spacing side; this effect is not so apparent with freshly-prepared SIGNER specimens. Despite these minor differences, the spacings corresponding to M_1 do not depend to any appreciable degree on the nature of the sample but only on the concentration of the specimen. The nature of the inner band M_2 is on the contrary greatly affected by both factors. Its spacing relative to concentration is considerably higher for the SIGNER sample than for the other two, and its intensity and definition are also more pronounced.

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TABLE II

DESCRIPTION OF THE LOW-ANGLE BANDS GIVEN BY MICELLAR SYSTEMS OF VARIOUS CONCENTRATIONS PREPARED FROM THREE DIFFERENT SAMPLES OF NUCLEIC ACID

Sample	Specimen	Volume Concentration c	Spacings in A.			Relative Intensities	Sharpness		Other Features
			Band M_1 d_1	Band M_2 d_2	Ratio d_2/d_1		Band M_1	Band M_2	
	1	0.351	21.0	77	3.67	$I_1 = 2I_2$	Quite sharp, symm.	Quite sharp, symm.	
	2a	0.236	27.0	102	3.78	$I_1 = I_2$	Quite sharp, symm.	Quite sharp, symm.	
	b		26.0	62	2.38				
SIGNER	3	0.182	30.5	116	3.80	$I_1 = 20I_2$	Sharp, unsymm.	Very broad	
AND	4	0.099	42.5	160	3.77	$I_2 = 4I_1$	Quite broad	Quite broad	
SCHWANDER	5	0.067	53.0	187	3.53	$I_2 = 6I_1$	Broad	Broad	
	6	0.032	73 (65, 82)			$I_2 = 10I_1$	Broad	Very broad	Background
	7	0.028	93 (80, 112)				Very broad	Not distinguishable	Strong background
	1	0.362	22.0	49 (42, 58)	2.23	$I_1 = 10I_2$	Quite sharp, symm.	Very broad	
	2	0.250	26.0	58 (48, 73)	2.23	$I_1 = 10I_2$	Sharp, unsymm.	Very broad	
BUTLER AND SMITH	3	0.243	27.5	64 (55, 77)	2.32	$I_1 = 10I_2$	Sharp, unsymm.,	Very broad	
	4	0.131	39.0	90	2.30	$I_1 = 5I_2$	Very broad	Very broad	Background
	5	0.069	50.5 (45, 57)	{118}	2.34	$I_1 = 2I_2$	Very broad	Very broad	Strong background
	1	0.257	28.0	70 (50, 103)	2.50	$I_1 = 6I_2$	Sharp, unsymm.	Very broad but well defined	Background
Commercial (BDH)	2	0.235	29	{115}		$I_1 = 2I_2$	Broad, unsymm.	Very broad	Background
	3	0.193	34	{115}		$I_1 = I_2$	Very broad	Very broad	Background
	4	0.226 (10% NaCl)	30	{115}		$I_2 = 4I_1$	Very broad	Very broad	

* Spacings in round brackets give the rough limits of the band. Curly brackets indicate that the value given is very approximate. d_1, I_1 and d_2, I_2 refer to the bands M_1 and M_2 respectively. Relative intensities are very approximate.

In three cases, the specimens were re-photographed after being kept untouched at room temperature for several weeks in their original sealed capillary tubes. The BUTLER samples (2 and 3) showed no significant differences after 8 weeks, whereas the SIGNER specimen (2) after 16 weeks gave a substantially altered photograph (see Table II). Whereas originally the inner band M_1 was about equal in intensity and sharpness to M_2, on keeping it had become much weaker and more diffuse. The spacing of M_1 had only slightly changed but that of M_2 was considerably lower than at first. In short, the diffraction effects were similar to those given by BUTLER AND SMITH specimens of about the same concentration.

The characteristics of the inner band are also dependent on the way in which a specimen is prepared. In general, the specimens were prepared without the use of any mechanical agitation, equilibrium being reached in a sealed tube merely by keeping for 2 to 3 days. If, however, gels of the SIGNER sample be made up by stirring in small containers and, when visibly homogeneous, sealed up in the usual tubes or in the vacuum-tight cell, the inner band is again weaker, more diffuse and of lower spacing. A typical specimen of this type gave spacings of 45 A. and 98 A. for M_1 and M_2; the ratio $d_2/d_1 = 2.15$ is substantially lower than in the unstirred specimens and resembles those found with the BUTLER samples.

One other isolated experiment needs to be mentioned. In an attempt to see whether any very weak bands were escaping observation, a very over-exposed photograph was taken of a weak gel (stirred-up SIGNER sample). The result is reported in Table I, column 7, and it will be seen that a third very weak band was present. The concentration of the specimen can be roughly gauged from the spacing of M_1 (35.8 A.) and is 0.15. The ratio of the spacing of M_1 to that of the new band is 1.69. There is a rough correspondence to the ratio, $\sqrt{3}$, of the spacings of the 10 and 11 planes in a two-dimensional hexagonal array.

STRUCTURE OF THE MICELLAR SYSTEMS

In the discussion which follows, it will be taken that BRAGG's law holds for the spacings of the low-angle bands. We have elsewhere[13,14] given theoretical arguments in favour of this supposition in certain cases. In the nucleic acid systems, the fact that strongly birefringent domains are visible in transparent specimens under static conditions argues a high degree of crystallinity and strengthens the BRAGG law assumption.

In order to illustrate the way in which the spacings d_1 and d_2 of the bands depend on concentration, the relevant data of Table II have been plotted on a double logarithmic scale (Fig. 4). The main features of the graph are

a. the points corresponding to d_1 all lie on or near the same straight line (C), no matter which sample is considered or how the specimen was prepared

b. the points corresponding to d_2 lie on one of two straight lines (A or B) according to the source of the nucleic acid sample and the method of preparation of the specimen

c. all three lines are parallel and have a slope of $-\frac{1}{2}$.

This value for the slope of the plots means that in each series the spacings are inversely proportional to the square root of the concentration. The expansion on dilution is therefore two-dimensional in each case and consequently neither spacing can relate to a simple repeat distance along the molecular axis.

The observation that d_1 is dependent only on the concentration of the specimen

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and not, or only slightly, on the source of the nucleic acid or the way in which the specimen is made up, suggests that it is related to some fundamental dimension in the molecule itself. All three samples, when made up into specimens of the same concentration, will give approximately the same value for d_1. The most probable explanation of these facts is that d_1 is simply related to the distance of separation s of neighbouring molecules and therefore to the molecular diameter σ.

The larger spacing d_2 derives from a further periodicity which is also subject to expansion in two dimensions. It is much more marked in the SIGNER specimens (when unstirred and fresh) than in the others. The SIGNER specimens clearly have a relatively very ordered structure. The larger periodicity is less perfect in the other specimens, and

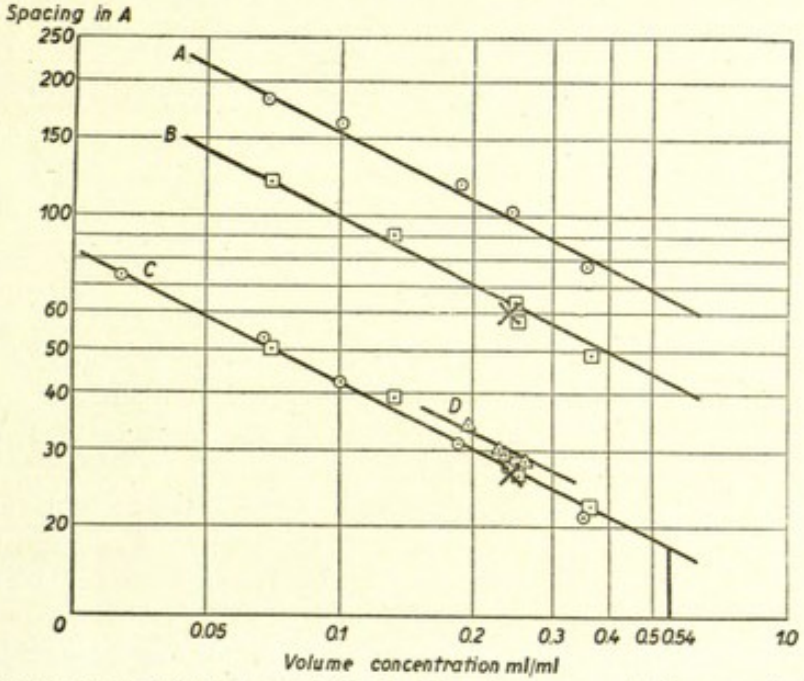

Fig. 4. Plots of the spacings d of the two micelle bands against concentration on a double logarithmic scale.

o SIGNER AND SCHWANDER specimens (fresh and unstirred) (d_2 on line A, d_1 on line C)
□ BUTLER AND SMITH specimens (d_2 on line B, d_1 on line C)
△ Commercial (BDH) specimens (d_1 on line D)
× SIGNER AND SCHWANDER specimen, after several months.

its mean value is lower. It is probably related to the micelle diameter δ but other hypotheses will first be discussed.

At first sight, the two spacings d_1 and d_2 might derive from two separate phases and to two different nucleic acid molecules. With complex macromolecules, this duality is not an impossibility but the hypothesis can be excluded by a simple argument. Both spacings are observed to be proportional to $1/\sqrt{c}$ where c is the measured concentration of the whole system. In any two phase system, each spacing would vary as $1/\sqrt{c}$ only if c were expressed as the concentration in the phase concerned. It follows that the variation of the two spacings must be different aspects of one phenomenon. The expansion of d_2 must be a consequence of the increase in d_1 on dilution and not a separate effect. Only two hypotheses meet the situation. One is that of micelle formation, and the other, of regular molecular folding.

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The expansion of d_1 is, in the first case, intramicellar while that of d_2 is intermicellar. The micelles consist of regular bundles of long rod-like molecules and are themselves arranged in a semi-regular 2-dimensional pattern. On addition of water the constituent molecules of the micelles move further apart and hence the micelles swell laterally. As a result of this intramicellar swelling, the centre-to-centre distance between neighbouring micelles shows a corresponding increase. d_1 and d_2 would thus always be in the same proportion at any stage of dilution, as observed experimentally.

The second hypothesis is that the long molecules are regularly coiled or folded in such a way that two-dimensional intramolecular swelling can take place as between different longitudinal sections. As a result, the overall lateral dimensions of the molecule increase and give rise to an increase in the intermolecular spacing d_2. The scheme is best exemplified by a regular cylindrical helix. Suppose the molecules in a concentrated specimen to be as in Fig. 5a. The longitudinal axis of the molecular chain (pictured as having a circular cross-section) is coiled into a regular helical form and there is an intramolecular periodicity d_1 corresponding to t, the distance between one coil and the next. The effective diameter of the molecule is not τ, the diameter of the chain, but σ, the diameter of the helix. There is therefore a further intermolecular periodicity d_2 corresponding to the distance of separation s of neighbouring helices in a regularly packed two-dimensional assembly. In order that the intramolecular swelling be two-dimensional, it is necessary that the diameter of the helix σ expand as well as the repeat distance along its axis t. The diametral expansion of σ causes a corresponding increase in s, and therefore in d_2 (Fig. 5b). This postulated intramolecular swelling is the equivalent of uncoiling the helix in a certain way. It is difficult to give an exact treatment of the dependence of d_1 and d_2 on concentration in such a case, as the various parameters defining the helix are unknown. In short, the helical hypothesis is a very flexible one, but, because of that, is difficult to use numerically. It is, in particular, difficult to employ an extrapolation procedure to derive τ and σ from the values of d_1 and d_2 over a range of concentrations. If this type of molecular model is correct, the interesting corollary follows that the molecule really possesses two diameters, τ and σ, which might be revealed differently by different physical methods.

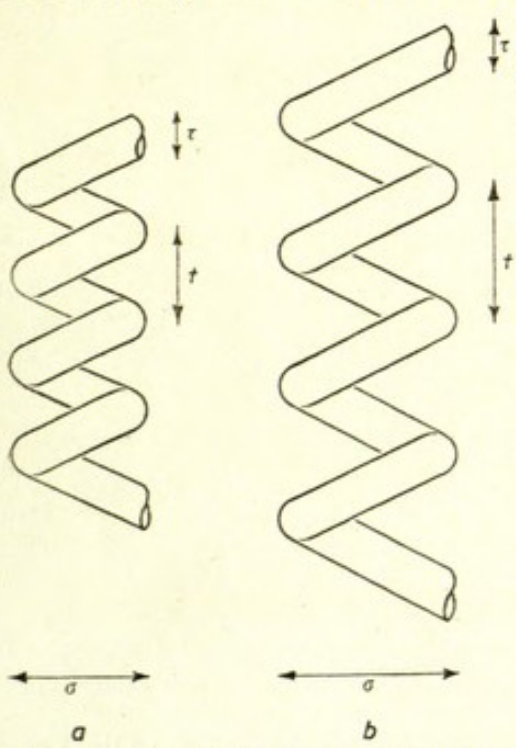

Fig. 5. Schematic picture of helical molecule at two stages of uncoiling on dilution. τ, true molecular (fibre) diameter; σ, effective molecular (helix) diameter; t, repeat distance along long axis of helix.

The alternative hypothesis of micelle formation has been subjected to detailed analysis in the case of the highly ordered SIGNER specimens. Two types of structure are possible and one may pass into the other on change of concentration. In one type, each micelle of diameter δ is free to rotate about its longitudinal axis; in the other, neighbouring micelles register one against the other and do not rotate. In both types, the molecules are free to rotate about their long axes. That they do so rotate, or at least

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oscillate, is suggested by the disappearance of the high spacing lines of the crystalline material when wetted. It seems reasonable to suppose, from the repeated wetting and drying experiments described earlier, that some components of the 10.9 A. and 15.7 A. composite bands are the spacings of vertical sets of planes defined by the precise location and orientation of molecules in the crystalline case. On addition of water, the molecules start to rotate and the perfection of the crystalline structure is destroyed. A para-crystalline system results in which the molecules can be approximated by cylinders of rotation and which therefore gives rise to a single intermolecular diffraction band in place of the richer crystalline line spectrum. Extrapolation of d_1 to a volume concentration of unity will give a rough value for the molecular diameter. A more accurate value can only result from an extrapolation procedure which takes into account the actual structure of the micellar systems. In Fig. 4, the value of d_1 extrapolated to $c = 1$ is 13 A. and this figure may be taken as a lower limit for σ, the diameter of the unhydrated molecule. Another value is given by the lowest observed value for d_1, which is *ca* 19 A. at $c = 0.44$. On further drying, the band disappears altogether and the lines characteristic of the crystalline state appear. This value of d_1 is presumably related to the diameter of the unhydrated molecule in a way discussed in detail below. Further, from Table II, it will be noted that at any concentration $d_2 = 3.7\ d_1$, approximately.

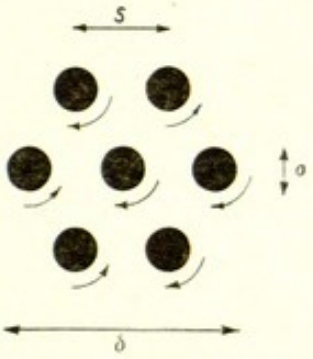

Fig. 6. Hexagonal 7-molecule micelle. Rotation of molecules indicated by arrows. σ, molecular diameter; δ micelle diameter; s, distance separating centres of neighbouring molecules.

A structure therefore needs to be proposed in which the micellar diameter δ' in the hypothetical dry state, is roughly three times the molecular diameter σ. The micelles are probably roughly cylindrical in shape and Fig. 6 shows the obvious model to adopt. Six cylindrical molecules are grouped hexagonally around a central one and the whole structure expands laterally on addition of water.

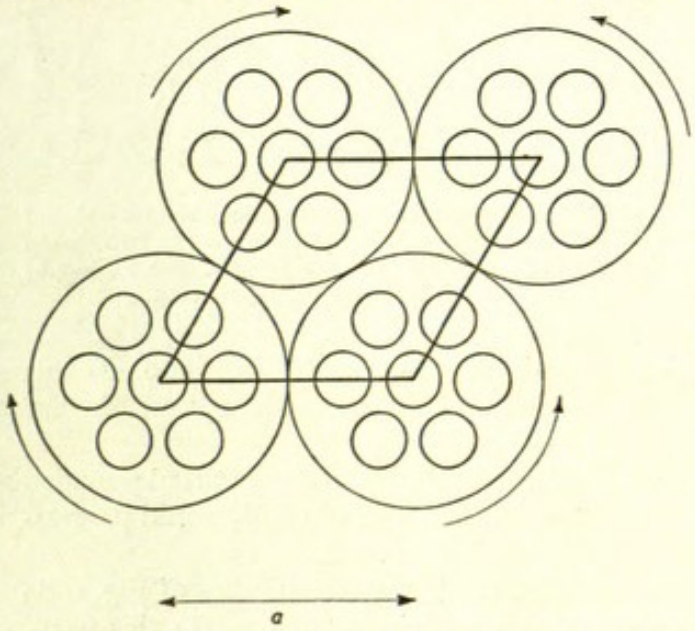

Fig. 7. Structure consisting of freely-rotating micelles in a regular hexagonal array. Rotation of micelles depicted by arrows. a, unit-cell edge.

The next step is to consider ways in which these 7-molecule micelles can be arranged in 2-dimensional patterns and any proposed structure must explain why d_2 can be more intense than d_1 in the SIGNER specimens. It must also satisfy the concentration conditions for micelle formation, as the typical bands do not appear at concentrations above about 0.44.

As mentioned above, one possibility is that the micelles are free to rotate. The depiction of this scheme in two dimensions is given in Fig. 7, which roughly reproduces the concentration conditions in the

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concentrated SIGNER specimens. If the micelles are placed in a regular hexagonal 2-dimensional array, the spacing d_2 would be pronounced, as it would correspond to the 10 planes of the statistical unit-cell of edge a outlined in the drawing. The spacing d_1, on the other hand, could not be crystallographic but would arise from purely intra-micellar interferences. As regards d_1, the structure consists of micelles which are independent scatterers, there being no coherence between radiation scattered by one micelle and the next. The problem of evaluating the intensity of scattering by assemblies of parallel cylindrical molecules, assumed to be of infinite length, has been treated by us elsewhere[14]. The normalized intensity (*i.e.* per molecule) of radiation scattered by the 7-rod micelle of Fig. 5 is

$$I \propto \frac{1}{49}\left[\frac{2J_1(kR)}{kR}\right]^2\left[7 + 24J_0(x) + 6J_0(2x) + 12J_0(\sqrt{3}x)\right] \qquad (1)$$

where $J_0(z)$ and $J_1(z)$ are the Bessel functions of order zero and unity respectively, and $k = \frac{4\pi\sin\Theta}{\lambda}$, where λ is the X-ray wavelength and 2Θ the scattering angle. $x = ks = 2\gamma kR$, where $R = \frac{1}{2}\sigma$ is the radius of the cylindrical molecules and $\gamma = s/\sigma$ is a factor introduced to make the expression apply to micelles of any degree of lateral dilution-expansion. Fig. 8 gives curves of I plotted against the dimensionless variable ks for two values of γ. It is evident that the diffraction bands are more pronounced the larger the gap between the molecules; at the same time, the maximum of the main band A moves towards a spacing corresponding to that of the 10 planes in a 2-dimensional hexagonal lattice of infinite extent. The subsidiary maximum B, which has no equivalent in the case of the infinite lattice, is a result of the small number of molecules in the micelle.

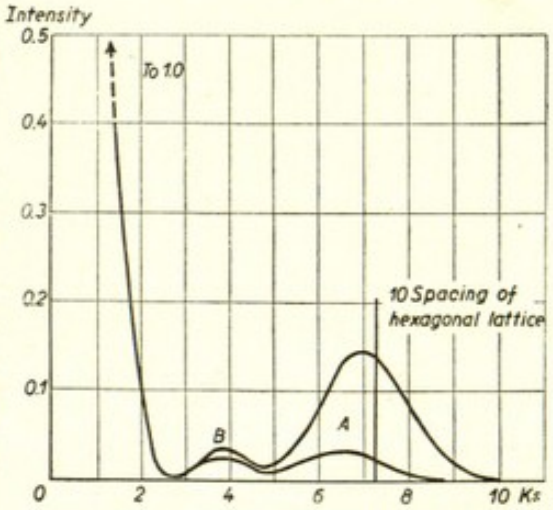

Fig. 8. Intensity curves calculated for isolated 7-molecule micelles (see Fig. 6) at two stages of dilution-expansion. Upper curve, $s/\sigma = 2.0$; lower curve, $s/\sigma = 1.25$.

While a structure of freely-rotating micelles may apply in dilute solutions, we exclude it in the case of the concentrated systems for the following reasons:

a. the observed band d_1 appears to be sharper than the band A in Fig. 8. On this hypothesis the band is most diffuse in the concentrated systems—the reverse of what is observed;

b. on dilution, the spacing of the band A decreases relative to the standard provided by the intermicellar spacing d_2. Thus, on this hypothesis, the ratio d_2/d_1 would progressively increase whereas it is observed to remain sensibly constant.

We need now to consider the hypothesis that the hexagonal micelles of Fig. 6 do not rotate but are arranged in a quasi-regular 2-dimensional structure. On this basis, the problem reduces to one of solving a 2-dimensional crystal structure which is partly disordered.

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The procedure adopted was to calculate the structure amplitudes F_{hk} of the principal sets of planes hk for a large number of structural models. Micelles other than that shown in Fig. 6 were tried in this work and a variety of structural types involving them were tested.

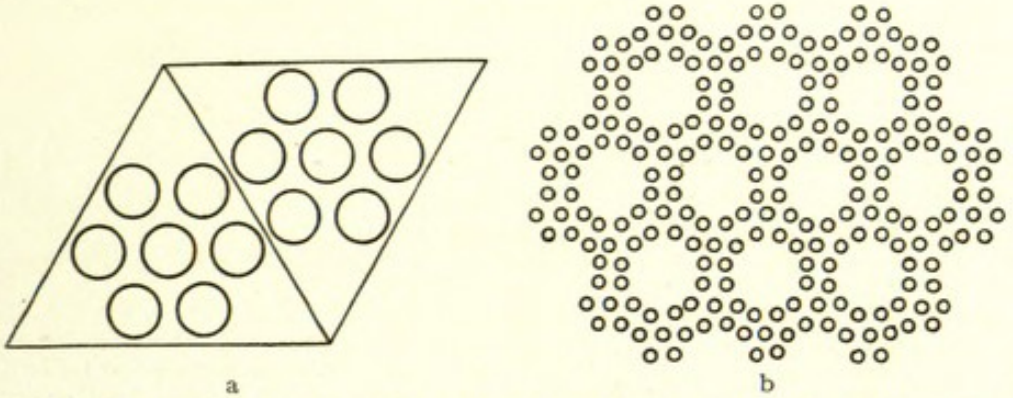
If the system can be considered as a two-dimensional crystal, the intensity of scattering by any set of planes hk is given by

$$I_{hk} \propto f^2 \mid F_{hk} \mid^2 \tag{2}$$

where f is the molecular scattering factor for cylinders, $2J_1(kR)/kR$, given in equation (1). We have considered only centro-symmetrical structures, in which case F is no longer complex and may be expressed as

$$F = \sum \cos 2\pi (hx + ky) \tag{3}$$

where x and y represent the positions in the unit-cell of the different molecules, expressed as fractions of the unit-cell edges.



a b

Fig. 9. Micellar structure proposed for fresh, unstirred SIGNER AND SCHWANDER specimens. (a) detail of hexagonal unit-cell containing 2 7-molecule micelles, for a concentrated specimen (b) general picture of structure showing honeycomb pattern with regularly disposed holes.

In the crystalline case, both d_1 and d_2 must refer to the spacings of crystallographic sets of planes or, when there is some disorder, to unresolved combinations. Owing to the rapid way in which the factor f^2 diminishes with angle (*i.e.*, k), only planes of low indices need be considered and this is obviously even more true in partly disordered structures. It is probable that the averaged overall symmetry is hexagonal, in which case simple relations exist between the interplanar spacings d_{hk} and the a dimension of the hexagonal unit-cell. From the relations it is seen that the ratio d_{10}/d_{31} is 3.61, whereas the observed ratio d_2/d_1 is *ca* 3.7. The ratio d_{10}/d_{40} is, obviously, 4.0. If, as seems probable, d_2 corresponds to d_{10}, d_1 must correspond to d_{31} or to an unresolved combination of d_{31} and d_{40}. It is a simple matter to derive structures in which 31 and 40 are strong reflectors. In order to make 10 strong, any proposed structure must have substantial discontinuities on the scale of the unit-cell edge a, *i.e.*, the structure must be of an open honeycomb pattern. Fig. 9 shows the structure finally adopted for which Table III gives the calculated F values for planes of low indices. For convenience in calculation, the parameters defining the positions of the molecular centres were taken to have exactly the following fractional values

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$$\pm\left[\frac{4}{9}\,\frac{1}{9},\ \frac{2}{3}\,\frac{1}{9},\ \frac{4}{9}\,\frac{1}{3},\ \frac{2}{3}\,\frac{1}{3},\ \frac{1}{9}\,\frac{4}{9},\ \frac{1}{9}\,\frac{2}{3},\ \frac{1}{3}\,\frac{4}{9}\right]$$

which makes the intermicellar gap slightly less than that between molecules within a micelle, whereas in the proposed structure, as drawn in Fig. 9, these two gaps are equal. Table III also gives the relative multiplicity factor p, *i.e.* the relative frequency of occurrence of reflecting planes related by symmetry. The theoretically estimated intensity is therefore pF^2 multiplied by the appropriate value of f^2 for the particular angle of reflection. As the influence of f^2 will vary with the degree of dilution-expansion, we have preferred to plot pF^2 only in exact form. Fig. 10 shows lines of length proportional to pF^2 drawn at positions given by the reciprocal of their spacings. This method of presentation is the pictorial equivalent of an actual low-angle X-ray photograph. The smooth curve shows roughly the effect of amalgamating the broadened lines which would be the result of structural disorder, and also allows for the falling-off effect

TABLE III

GEOMETRICAL STRUCTURE FACTORS F_{hk} CALCULATED FOR THE MODEL SHOWN IN FIG. 9 WHEN ADJUSTED TO A $\frac{1}{9}:\frac{1}{9}$ NET, TOGETHER WITH THE CORRESPONDING RELATIVE MULTIPLICITY FACTORS p

hk	F	p	pF^2
10	−3.68	1	13.5
20	+0.76	1	0.6
30	+2.00	1	4.0
40	−6.08	1	37.0
11	−0.40	1	0.2
22	−2.44	1	6.0
21	+1.54	2	5.7
31	−1.88	2	7.1

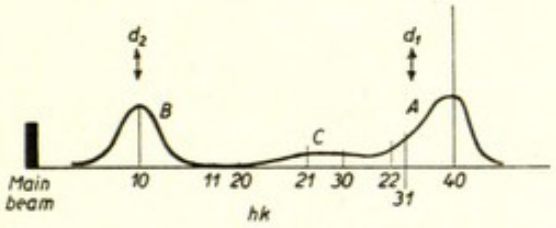

Fig. 10. pF^2 values (vertical lines) for the low-order hk planes plotted against $1/d$ for the structure drawn in Fig. 6. Smooth curve gives rough estimate of intensity distribution to be expected in a real specimen. Arrows indicate observed positions of diffraction bands.

of the f^2 factor. It will be seen that the diffraction consists of the two main bands A and B connected by a region of lower intensity, the plateau C. If B corresponds to d_2, the observed mean position of d_1 is shown by arrows. The position and intensity of the composite maximum A is thus in reasonable accord with experiment. Some of the SIGNER specimens do, in fact, show a slight tail on the low-angle side of the outer band as would be expected from Fig. 10 (see Fig. 3a). In the proposed structure, as stated above, the water-gap between the "contact" sides of the micelles has been taken to be the same as that between molecules within a micelle. On dilution, the whole structure expands uniformly in two dimensions and the ratio d_2/d_1 remains constant. At lower concentrations, however, the structure will become progressively more disordered and the diffraction pattern correspondingly more diffuse.

This suggested structure has been derived from purely crystallographic reasoning. It remains to be seen whether it satisfies the concentration conditions observed in practice. The volume concentration of the structure drawn in Fig. 9, assuming infinitely long cylindrical molecules, is 0.54 $(\sigma/s)^2$. At molecular contact ($\sigma = s$), therefore, the volume concentration would still be quite low, 0.54. The extrapolated value of d_1 at

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this limiting concentration is 18 A. which compares reasonably well with the lowest observed value, 19 A., which must embrace a small water-gap.

We are now in a position to derive a figure for the unhydrated molecular diameter σ and to compare it with the limiting values discussed earlier. The values of the relevant interplanar spacings d_{kk} in terms of the intermolecular distances s are

$$\left.\begin{aligned} d_{10} &= 4.20\ s \quad \ldots\ldots d_2 \\ d_{31} &= 1.17\ s \\ d_{41} &= 1.05\ s \end{aligned}\right\} \ldots\ldots d_1$$

As d_1 is composite, it is perhaps more satisfactory to work from the d_2 relation and employ the known ratio d_2/d_1 to derive the factor connecting d_1 and s. In this way, we find that $d_1 = 1.14\ s$. It follows, from the extrapolated-to-dryness value of 18 A. for d_1, that the unhydrated molecular diameter σ is 16 A. The diameter of the dry micelle δ' is therefore approximately 3 times this figure, which agrees with the value of *ca* 50 A. observed in the electron microscope.

The structure of the micellar systems prepared from the other two samples of nucleic acid (BUTLER AND SMITH; BDH) is less definite. Both samples give strong and sharp d_1 bands whose spacings, as a function of concentration, are almost identical with those given by the SIGNER specimens. The inner d_2 bands, on the other hand, are weak and very diffuse. In the case of the commercial sample, it is difficult to assign a significant mean spacing to the d_2 band. The long-range order in these specimens must therefore be considerably less than that existing in the SIGNER systems.

In the case of the commercial sample, it is probable that there is only a small tendency to micelle formation. This is shown, first of all, by the extreme lack of definition of the inner band. In the most concentrated specimen, d_1 is, on the contrary, very sharp and intense. This would be in accord with a simple hexagonal arrangement of molecules uniformly in two-dimensions. In such a structure, the volume concentration $c = \frac{\pi}{2\sqrt{3}}\left(\frac{\sigma}{s}\right)^2$ and s is now given by the crystallographic relation $\frac{2}{\sqrt{3}}\ d_1$ for 10 planes in a simple hexagonal array. Substitution of the known values for d_1 and σ into these expressions ($d_1 = 28$ A., $\sigma = 16$ A.) gives $c = 0.228$, which, when compared with the measured value of 0.257, suggests only slight micelle formation. However, a numerical argument cannot be pushed too far because of the effect of small amounts of impurity or of degraded molecules of nucleic acid which are probably present. The fact that the values for d_1 for this sample lie above the others in Fig. 4 is in agreement with only slight micelle formation (line D compared with line C).

The position of the BUTLER AND SMITH specimens is intermediate. There is certainly a tendency to micelle formation as the inner band, although very broad and weak, nevertheless exists and moves rationally with change of concentration. The mean spacing of this band is considerably less than in equivalent SIGNER specimens. There is also the significant observation that SIGNER preparations, on stirring or keeping, give diffraction patterns which are practically identical with those given by the BUTLER samples. This is strong evidence that the highly organized SIGNER systems are formed only under exceptional conditions and are easily transformed into the more disordered BUTLER structures.

The essential difference between the two types would appear to be the occurrence

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of large and regularly disposed water gaps in the SIGNER structures. Only by having an open honeycomb pattern as shown in Fig. 9 is it possible to make d_2 sufficiently intense and well defined. It is obvious that this band will be less intense for any structure in which the molecules tend to fill space uniformly and, in the limiting case of a simple hexagonal network, it will not occur at all. In an attempt to elucidate the nature of the BUTLER specimens, we have considered possible micellar structures of intermediate density (Fig. 11), While the general trend of the pF^2 plots (*cf.* Fig. 10) is in the right direction, in no single instance is it possible to arrive at close agreement with experiment. It would therefore appear that the real structure is a semi-ordered mixture of micellar structures of the sort shown in Fig. 9 and 11. An important result of the calculations is that the intense band, corresponding to the d_1 spacing, does not change greatly in position under equivalent concentration conditions.

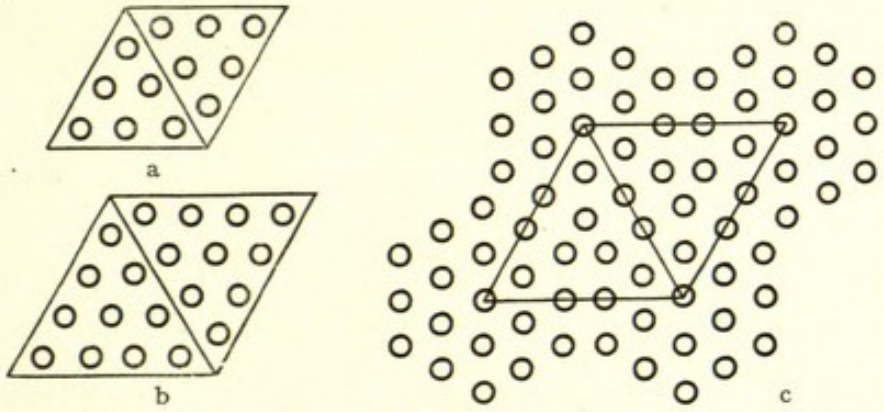

Fig. 11. Some possible micellar structures considered for the less-ordered systems.

GENERAL CONCLUSIONS

Our X-ray investigations have shown that desoxyribonucleic acid in solution is a system of considerable complexity. Fortunately, since the samples show relatively large crystalline domains, we are justified in applying crystallographic principles to the treatment of the data.

The nature of the variation of the two large spacings with concentration suggests immediately a two-dimensional swelling. This swelling is, however, of a higher degree of complexity than that reported for tobacco mosaic virus solutions by BERNAL AND FANKUCHEN[15] where the molecules simply fill space uniformly. In Fig. 9 is given a possible structural model of a nucleic acid gel which is consistent with the observed data for the sample of highest molecular-weight. The more degraded samples of nucleic acid in solution exhibit less long-range order, indicating that the proposed honeycomb structure is critically dependent on the length of the fibres. However, with all three samples studied, small local micelles of the fibrous molecules are always present except at extreme dilutions. Apparently the regular honey-comb structure is not very rigid since it can be broken down by mere mechanical agitation. This may help to explain the unusual mechanical properties of nucleic acid gels.

We cannot at the present time attempt to explain in rigorous fashion the nature of the forces acting between nucleic acid molecules in the manner applied in the case of tobacco mosaic virus[16]. What are lacking are thermodynamic measurements made

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on concentrated solutions of nucleic acid in the presence of varying amounts of salts, in order to ascertain the role of electrostatic forces.

From consideration of the micellar systems, we arrived above at a value of 16 A. for the diameter of the unhydrated molecule. In fact, the molecule is unlikely to be precisely cylindrical and this figure will more exactly have reference to its largest lateral dimension. It is perhaps significant that the value is close to the largest crystalline spacing of 16.5 A. This spacing is probably the same as ASTBURY's 16.2 A. side-spacing and may be defined, as was suggested earlier, by the side-by-side packing of molecules along one direction. From the perfection of the diffraction pattern given by the crystalline specimen, it is clear that some hydrate of nucleic acid is formed when a critical minimum amount of water is added corresponding to a volume concentration of 0.44 (80% wt/vol). Vapour pressure measurements of this hydrate might prove instructive.

The fact that the main low-spacing diffraction regions persist, albeit sometimes blurred, in all the specimens examined, wet or dry, is strong evidence that the nucleic acid molecule is very rigid. The internal structure of the molecule must possess pronounced regularity for its principal features are still detectable in a dilute wet gel ($c = 0.15$ or 3% wt/vol approximately) as is shown in column 8 of Table I. In a dilute sample such as this, each molecule is an independent scatterer as regards its internal periodicities in structure.

We should not like, from our data, to draw any positive conclusions regarding the detail of the internal structure of the molecule. Our results, in the main, are nevertheless not inconsistent with ASTBURY's general picture of the nucleic acid structure. In his view, the molecule consists of a long column of flat nucleotide discs piled vertically one above another, each nucleotide having linear dimensions 15 A. × 7.5 A. approximately, and being joined to its neighbours through phosphoric acid links. Astbury places great emphasis on the meridional 3.34 A. spacing he observes and assigns it to the distance separating neighbouring parallel nucleotide discs along the fibre axis. Our results, as summarised in Table I, place less emphasis on this spacing. A marked spacing at 3.25 A. occurs in the air-dry fibres, but in the fully-ordered crystalline specimens there is no uniquely outstanding spacing in this region. There is a line actually at 3.34 A. but there are several others, notably at 3.15 A. and at 4.04 A., which are as strong or stronger. Further, we have discovered no evidence of the suggested true repeat distance along the fibre axis of 27 A. or of a still higher multiple of the 3.34 A. spacing (16 times) as later stated by ASTBURY[12]. We have examined the high-spacing region carefully and have observed no diffraction lines for the crystalline material with spacings greater than 16.5 A.

It is clear from the data presented in Table I, columns 4 and 5, that the unit-cell cannot be very large even if some of the lines are second-order reflections with the first-order absent for reasons of symmetry. Some of the lines can be tentatively indexed on the basis of an orthorhombic cell but we have not pursued the matter. Comparison of our crystalline data with ASTBURY's should not be carried too far as his specimens were prepared in a quite different way. Nevertheless, our crystalline specimens contain undegraded nucleic acid molecules in a nearly natural condition and the data should reflect some of the principal characteristics of their structure. A detailed analysis of the molecular structure of nucleic acid will require, in addition to the results presented here, a thorough examination of the diffraction spots obtainable from highly oriented specimens.

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SUMMARY

Aqueous systems of desoxyribonucleic acid (Na salt), over a range of concentrations from about 0.03 to 1.8 g/ml (2% to 100% by volume) have been submitted to X-ray diffraction analysis. The structure of the specimens is markedly dependent on the quantity of water present and on the method of preparation. Over a narrow concentration range, the material appears to be really crystalline and, on further dilution, the systems are paracrystalline over a wide range. A detailed analysis of the micellar structures is given for the paracrystalline specimens. These structures are more complex than in the case of tobacco mosaic virus, but also exhibit two-dimensional regularity and swell on dilution. The molecular fibre itself is rigid and has a diameter of about 16 A. The most-favoured micelle consists of 7 molecules in a centred hexagonal arrangement. In the most ordered micellar systems, the structure possesses regularly disposed holes which produce an open honeycomb effect.

RÉSUMÉ

Des systèmes aqueux d'acide desoxyribonucléique (sel de sodium), de concentrations comprises entre 0.03 et 1.8 g/ml (2% à 100% en volume) ont été soumis à l'analyse par diffraction des rayons-X. La structure du spécimen dépend considérablement de la quantité d'eau présente et de la méthode de préparation. Dans un domaine de concentration restreint la matière se montre réellement crystalline; lorsque la dilution augmente, les systèmes sont paracrystallins dans un large domaine. Les auteurs donnent une analyse détaillée des structures micellaires des spécimens paracrystallins. Ces structures sont plus complexes que dans le cas du virus de la mosaïque du tabac, mais elles montrent aussi une régularité à deux dimensions et elles gonflent à la dilution. La fibre moléculaire elle-même est rigide; son diamètre est de 16 A environ. La micelle la plus favorisée consiste en 7 molécules en disposition hexagonale centrée. Les systèmes micellaires les plus ordonnés possèdent des trous disposés régulièrement qui produisent un effet de rayon de miel ouvert.

ZUSAMMENFASSUNG

Wässrige Systeme von Desoxyribonukleinsäure (Natriumsalz), in einem Konzentrationsbereich von ungefähr 0.03 bis 1.8 g/ml (2 bis 100 Volumprozent) wurden der X-Strahlendiffraktions-Analyse unterworfen. Die Struktur der Proben ist deutlich abhängig von der Wassermenge und der Herstellungsmethode. In einem kleinen Konzentrationsbereich erscheint das Material wirklich kristallin; bei weiterer Verdünnung sind die Systeme in einem grossen Bereich parakristallin. Für die parakristallinen Proben wird eine ausführliche Analyse der Mizellenstrukturen dargelegt. Diese Strukturen sind komplexer als im Falle des Tabakmosaikvirus, aber sie zeigen auch eine zweidimensionale Regelmässigkeit und schwellen bei Verdünnung. Die molekulare Faser selbst ist steif und hat einen Durchmesser von ungefähr 16 A. Die meist bevorzugte Mizelle besteht aus 7 Molekülen in hexogonal zentrierter Anordnung. Die Struktur der regelmässigsten Mizellensysteme enthält regelmässig angeordnete Löcher, welche an eine offene Honigwabe erinnern.

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Received June 18, 1951

	mm	From chart $\frac{1}{3}$	$\frac{1}{5}$
Equator	9·5	0	·16
	14·5		·235
	19·6		~~·3~~·32
	24·5		·40
	(35·5)		(·58)
1st long line	5·3	·63	09
	9·4		·16
	10·8		·18
2nd " "	5·0	·125	·085
	6·2		·11 ~~·185~~
	10·7		·18
	12·5		·205
	14·5		·24
	18·8		·31
	19·9		·33
4th	16·0	·25	·235
	18·0		·285
5th	12·5	~~·375~~ ·315	·20
6th	10·5 11·5	~~·45~~ ·375	~~·25~~ ·21
7th	10·0 11·5	·45	~~·285~~ ·23
8th	9·5	·52	~~·30~~ ·25

Observed Spots

θ		2x cms	d	Intensity	2x cms.	$\lambda_{K\dot{\alpha}_{12}}$ dÅ°
2·72	1	0·55		Weak	0·55	19·5Å°
4·54 ·0790	3	0·95		Very Strong	0·95	11·3
5·35 ·0932	4	1·12		" Very Weak	1·10	9·8
6·925 ·1206	5	1·45.		Fairly Strong.	1·45	7·4
8·12 ·1412	6	1·70.		Very Weak.	1·70 (5·72)	6·53
					1·83	5·88
9·31 ·1618	7	1·95		Strong	1·917	5·63
					1·99	5·40
10·18 ·1768	8	2·13		Weak.	2·15. 2·25	5·01 4·78
11·27 ·1954	9	2·36.		~~Fairly Strong~~ Weak	2·35	4·59
11·79 ·2042	10	2·47		Fairly Strong	2·47	4·37
12·32 ·2133	11	2·58		Weak.	2·59	4·17.

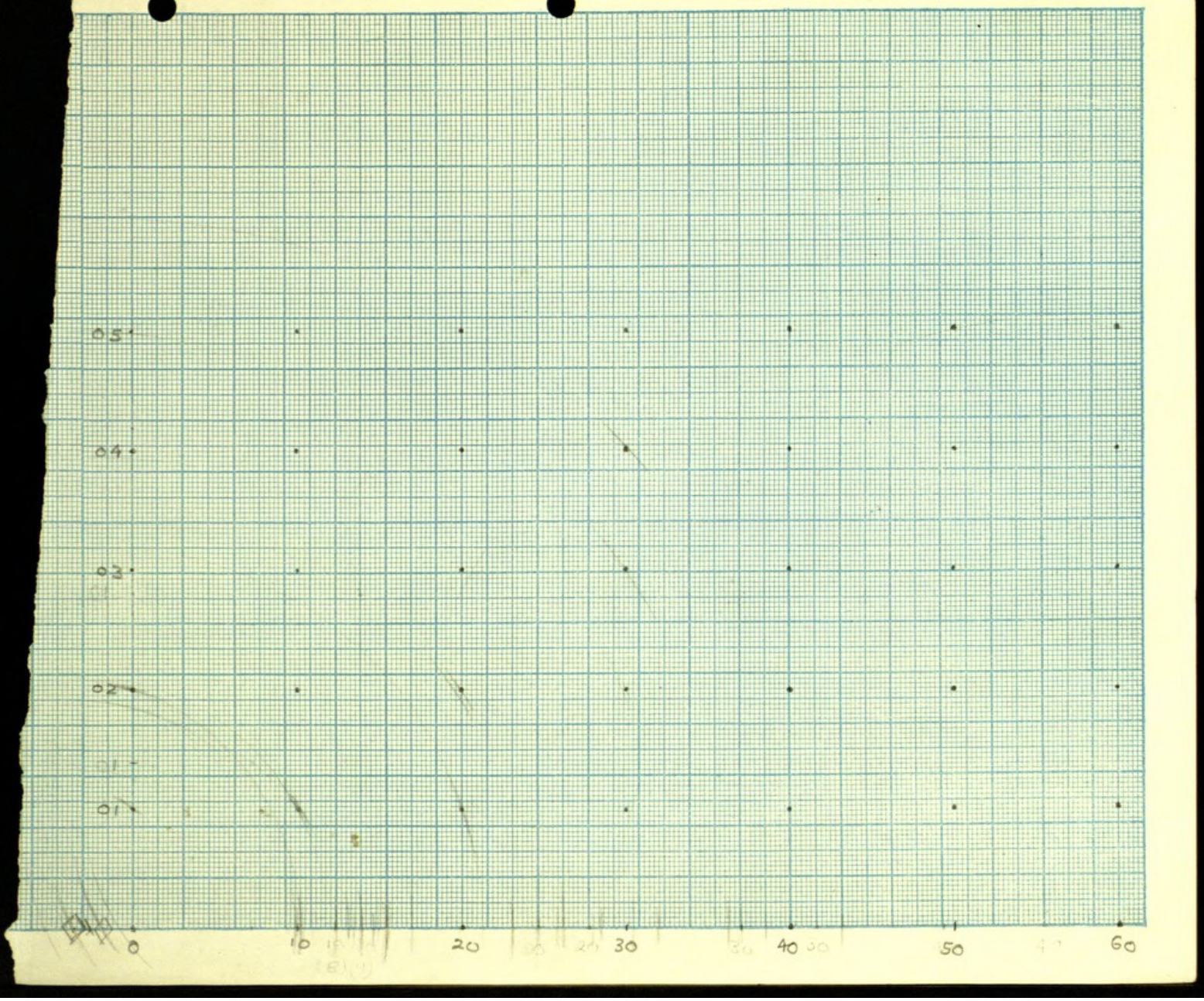
Camera radius 3 cm

∴ for $2l = 30\pi$ mm $\quad \theta = 45°$

1 mm in $2l$ = ~~·4775°~~ ·4775°

$$Q = \frac{1}{a^2} \cdot \frac{4}{3}(h^2+k^2+hk) = s^2 = \frac{1}{d^2}$$

$\varepsilon_{1150} = 6.5\ 10^{-2}$



Hexagonal close-packing

10	1
11	$\sqrt{3}$
20	2
21	$\sqrt{7} = 2.65$
22	$2\sqrt{3} = 3.46$
31	$\sqrt{13} = 3.61$
32	$\sqrt{19} = 4.36$

$\frac{5}{4}$ equatorial spots on 51c are fitted to this, they are:—

100	v v strong
110	v weak
(200	absent)
210	weak
320	strong but diffuse

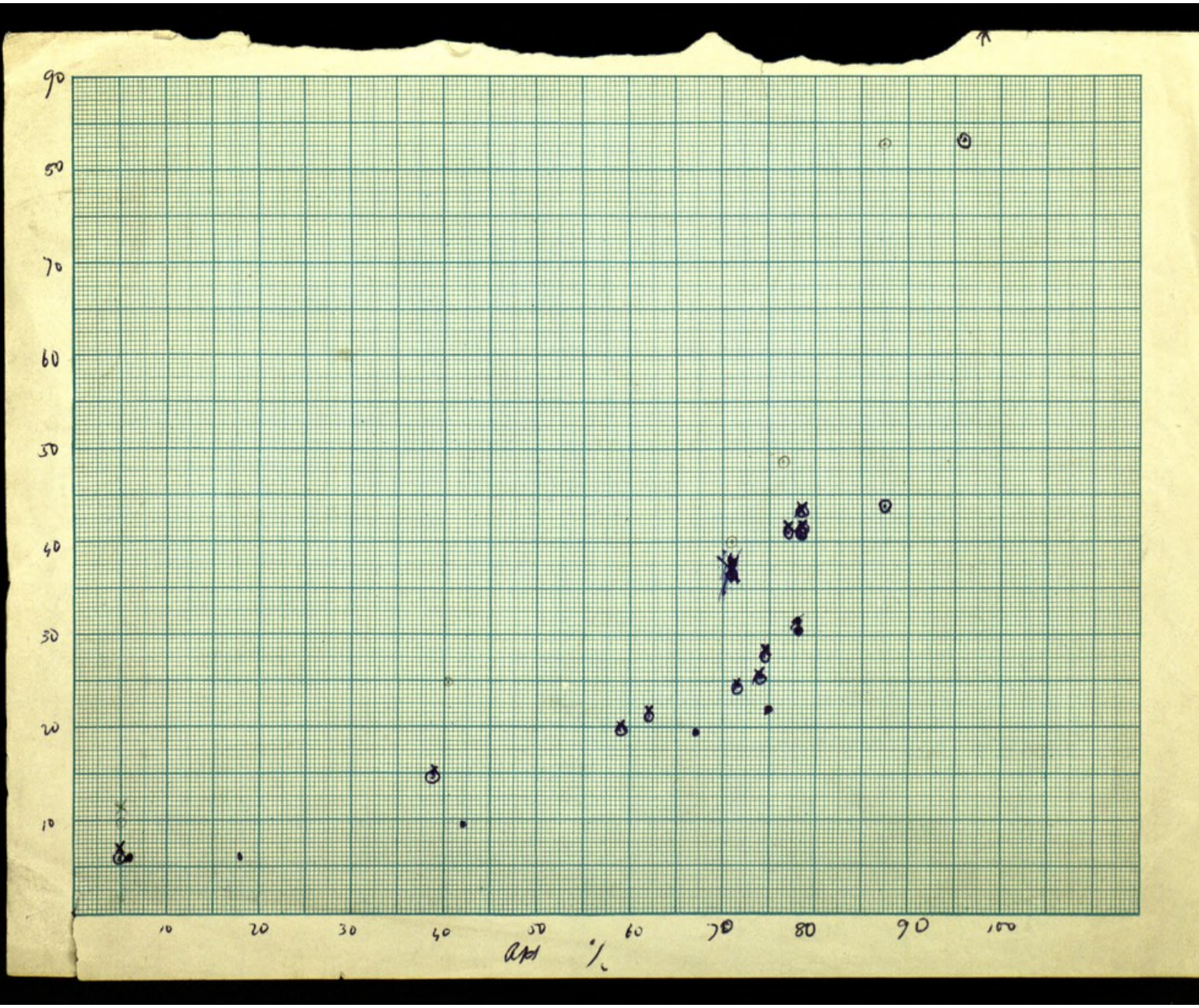
$\rightarrow$ 'a' : $\frac{2}{\sqrt{3}} \times 24 : 28A$

Cylindrical Patterson syntheses for the investigation of chosen directions in crystal structure analyses

Booth TFS 48 197 (1952)

Expressions not involving Bessel functions but requiring previous knowledge of Fiel indices

1cm : 5A


90
80
70
60
50
40
30
20
10
10
20
30
40
50
60
70
80
90
100

X-ray studies of Na thymonucleate fibres

III The 3-dimensional Patterson Function

~~Intensities~~ Intensities measured & corrected & reflections indexed as described in all & space group quoted.
in II. & Resulting data set out in Table

Table

Layer line	Measured I	I corr. for geom. factors	$I \times e^{-a^2 s^2}$	Indices	I used

Ambiguous indexing of outer reflections discussed in II. Where double wide photos (II plate I) gave indication of which more important, weighted accordingly, otherwise equally shared. Intensity of strong doublet in equator & 2nd layer-line were measured as one, & number weighted as indicated by special high-resol photos in wh. doublets shown resolved.

Patterson calculated . . . . a-b section - - - shown in Fig

Description of Patterson

Rel. single but prob. ∴ few terms
- discussion of effect of few terms on no. & rel. heights of peaks, esp. when many non-systematic absences

Outstanding features
- pseudo-helix, suggests part of molecule repeats ~ ½ c
- peaks on section 2
  → P-P distances?

Notes from

The Chemistry & Biology of Proteins – Felix Haurowitz

(Academic Press Inc. Publishers)

P. 224-5

Difficulty of distinguishing between true nucleo-protein & artefact formed by combⁿ of nucleic acid & protein

(suggested this latter is ppt'd by Ca salt, former not)

P. 334

Extraction of calf with molar NaCl gives DNA fraction,

mainly nucleo-protamines and nucleo-histones

(N.B. nuclei contain protamines or histones but not both)

RNA is not extracted by molar NaCl, & exists in form of true nucleo-protein

Probably RNA important in protein synthesis

P. 535

Throws doubt on question of DNA being involved in ~~chromosome~~ heredity

∴ structure of histones too simple

8μ slits tungsten pins w screw driver tips, 1mm depth

11m achieved by hand. Max. intensity w counter

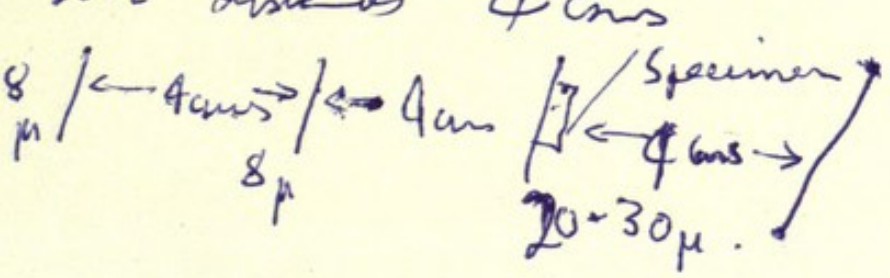
Slits and film on arms, can be taken out

of beam & re-placed & used to re-line up

during exposure

Run tube a 4 hrs before starting

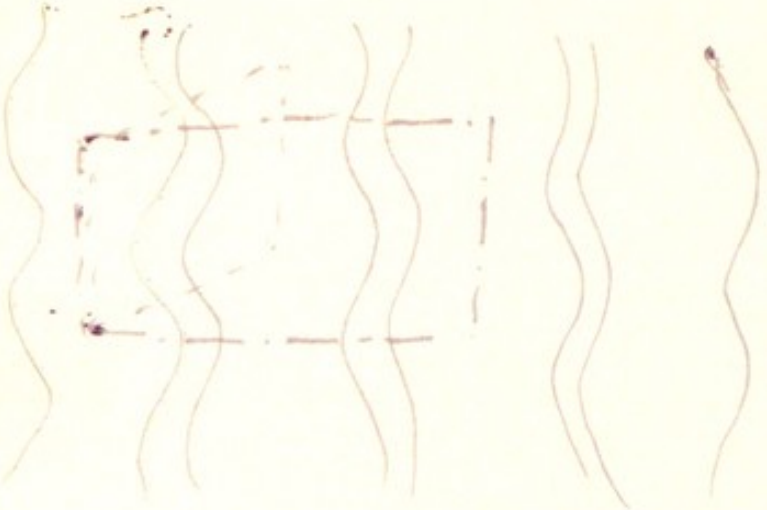
Slit distances 4 cms

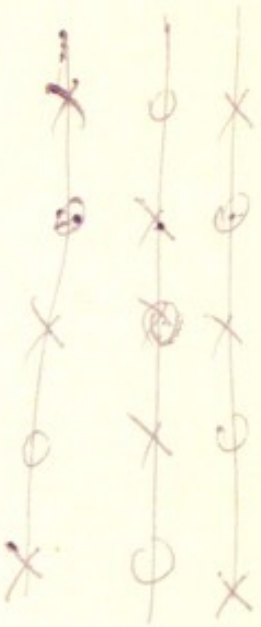


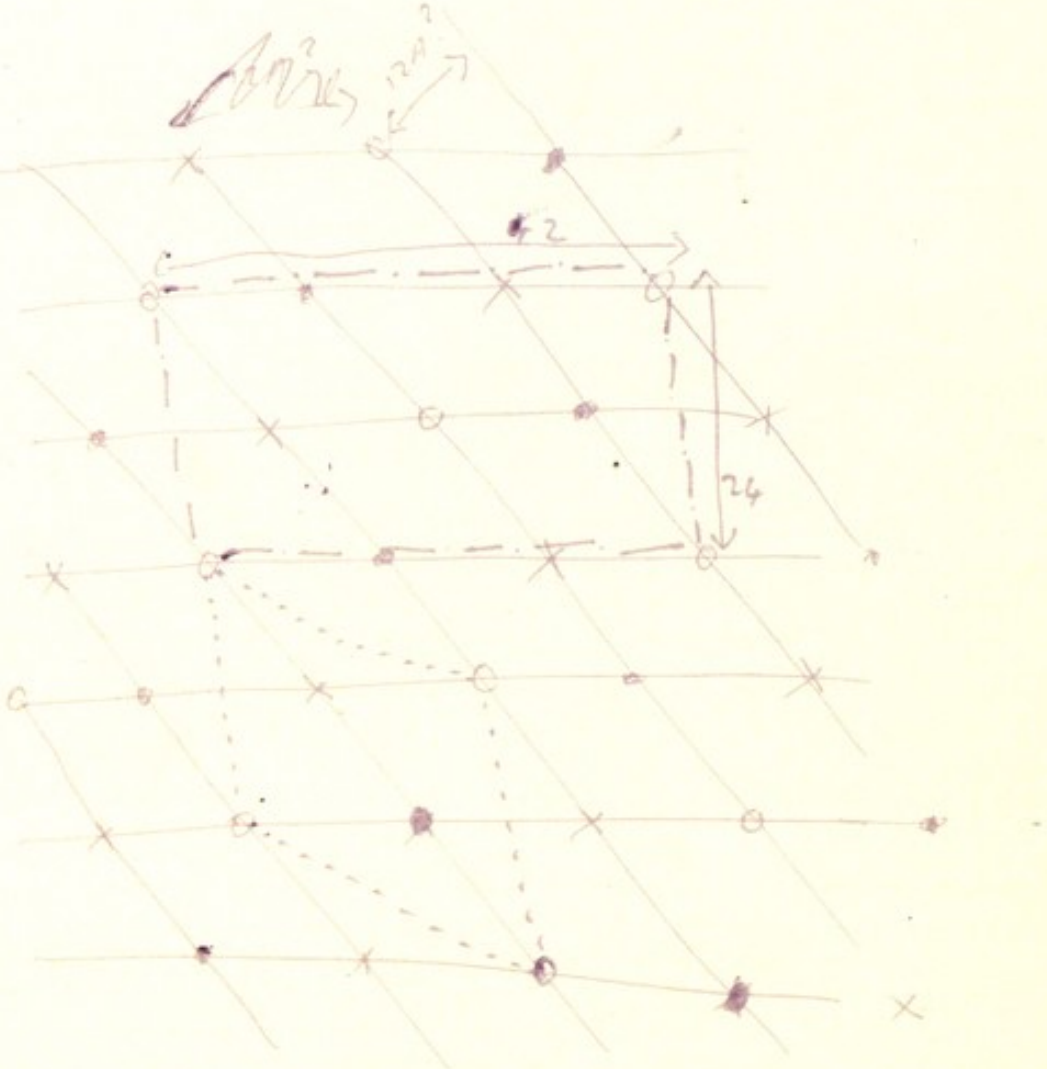
Windows AR Alclite - thermosetting resin

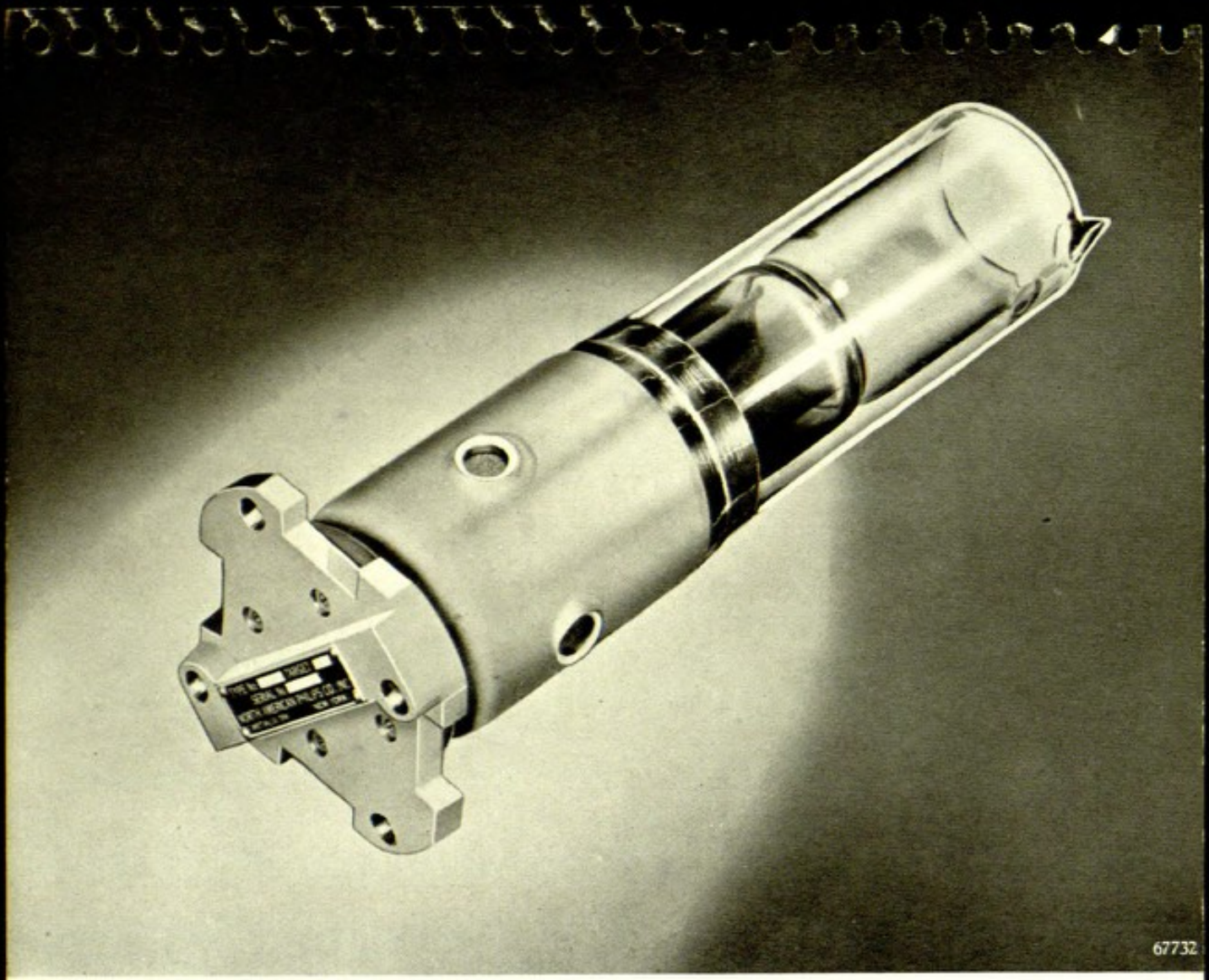
: wax softens

1mm-diameter specimen. 20hrs. exposure.

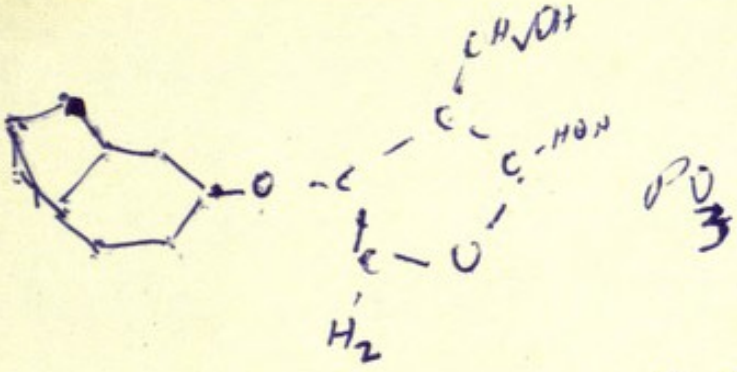





42
24



Four window X-ray diffraction tube. A new focal spot design lends a highly concentrated radiation output to these tubes. Focal spot size and design permits use as a source slit — providing higher resolution and higher peak intensities. Available with a variety of anode materials.
(North American Philips Co. Inc., New York).



MW 300 – 400

6×10^{23} residues weigh ~~300~~ 330 g

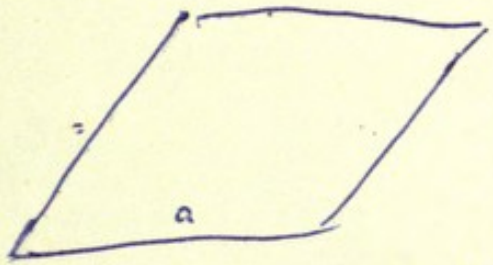
1 cc weighs 1.6 g

∴ 1 residue ~~occupies~~ weighs $\frac{300}{6 \times 10^{23}}$ g

1 occupies $\frac{\cancel{300} 330 \times 10^{24}}{6 \times 1.6 \times 10^{23}}$ A^3

$= 300\ A^3$

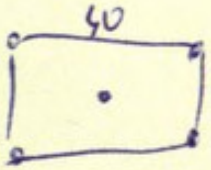
area ~ $1000 A^2$



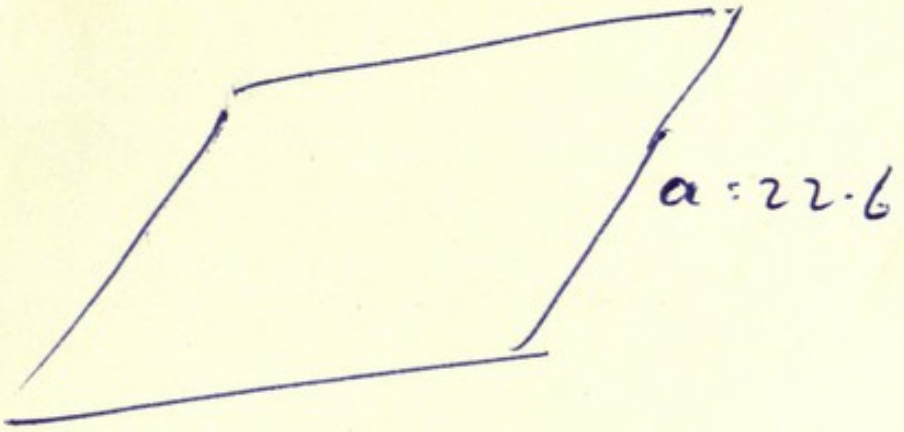
hexag. all for projects

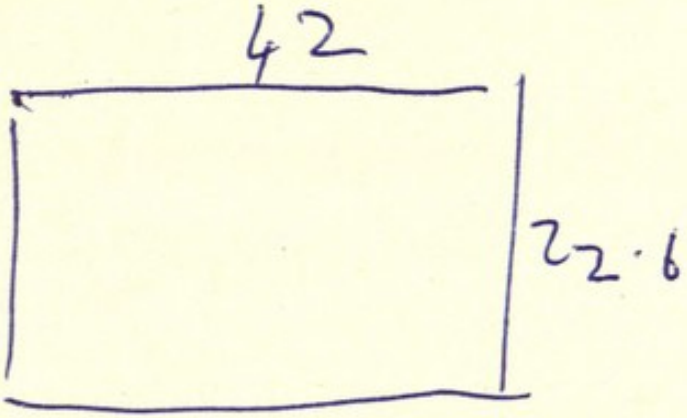
area : $a^2 \frac{\sqrt{3}}{2}$ ~ $500 A^2$?

orthorhombic



~ $40 \times 24 = 1000 A^2$


a : 22.6


42
22.6