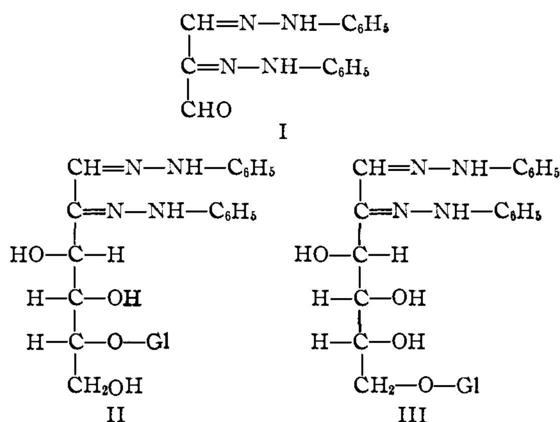
only 1% hydrolysis is observed in 24 hours at room temperature in 0.2 *N* HCl.

A tentative conclusion as to the point of attachment of the glucosyl group has been based on the following observations: Chargaff and Magasanik[1] noted that glucose phenylosazone was rapidly cleaved by sodium periodate to give a precipitate of compound I in 85% yield. We have extended this technique to the disaccharides and find it useful in the determination of the point of union of the component parts, since glycosidic linkages in the 3- and 4-positions block the reaction. The method should be applicable to any oligosaccharide giving an osazone.

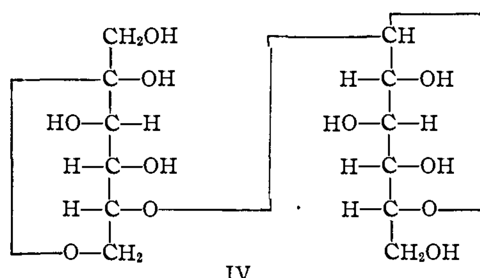


Under the conditions used by those workers we found that the phenylosazone of the new sugar as well as that of isomaltose (6-(α-D-glucopyranosyl)-D-glucose) and of gentiobiose (6-(β-D-glucopyranosyl)-D-glucose) gave rapid precipitation of compound I in yields of 70–80%. On the other hand, the phenylosazones of turanose (3-(α-D-glucopyranosyl)-D-fructose), laminaribiose (3-(β-D-glucopyranosyl)-D-glucose), maltose (4-(α-D-glucopyranosyl)-D-glucose), and cellobiose (4-(β-D-glucopyranosyl)-D-glucose) gave no precipitates (other than colorless inorganic salts) even on standing overnight. Such behavior is compatible only with structures II and III having the glucosidic linkage on positions 5 or 6, respectively. This conclusion is further supported by the fact that the phenylosazone of the new sugar gives an X-ray diffraction pattern readily distinguishable from that obtained with the phenylosazones of turanose, laminaribiose, maltose, or cellobiose.

The possibility of a 1,6 linkage was also rendered unlikely by the following considerations: The X-ray pattern of the phenylosazone of the new sugar was found to differ from that of gentiobiose phenylosazone; the pattern given by isomaltose phenylosazone, however, was so similar to that of the new phenylosazone that conclusive differentiation was not possible. Fortunately, identity could be ruled out by the melting point of isomaltose phenylosazone (205–207°) and the fact that it forms a crystalline phenylosotriazole (m.p. 179–180°).

Preliminary evidence, then, suggests that the new sugar may be a 5-(D-glucopyranosyl)-D-fructopyranose, a possible form of which is shown in formula IV.

(1) E. Chargaff and B. Magasanik, THIS JOURNAL, **69**, 1459 (1947).



Apparently no 1,5-phenylosazones of the proper configuration are available for comparison. Freudenberg and v. Oertzen[2] recently synthesized 5-(β-glucosido)-glucose but were unable to obtain pure osazones.

Our work on the mechanism of dextran formation indicates that the new sugar plays a role in the polymerization process; we believe it advisable, therefore, to assign it the common name of "leucrose" which is suggested by its particular microbial origin.

Further structure studies are in progress.

We are indebted to Prof. E. L. Hirst and Dr. V. C. Barry for laminaribiose samples, to Dr. Allene Jeanes for the isomaltose, to Drs. N. K. Richtmyer and C. S. Hudson for the turanose, and to Dr. N. Hellman and Mr. H. F. Zobel for the X-ray determinations.

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(2) K. Freudenberg and K. v. Oertzen, *Ann.*, **574**, 37 (1951).

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THE MOLECULAR WEIGHT AND SHAPE OF DESOXYPENTOSE NUCLEIC ACID

Sir:

The uncertainty which persists with regard to the molecular weight and shape of desoxypentose nucleic acid, DNA, from calf thymus appears to be due to the inadequacy of most macromolecular techniques in characterizing very large, charged polyelectrolytes and to the varying degrees of degradation inherent in the different methods of preparation. In a current paper[1] it is shown that the measurement of the angular distribution of scattered light from DNA solutions in the concentration range of 1 to 10 mg./100 cc. leads to a determination of the molecular weight as well as to some definite conclusions about the size and shape of several different samples. The sample having the highest molecular weight was that prepared by Schwander and Signer.[2] We wish to report measurements on a sample we have prepared by the Signer method which indicate the reproducibility of this preparative method and provide new information on the structure of the DNA molecule.

Light scattering measurements on the new preparation in 0.2 *M* NaCl show the molecular weight to be 7,700,000 in comparison with 6,700,000 found[1]

(1) P. Doty and B. H. Bunce, THIS JOURNAL, in press.

(2) H. Schwander and R. Signer, *Helv. Chim. Acta*, **33**, 1521 (1950).

for the Signer sample (VII). This difference is somewhat outside probable error, and the intrinsic viscosities at 1000 sec.$^{-1}$ of 22.2 and 19.9 (100 cc./g.) support this view, but the difference is marginal in comparison with the molecular weights of DNA prepared by other methods.[1] Further evidence of the reproducibility of the DNA samples prepared by the Signer method lies in the work of Katz[3] who prepared independently another sample and using the techniques previously worked out[1] found the molecular weight to be 8,000,000.

Information on the size and shape of the DNA molecule can be derived from the angular distribution of the reduced intensity extrapolated to zero concentration. Our results show that the molecule is not at all rod-like at *p*H 6.5 but that it has a three-dimensional structure only slightly more asymmetric than a random coil. The maximum dimension of the molecule is about 6500 Å.

Two other studies indicate that the molecule is not a linear, random coil but rather a lightly branched or cross-linked polynucleotide as suggested elsewhere.[1] One of these is the investigation of the changes produced upon lowering the *p*H of the 0.2 *M* NaCl solution of DNA to 2.6 by dialysis. It is found that the molecular weight does not change but that the molecule collapses into a rod 3000 Å. long, as shown by the radical change of the angular intensity distribution to that corresponding to a rod. These results are summarized in the accompanying figure which shows the intensity distribution before, during, and after the exposure to *p*H 2.6.

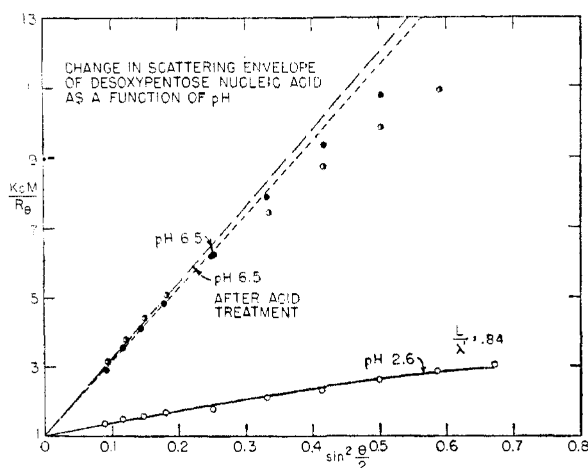

Fig. 1.

If this were a linear polynucleotide coil, it would, due to its charge, expand greatly upon removal of the salt from its solution and would exhibit an increase of the reduced specific viscosity upon dilution of its aqueous solution. In view of what has been found, for example with polymethacrylic acid,[4] this would for DNA involve about a ten-fold increase in its maximum dimension and at least a hundred-fold increase in the reduced specific viscosity. Instead it is found that at *p*H 6.5 the maximum dimension only increases about 60% when salt is removed[5] and that the reduced specific viscosity only increases from 55 at 16 mg./100 cc. to 85 at 1 mg./100 cc. Thus the polynucleotide chain is under considerable restraint probably due to a small number of branch or network points. On the other hand, even this limited expansibility of the molecule eliminates the possibility of it being rod-shaped at neutral *p*H.

(3) S. Katz, THIS JOURNAL, **74**, 2238 (1952).

(4) A. Oth and P. Doty, *J. Phys. Chem.*, **56**, 43 (1951).

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(5) E. P. Geiduschek, Ph.D. Thesis, Harvard University, 1951.

(6) U. S. Public Health Post-doctoral Fellow.

(7) A fellowship from the American Cancer Society Institutional Grant is gratefully acknowledged.

NATURE OF MONOESTER PHOSPHATE GROUP IN COENZYME A[1]

Sir:

The coenzyme A molecule has been reported to contain three phosphate groups.[2] Two of these phosphate groups are in a pyrophosphate linkage, whereas the third phosphate has been ascertained to be in the monoester form, esterified with the ribose moiety of the adenylic acid portion of the coenzyme.[3] Prostatic phosphatase, a monoesterase, has been found to inactivate coenzyme A by removal of one phosphate.[2] We have found that an enzyme from barley,[4] which splits only "b" nucleotides, also will remove the monoester phosphate group and inactivate the coenzyme.[5] Table I shows the action of the "b" nucleotidase on the coenzyme.[6]

TABLE I

SPLITTING OF COENZYME A BY "B" NUCLEOTIDASE

	Inorg. P, μM	Arsenolysis of acetyl-PO_4 units	Acetylation of sulfanilamide units
CoA (0.05μM.)	0	14.8	14.8
CoA (0.05μM.) + "b"-Nucleotidase	0.052	0	13.9

The "b" nucleotidase has also been found to attack coenzyme A at about the same rate as it attacks adenylic acid "b," whereas the prostatic enzyme splits coenzyme A at a much slower rate than adenylic acid "b."

Kornberg and Pricer[7] have reported that the

(1) Contribution No. 28 of the McCollum-Pratt Institute. This work was supported in part by grants from the American Cancer Society as recommended by the Committee on Growth of the National Research Council, and the Williams-Waterman Fund.

(2) J. D. Gregory, G. D. Novelli, and F. Lipmann, THIS JOURNAL, **74**, 854 (1952).

(3) J. Baddiley and E. M. Thain, *J. Chem. Soc.*, 3421 (1951); G. D. Novelli, personal communication.

(4) L. Shuster and N. O. Kaplan, *Fed. Proc.*, **11**, 286 (1952).

(5) Coenzyme A was assayed by its activity in the arsenolysis of acetyl phosphate by transacetylase (E. R. Stadtman, G. D. Novelli and F. Lipmann, *J. Biol. Chem.*, **191**, 365 (1951)). Coenzyme A, inactivated by "b" nucleotidase or prostatic phosphatase, can be rephosphorylated to form active coenzyme with ATP and pigeon liver extract, so that no inactivation is apparent when the pigeon liver assay system (containing ATP, acetate and sulfanilamide) is used.

(6) The coenzyme A preparation used was approximately 90% pure (370 units per mg.), and was generously supplied by Drs. G. D. Novelli and Fritz Lipmann.

(7) A. Kornberg and W. E. Pricer, Jr., *J. Biol. Chem.*, **186**, 557 (1950).