Evidence from Light-Scattering Studies for a Dimeric Structure for Deoxyribonucleic Acid in Solution

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The investigation of the size and shape of the sodium salt of deoxyribonucleic acid (DNA) in solution has attracted much attention both because of its inherent interest and its great biological importance. But the complications of its poly-electrolyte character and its very great size, both of which contribute to the marked intermolecular interactions, make the interpretation of the experimental results difficult. The least ambiguous method, light scattering, indicates that the nucleic acid derived from calf thymus is best regarded as a stiffened random coil with a molecular weight of about 6×10^6 , and using the formulae appropriate to coiling polymer chains, Doty, Reichmann, Rice & Thomas (1954) have shown that the results from measurements of viscosity and sedimentation are consistent with this value.

Crick & Watson (1953, 1954) have proposed recently that the analytical data for DNA and the X-ray diffraction diagrams by Franklin & Gosling (1953) obtained for DNA fibres can best be explained by a model in which two strands of nucleic acid intertwine to form a double helix held together by hydrogen bonds between the amino and hydroxyl groups of the purines and pyrimidines. X-ray diagrams indicating the presence of such structures have also been obtained from DNA in native materials (Wilkins, Stokes & Wilson, 1953). Since it is unlikely that such a specific structure can be formed in the process of producing the fibres, it follows on this hypothesis that DNA in solution must already be associated as a double molecule.

To test this hypothesis we examined by light scattering the size and shape of DNA in concentrations of urea which we had previously shown to dissociate hydrogen-bonded aggregates of dyes (Alexander & Stacey, 1952) and of polyvinyl alcohol (Stacey & Alexander, 1954*a*). The dissociation by 4*M* urea of the haemoglobin molecule into two halves by the breaking of hydrogen bonds was clearly demonstrated in the ultracentrifuge by Steinhardt (1938). The results of preliminary experiments (Stacey & Alexander, 1954*b*) indicated that there was a dissociation of the DNA molecule, although it appeared to retain its linear dimensions.

Greenstein & Jenrette (1941) had observed that urea decreases the viscosity of solution of DNA, and this observation was confirmed by Conway & Butler (1952), who interpreted this change as a loss of the rigidity of the DNA molecule and also found indications for a halving of the molecular weight.

The ionization of the amino groups of cytosine and adenine which are involved in the hydrogen bonds should also produce a dissociation of the twin spiral structure, and for this reason the effect of adding acid on the size and shape of DNA has also been examined. Cecil & Ogston (1948) found in the ultracentrifuge that in solutions of nucleic acid acidified to pH 3.5 a second heterogeneous component appeared with a lower sedimentation constant and increased rate of boundary spreading, but Doty, Bunce & Reichmann (1953) have shown that it is possible to titrate DNA to pH 2.6 and back to pH 5.8 without degradation. From the change in the angular distribution of the light scattering they concluded that the well-known decrease in viscosity on acidification was due to the contraction of the coil to a much more compact structure. Pouyet & Sadron (1954) have challenged this interpretation because they find no change in the light scattering on adding acid to pH 3.8 and suggest DNA is rather more rigid. We have studied the light scattering of nucleic acid from herring sperm, which appears to have the same properties as the more extensively investigated DNA from calf thymus.

EXPERIMENTAL

Isolation of nucleic acid

The soft roes of fresh herring were minced and dispersed in ice-cold water. After removal of the fibrous matter by filtration through muslin, the suspension was mixed in a Waring Blendor at high speed for 1 min. and centrifuged. The precipitate was re-suspended in 0.01 *M* sodium citrate solution and centrifuged again. An oily layer on top of the precipitate was removed and this process repeated twice. The final precipitate was washed with ethanol and ether and dried in a vacuum desiccator. The analysis ($N=19.9$; $P=5.81\%$) was the same as that found by Felix, Fischer, Krekels & Mohr (1951) for the nucleoprotamine complex they isolated in a similar manner from trout sperm. They

demonstrated, and we have confirmed, the absence of an appreciable quantity of any other component.

The nucleoprotamine was suspended in water by stirring in a Waring Blendor for 1 min. and added very slowly with continuous vigorous agitation to 5M-NaCl until a solution containing 0.6% nucleic acid was obtained. This was shaken for several hours and then saturated with salt and allowed to stand in the refrigerator with an occasional agitation for 12 days. The denatured and precipitated protein was filtered off on Celite (Johns-Manville No. 545) after dilution to twice the volume with saturated salt solution, and the filtrate diluted. The nucleic acid was precipitated with ethanol, washed with 70% (v/v) ethanol and dried with ethanol and ether and stored in a vacuum desiccator. This method, although slow, was found to yield the best preparations, and gave a product with a negative Sagakuchi reaction for arginine. The Sevag method in which the protein is denatured by shaking with chloroform-butanol mixtures and removed by centrifuging, gave in our hands, products of lower molecular weight. The molecular weights of the DNA obtained by the present method were consistent and agreed with those of Doty *et al.* (1954) (see also Frick, 1954).

Although it is known that the use of the Waring Blendor can cause degradation of macromolecules (Alexander & Fox, 1954) it is felt that no damage results from its use in preparing the dispersion because the DNA is not then in solution but is contained within the sperm heads from which no detectable quantities of DNA were released during the short periods of stirring.

Light scattering

The apparatus, method and calibration is described elsewhere (Alexander & Stacey, 1955). The scattering was measured with both the blue (436 m μ .) and green (546 m μ .) mercury lines, isolated by interference filters supplied by Messrs Barr and Stroud. Dust was removed from the solutions by spinning them in an M.S.E. centrifuge at 13 000 rev./min. (20 000 g) for at least 4 hr. It was found essential to add some salt to the stock solutions being centrifuged otherwise the clarification was far from satisfactory. Almost invariably a small pellet of gel was deposited. A gel component was frequently observed by Cecil & Ogston (1948) in solutions of nucleic acid, and similar highly swollen gel particles were found in aqueous solutions of high molecular weight polymethacrylic acid. The presence of these gels may be a consequence of the slow rate of solution of these high molecular weight materials, and in the case of DNA, cross-linking by heavy metal ions may also be a contributing factor. The variation from sample to sample of the viscosity of DNA prepared without filtration or centrifuging may be caused by small amounts of these gel particles, which would not be detected other than by centrifuging, and quite reproducible viscosity measurements are obtained in their presence.

Most experiments were carried out in solutions of 0.1M-NaCl. In the experiments in which the pH was taken to 2.2 the salt was added after reneutralization because at this pH DNA is insoluble in salt solution. The nucleic acid concentrations used for the light-scattering measurements were in the range $1-40 \times 10^{-5}$ g./ml.

The molecular weight, M , and the radius of gyration of the particle have been derived from the light-scattering data by Zimm's (1948) method in which the reciprocal of the reduced scattering, K_c/R_θ is plotted against $\sin^2(\theta/2) + kc$,

where k is an arbitrary constant. This produces a grid-like plot which can be simultaneously extrapolated to zero concentration and zero angle. If it is assumed that the molecules exist in solution as polydisperse random coils this is a linear extrapolation and the weight-average molecular weight, M , is obtained from the intercept and the radius of gyration r is given by the ratio of the initial slope of the zero angle line to the intercept by these relations:

$$\left(\frac{K_c}{R_\theta}\right)_{c \rightarrow 0} = \frac{1}{M}; \quad K = \frac{2\pi^2 n^2}{N\lambda^4} (dn/dc)^2, \quad (1)$$

and

$$\frac{\text{initial slope}}{\text{intercept}} = \frac{16\pi^2 r^2}{3} (n/\lambda)^2, \quad (2)$$

R_θ is Rayleigh's ratio for the angle of observation; c is the concentration in g. ml.⁻¹; λ = wavelength of light *in vacuo*; n = refractive index of the solvent; N = Avogadro's number; dn/dc = specific refractive index.

A typical Zimm plot is shown in Fig. 1, but for clarity in the other figures the reciprocal reduced intensity

$$[(K_c/R_\theta)c \rightarrow 0]$$

as a function of $\sin^2(\theta/2)$ only has been drawn.

The treatment of the light-scattering data of the solutions containing 4M urea is more complicated because additional terms for the fluctuations in concentration with respect to the additive (urea) must be considered.

The theory relating to multi-component systems, as elaborated by Kirkwood & Goldberg (1950) was used successfully by Badger & Blaker (1950) to explain the interaction of nitrocellulose with a variety of mixed solvent-precipitant systems. They used the equation

$$\frac{K_{c_2}}{R_\theta \rho_0} = \frac{1}{M} (1 + G_{10}c_1 + G_{01}c_2^2 + G_{20}c_1^2 + G_{11}c_1c_2 + G_{02}c_2^2),$$

where c_1 and c_2 are the concentrations of solute and additive respectively in g./g. of primary solvent, ρ_0 is the mass of primary solvent per unit volume of solution. G_{10} etc. are the thermodynamic interaction functions, which in this case it was impossible to measure, both because the molecular weight was not accurately known and because it proved impossible to measure the refractive index increment of DNA in 4M urea; but it is probable that they are too small in this system to affect seriously the calculated molecular weights. Experimental support for this argument was obtained by us (unpublished results) by measuring the light scattering of polymethacrylic acid in three different concentrations of urea, when this equation accounted satisfactorily for the changes in the turbidity observed.

The concentrations of DNA were always determined in a Unicam spectrophotometer in 0.2M-NaCl and at a pH near 6.5, since it is known that the extinction coefficient varies with both the concentration of added salt and the acidity. The value of the extinction coefficient $E_{1\text{cm}}^{1\%} = 211$ at 259 m μ . which was based on phosphorous analyses is very close to 213 given for calf thymus DNA under the same conditions (cf. Doty *et al.* 1954).

Specific refractive index increment

There is some uncertainty in the value of dn/dc for nucleic acid for the 436 m μ . mercury line. Doty *et al.* (1954) report 0.188 ml. g.⁻¹ based on measurements of the dry weight, and Northrop, Nutter & Sinsheimer (1953) give 0.201 ml. g.⁻¹ based on phosphorous analyses. We have measured the

difference of the refractive index Δn of several solutions of nucleic acid from water in a double prism differential refractometer having a sensitivity of 2×10^{-6} (Alexander & Stacey, 1955) and calibrated with solution of KCl (Stamm, 1950). The concentrations were determined as given below. The average of the values given in Table 1 is $0.191 \text{ ml. g.}^{-1}$.

Table 1. *Refractive index increments of DNA at various concentrations*

DNA concentration (g./ml. $\times 10^4$)	$\Delta n \times 10^6$	$\Delta n/c$ (ml. g. $^{-1}$)
11.4	219	0.192
7.7	147	0.191
5.5	104	0.189
5.3	100	0.189

Fig. 1. The Zimm plot for herring sperm deoxyribonucleic acid at pH 6.8. The intercept gives a molecular weight of 5.9×10^6 and the ratio of the initial slope of the zero concentration line to the intercept gives a radius of gyration of $1980 \text{ \AA}$. The dashed line indicates the theoretical curve of the zero-angle line for a solution of poly-disperse random coils of this radius of curvature.

RESULTS

Molecular weight. In the best examples so far examined, the molecular weight found was very nearly 6×10^6 (5.6 , 5.7 and 5.9×10^6) (Fig. 1), which compares well with the results given by Doty *et al.* (1954) for a variety of preparations of calf thymus nucleic acid (5.8 – 6.8×10^6), if allowance is made for the slight difference in the values of the specific refractive index increment used in the calculation. As would be expected the radii of gyration were of the same order, but this may be a consequence of the very similar methods of preparation, since these

values are very sensitive to the conditions of the preparation and the presence of residual protein. Even in the presence of 2 M-NaCl , the molecular weight of 14 – 15×10^6 was found for a sample containing 10% protamine.

Fig. 2. The reduced scattering in arbitrary units of DNA as a function of $\sin^2(\theta/2)$ for solutions of different ionic strength showing that it is impossible to make the correct extrapolation for ionic strengths below 0.1 M . The concentration of DNA in each case is $1 \times 10^{-4} \text{ g./ml.}$ (a) in 0.06 M-NaCl , (b) in 0.1 M-NaCl and (c) in 0.3 M-NaCl . The same set of points as the last is obtained over the range 0.1 – 0.3 M-NaCl .

Flexibility of the DNA molecule. In synthetic linear polyelectrolytes there is a decrease in dimensions on the addition of salt (Alexander & Stacey, 1955) owing to the reduction in electrostatic repulsion of the charged groups. Most experiments were made in solutions of 0.1 – 0.2 M-NaCl but there was a small decrease in the radius of gyration observed in 0.9 M-NaCl which does indicate a limited flexibility in the chain. Unfortunately, in solutions of low ionic strength the intermolecular repulsions of highly charged molecules produce a certain degree of order in the solution and the light scattering is reduced by destructive interference particularly in the forward direction (Doty & Steiner, 1952). Although it would be of interest to establish the dimensions in solutions of lower ionic strength than 0.1 M-NaCl , owing to this charge effect the decrease in the forward and total scattering is appreciable (Fig. 2) even in 0.08 M-NaCl .

Table 2. Influence of urea and acid on the size and shape of DNA in solution

Treatment	$10^{-6} \times$ molecular weight	Radius of gyration ($\text{\AA}$)
None (pH 6.8)	5.9	1980
Addition of urea to make solution 4M	2.7	2100
Removal of urea by rapid dialysis from the above material	2.5	1180
Addition of 0.01M-HCl to make solution pH 4.0	5.9	1610
Addition of acid to make solution pH 3.0 (measured after 15 hr.)	6.6	1520
Reneutralization to pH 7.0 from 3.0	5.8	1870
Addition of acid to pH 2.6	15.9	1040
Reneutralization to pH 7.0 from 2.6	5.9	920
Reneutralization to pH 7.0 from pH 2.2	2.45	800
Addition of urea to the above material to make solution 4M	2.0	1360

When this interference effect occurs it is necessary to go to much lower concentrations before the Zimm extrapolation is valid and for DNA this, so far, has been experimentally impracticable because of the very low level of the scattering.

A moderate flexibility of the molecule is also shown by its coiling up on the addition of acid. It can be seen from Table 2 that there was a progressive decrease in the radius of gyration from pH 6.8 to 3.0 without any alteration in the molecular weight. This confirms the findings of Doty *et al.* (1953) that partial ionization of the amino groups and the consequent reduction in the net charge reduces the electrostatic repulsion and therefore the degree of extension of the coil.

In more acid media, there was rapid aggregation until precipitation occurred. At pH 2.6 apparently stable aggregates with molecular weights of about 15×10^6 were produced (Fig. 3). This material is polydisperse and no significance can be attached to this numerical value since the weight-average molecular weight varies somewhat with slight changes of experimental conditions in this pH range.

Even after prolonged exposure to pH 3.0 (15 hr. at room temperature) the coil extended on reneutralization to very nearly its previous extension. This is remarkable because even at this pH one-half of the amino groups have acquired a proton (Peacocke & Lea, 1953). On reneutralization from pH 2.6 the radius of gyration returned to not more than half its original value, although the molecular weight had not altered (see Table 2). On reneutralization after exposure to more acid conditions rather more drastic alterations became apparent. Exposure to pH 2.2 for 3 min. followed by rapid reneutralization produced a marked decrease in the molecular weight, and it is tempting to suggest that this was due to the dissociation of the twin helix. The angular scattering of the acid-treated material indicated an increase in the polydispersity, and the hydrolysis of some labile bonds would produce a

similar effect (Overend, Stacey & Webb, 1951). The possibility that the decrease in molecular weight was entirely due to acid hydrolysis seems unlikely since the same reduction was obtained when the DNA was left at pH 2.2 for much longer periods.

Dissociation by urea. Evidence for the hypothesis that native DNA is a hydrogen-bonded dimer was

Fig. 3. The reciprocal reduced intensity as a function of $\sin^2(\theta/2)$ for DNA after different treatments (see Table 2): (a) None at pH 6.8; (b) pH 4.0; (c) pH 3.0; (d) pH 7 after reneutralization from pH 2.6; (e) pH 2.6. The intercept is equal to the reciprocal of the molecular weight and the slope is proportional to the square of the radius of gyration.

obtained by the addition of concentrated urea (Stacey & Alexander, 1954*b*), since it is known that this agent does not rupture covalent bonds (Neurath, Greenstein, Putnam & Erickson, 1944). In 4*M* urea the molecular weight fell from 5.4×10^6 to 2.7×10^6 while the initial slope increased slightly (Fig. 4). The fall in molecular weight might be due to the rupture of a weak link but it seems improbable that the

Fig. 4. The reciprocal reduced intensity plotted as a function of $\sin^2(\theta/2)$ for DNA after treatments with urea and acid: (a) in 4*M* urea; (b) in 4*M* urea after exposure to pH 2.2; (c) at pH 7.0 after exposure to pH 2.2; (d) at pH 7.0 in 0.1*M*-NaCl after treatment with 4*M* urea followed by rapid dialysis.

resulting particles would have a radius of gyration so near that of the original. However, the approximate halving could be regarded as the result of the dissociation of the hydrogen bonds along the length of the chain which is then maintained in its extended form by the electrostatic repulsion of the phosphate groups. This effect of urea in lowering the molecular weight has been observed in solutions of aggregated polyvinyl alcohol when in 4*M* urea the aggregates are dispersed: the molecular weight after the addition of urea corresponded to that found for the unaggregated material (Stacey & Alexander, 1954*a*). Measurements on DNA after the urea has been removed by dialysis showed that the molecular weight had only slightly changed (2.5×10^6) and, owing probably to the formation of intramolecular hydrogen bonds, coiled into a much more compact structure (see Table 2). The addition of *M* urea did

not produce this change in molecular weight. The small change of molecular weight on dialysis and the fact that these values are not exact multiples cannot wholly be explained by the experimental error of the light-scattering measurements ($\pm 5\%$) and are probably the result of a small amount of degradation occurring during the time of the experiment.

Preliminary experiments carried out in conjunction with M. de la Varr in the laboratory of Professor Desreux showed that the flow birefringence of a sample of this herring sperm DNA in 0.2*M*-NaCl was very similar to that found by Schwander & Cerf (1949) for calf thymus DNA. On the addition of 4*M* urea the rotatory diffusion coefficient (after taking into account the slight increase in viscosity) increased by 20% from 34 to 41 sec.^{-1} , in agreement with the suggestion that the coil is slightly more extended in urea after dissociation.

The molecular weight of a sample of DNA which has been acidified to pH 2.2 and then reneutralized was not significantly reduced by the addition of 4*M* urea (Fig. 3). This observation is consistent with the view that the fall in molecular weight produced by acidification is largely due to the dissociation of the two-stranded dimeric structure. It is also noteworthy that there is a considerable uncoiling of this molecule in urea (see Table 2) which would not be expected if the decrease in molecular weight were due solely to acid hydrolysis.

DISCUSSION

The close coincidence of the properties established here for herring sperm DNA with those of the material from calf thymus is remarkable but it also appears to be true for DNA from bacteria (Norman & Simmons, 1953) and bacteriophage (Doty *et al.* 1954). All of these appear to exist in solution as units of about six million. The value of the molecular weight and the dimensions of this unit is based on the assumption that a linear extrapolation of the Zimm plot is possible. This is strictly true only for polydisperse random coils with distribution of molecular sizes such that the ratio of the weight average (M_w) to the number average (M_n) molecular weight, M_w/M_n , is near 2. If, the polydispersity is less than this, the values given above are too high, but the fact that the same intercept is obtained with lines of very different slope (Figs. 3 and 4) does indicate that this error must be small. The same limiting slope was obtained for both the blue and green wavelengths, which further supports the view that this extrapolation is reliable as it can be seen from Zimm's equations that the function giving the radius of gyration is also dependent on the wavelength. But, despite these uncertainties

and that of the specific refractive index increment which affect the absolute value, the striking similarity between the materials isolated by different methods from different sources still holds, and we share the view of Doty *et al.* (1954) that in spite of the difficulties of interpreting the full angular distribution curve of the light scattering the values for the molecular weight and radius of gyration given by this method may be accepted as the most nearly correct in the absence of an adequate theory for stiff coiled polyelectrolytes in the other methods available for the determination of the size and shape of macromolecules.

The large radius of gyration in neutral solution points to a rather stiff molecule. The separation of the many factors which affect the shape of the reciprocal scattering curve is very difficult on the basis of light-scattering theory only. This function should be a straight line for polydisperse Gaussian random coils and the gentle deviation downwards at the higher angles (Fig. 2) may be due to a greater range of distribution in molecular weight, or that the molecules are too stiff to be considered Gaussian. It is unlikely that the polydispersity is great enough to cause this effect, although it is probably sufficiently great to make the application of Peterlin's (1952) treatment of the scattering of stiffened chains inapplicable. That the coils are somewhat non-Gaussian in character is indicated by light-scattering considerations. The r.m.s. end-to-end length of a random coil is $\sqrt{6}$ times the radius of gyration, and Benoit & Doty (1953) have shown that coils do not become Gaussian until the contour length is at least 4 times the end-to-end length. For DNA (Doty *et al.* 1954) have calculated the ratio of the contour length to be about 5 times, which suggests that at least one-half of the molecules are non-Gaussian.

Since the addition of acid causes a substantial reduction in size, this stiffness may in part be due to the repulsion between the phosphate groups by analogy with the synthetic polyelectrolytes, but the much greater degree of coiling in the dissociated molecule suggests that it is the stiffness of the structure of the double molecule which is mainly responsible.

The changes in the physical properties of DNA produced by high urea concentrations strongly suggests that it exists in solution as a double-stranded structure, held together, as suggested in general terms by Gulland, Jordan & Taylor (1947) and more explicitly by Crick & Watson (1953, 1954), by hydrogen bonds. Aggregates associated by secondary valency forces of other types are not dissociated by urea (Alexander & Stacey, 1952). Our results further indicate that when more than 75% of the amino groups are ionized by the addition of acid to pH 2.2 the dimer is also dissoci-

ated in agreement with the suggestion of the hydrogen bonded structure. Ionization of the amino groups produces a decrease in the net charge by internal neutralization of the positive groups by phosphate groups. This, coupled with the inherent resistance of a still partly organized structure, is probably the explanation why this splitting does not occur when less than about 80% of the amino groups are ionized, although it is clear that some disruption of the original structure does occur on ionization of the amino groups, since the exact dimensions are not regained on reneutralizing a solution acidified to pH 2.6 (see Table 2).

The light-scattering results indicate that the DNA in solution exists as a dimeric structure which can be readily dissociated by the rupture of the hydrogen bonds. These findings lend support to the suggestions of Crick and Watson for the structure of the molecules in the paracrystalline fibres, although the facile dissociation in solution may not be reconcilable with the exact stereochemical arrangement of the two strands proposed by these authors.

SUMMARY

1. The molecular weight and the radius of gyration of deoxyribonucleic acid prepared from herring sperm has been measured by light scattering under a variety of conditions. In neutral solution the molecular weight of the best samples is near to 6×10^6 , in good agreement with the results of Doty *et al.* (1954) for calf thymus nucleic acid.
2. On the addition of acid to pH 2.8 the coil decreased in size, although the molecular weight remained the same. Very nearly the original radius of gyration was recovered on neutralization.
3. On reducing the pH to 2.6 and below higher apparent molecular weights indicating considerable aggregation were obtained. On reneutralization it was found that an irreversible decrease in size had occurred.
4. The molecular weight of nucleic acid exposed to pH 2.2 for 3 min. before reneutralizing was reduced to 2.5×10^6 . It is unlikely that hydrolysis has contributed much to this decrease.
5. The addition of urea reduced the molecular weight to about one-half of its original value although the radius of gyration is slightly increased. On removal of the urea by dialysis there is only a small further drop in molecular weight but a marked increase in the degree of coiling. Urea has only a small additional effect on the molecular weight of nucleic acid reneutralized from pH 2.2.
6. These results are consistent with the view that DNA is present in solution as a two stranded structure held together by hydrogen bonds which is dissociated when these are broken by ionization of the amino groups or by the addition of urea.

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Estimation of Monosaccharides by the Orcinol-Sulphuric Acid Reaction

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Methods of estimating carbohydrates, based on the colour reaction with orcinol and sulphuric acid, have been described by Tillmans & Philippi (1929), Sørensen & Haugaard (1933) and Brückner (1943). This paper describes an improved method based on the same principle.

MATERIALS AND METHOD

Orcinol reagent. Orcinol (0.4 g.) was dissolved in 10 ml. of water. The orcinol and its solution should be colourless. If this is not the case, the orcinol must be repeatedly crystallized from water and/or CHCl_3 until a colourless product is obtained. Freshly prepared solutions should be used.

Sulphuric acid reagent (31.2N). This was prepared by mixing 800 ml. of analytical grade H_2SO_4 (sp.gr. 1.84) with 200 ml. of water and adjusting the normality of the solution.

Carbohydrates. Materials (whenever possible analytical reagents or C.P. grade reagents) were obtained from the following sources: D-glucose and D-mannose from British

Drug Houses Ltd.; D-fructose from Schering-Kahlbaum; D-galactose from Hoffman La Roche; L-sorbose, L-rhamnose, D-ribose, D-xylose and D-lyxose from the Pfanstiehl Chemical Co.; D-arabinose from Nutritional Biochemicals Inc. The carbohydrates were dried to constant weight in a vacuum desiccator over H_2SO_4 and NaOH pellets and used in 0.01% (w/v) solutions in water. D-Xylulose was prepared and kindly supplied by Mr R. G. Kulka, Department of Biochemistry, University of Otago. It was used in a solution of approximately 0.01% in water.

Test tubes. It is advisable to use uniformly made test tubes, approximately 17 cm. long, prepared from glass tubing of approximately 0.7 cm. internal diam. Thin-walled test tubes (glass thickness about 0.5–0.6 mm.) are preferred.

General method

The carbohydrate solution (1.0 ml.) and 1.0 ml. of orcinol reagent are mixed in a 100 ml. round flask. The flask is cooled in cold water and 8 ml. of sulphuric acid reagent added, drop by drop, while the flask is steadily rotated in