280. *The Sedimentation of Thymus Nucleic Acid in the Ultracentrifuge.*

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Measurements have been made of the sedimentation constant, over a range of concentrations, and of the diffusion constant of a sample of the tetrasodium salt of the deoxypentose nucleic acid prepared from calf thymus by Gulland, Jordan, and Threlfall. The results indicate that the sample is homogeneous, with a molecular weight of $8{\cdot}2 \times 10^5$, and that its particles are highly asymmetric.

The effects of treatment with acid and alkali have been studied. These cause important changes in the sedimentation properties of the material which are interpreted in terms of changes in its degree of molecular aggregation.

An examination of the tetrasodium salt of the deoxypentose nucleic acid of calf thymus (TNA), prepared by Gulland, Jordan and Threlfall (*J.*, 1947, 1129) was undertaken in order to compare its behaviour in the ultracentrifuge after treatment with acid and alkali with the changes in the titration curve (Gulland and Jordan, *J. Expt. Biol.*, in press) and in the viscosity of the solution (Creeth, Gulland, and Jordan, *J.*, 1947, 1141; Gulland and Jordan, *loc. cit.*). These included the appearance of new titratable groups and large changes of viscosity which suggested that changes in the state of aggregation or molecular symmetry might be occurring.

Experimental.

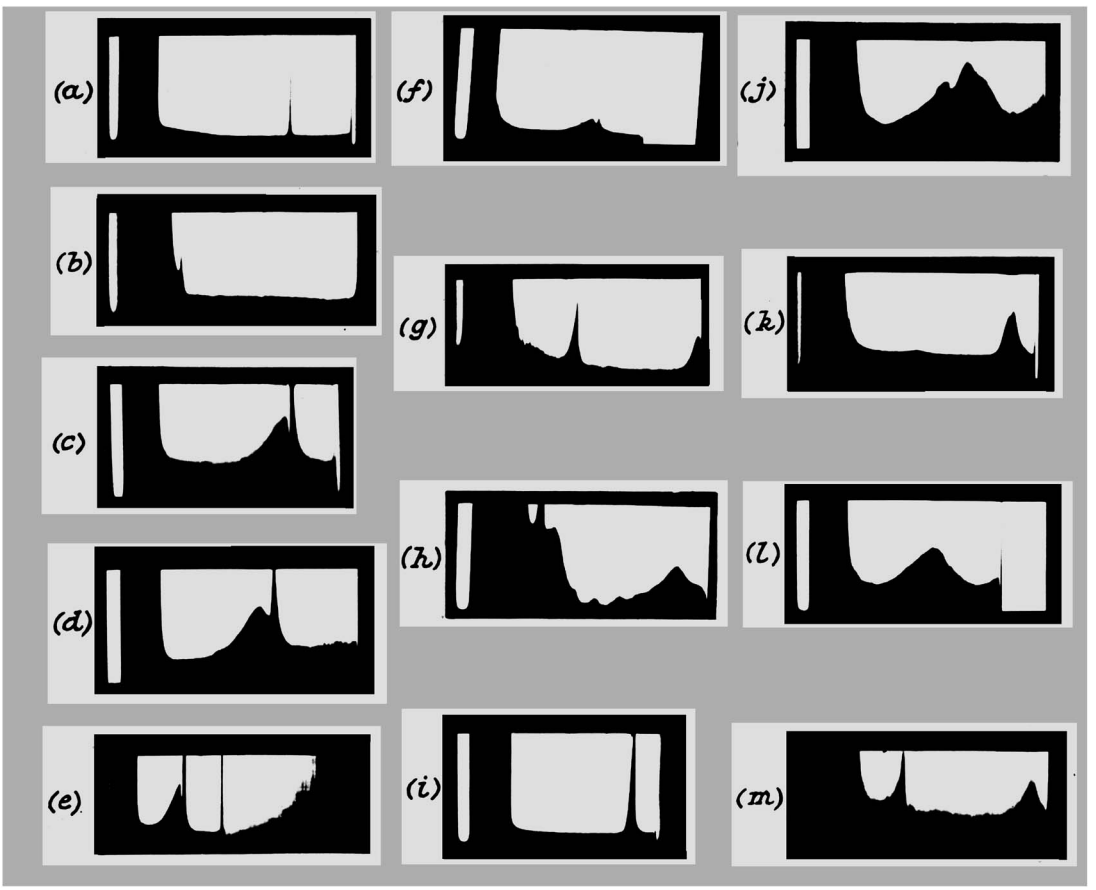
A sample of untreated TNA and samples which had been treated with acid and alkali and precipitated were supplied by the late Professor J. M. Gulland; these had been dried in a vacuum at 100°. Solutions were made up by weight, usually by dissolving the material in water and subsequently adding M-sodium chloride to produce a final concentration of 0·2M. Buffer solutions were not used, the solutions being brought to pH 3·5 or 12·5 and re-neutralised by adding N-hydrochloric acid or N-sodium hydroxide in amounts given by the titration data of Gulland, Jordan, and Taylor (*loc. cit.*). The pH after neutralisation was checked with a glass electrode cell and adjusted to $7 \pm 0{\cdot}1$.

Sedimentation was observed in a Svedberg oil-turbine ultracentrifuge by the diagonal-schlieren method of Philpot (*Nature*, 1938, **141**, 283). The speed was usually 1010 revolutions per second. The refractive increments of the solutions of TNA were determined against corresponding sodium chloride solutions. The integration of the schlieren boundaries was performed as described by Johnson and Ogston (*Trans. Faraday Soc.*, 1946, **42**, 789).

Results.

1. *Untreated TNA at* pH 7.—All solutions showed the presence of a component which gave a very sharp boundary (Fig. 1*a*). Its rate of sedimentation was measured over a range of concentration from 0·5 to 0·03 g./100 ml. Except at the lower concentration, the values for the sedimentation constant obtained from successive intervals in each run were reasonably constant. At 0·03 g./100 ml., however, a fall of sedimentation constant, as the run progressed, was observed in duplicate runs: we do not know the reason for this. The value of S quoted for this concentration is its highest initial value (see Discussion). The results are given in Table I*a*: Fig. 2*a* gives a plot of the sedimentation constant, corrected to water at 20°, against concentration. Extrapolation to zero concentration gives a value for $S_{20\ (corr.)}$ of $12{\cdot}6 \times 10^{-13}$.

FIG. 1.



In all diagrams, sedimentation occurs from right to left.

1*b*. *The gel component can be seen near the bottom of the cell before the normal* TNA *has separated from the meniscus.*
1*e*. *The cell leaked badly during this run. The meniscus can be seen half way down the cell.*
1*f*. *The small size of the boundaries is due to the use of a* 3*-mm. cell. The tilted appearance of the diagram is due to an accidental maladjustment of the cylindrical lens.*
1*g*. *Diagram taken during the early part of the run showing the gel component well down the cell, and the second component just separating from the meniscus.*
1*h*. *Diagram taken later during the same run as* g, *showing the heterogeneous component, with the gel component just visible at the bottom of the cell.*
1*m*. *The gel component can be seen near the bottom and the heterogeneous component near the top of the cell.*

[*To face p.* 1382.

TABLE I*a*.

Concn. of TNA, g./100 ml.	Concn. of NaCl, mol./l.	$S_{20\,(corr.)} \times 10^{13}$.	Remarks.
0·03	0·2	11·7	—
0·046	0·2	10·9	—
0·11	0·2	9·1	—
0·12	0·2	10·3	Gel component present. Integration gives 50% gel and 50% normal component. Value for $S_{20\,(corr.)}$ fits a concn. of 0·06%.
0·14	0·2	8·6	Gel component present, but integration not possible. Value of $S_{20\,(corr.)}$ lies above the average curve.
0·22	0·2	6·0	—
0·25	0·2	6·9	Gel component present, but integration not possible. Value of $S_{20\,(corr.)}$ lies above the average curve.
0·32	0·2	5·6	—
0·43	0·2	4·5	—
0·49	0·2	4·9	—
0·49	0·2	4·8	This solution was left for 13 days at 2° to find if the sodium chloride was responsible for gel formation. No gel was observed; the value for $S_{20(corr.)}$ agrees well with the preceding run at the same concentration.
0·054	0·01	8·0	Run at low NaCl concn. in order to estimate magnitude of salt effect.

TABLE I*b*.

Concn., g./100 ml.	D, cm.²/sec. $\times 10^7$.
0·187	0·48
0·123	0·62
0·054	0·71

TABLE II.

Treatment.	$S_{20\,(corr.)} \times 10^{13}$.	Remarks.
0·5% Solution brought to pH 3·5 and run.	7·0 7·9	Slow homogeneous component. Fast heterogeneous component.
0·5% Solution kept at pH 3·5 for 10 mins., returned to pH 7, and run.	8·1 9·3	Slow homogeneous component. Fast heterogeneous component.
0·5% Solution kept at pH 3·5 for 30 mins., returned to pH 7, and run.	6·4 7·6	Slow homogeneous component. Fast heterogeneous component.
0·5% Solution kept at pH 3·5 for 2 hrs., returned to pH 7, and run.	7·3 8·2	Slow homogeneous component. Fast heterogeneous component.
Solution brought to pH 3·5 for 10 mins., returned to pH 7, pptd., and dried. Made up to 0·5% and run.	10·9	Heterogeneous component accompanied by gel-forming component (see Fig. 3 for S_{20}).

TABLE III.

Treatment.	$S_{20\,(corr.)} \times 10^{13}$.	Remarks.
0·5% Solution brought to pH 12·5 and run.	3·2	Single boundary—fairly homogeneous.
0·5% Solution kept at pH 12·5 for 10 mins., returned to pH 7, and run.	12	Two very heterogeneous components. Only the slower could be measured.
0·5% Solution kept at pH 12·5 for 10 mins., returned to pH 7, and allowed to stand for 96 hrs. before running.	11 17	Two very heterogeneous components and a small amount of further material which could not be measured.
Solution brought to pH 12·5 for 10 mins., returned to pH 7, pptd., and dried. Made up to 0·37% and run.	11·4	Single heterogeneous component.
Solution brought to pH 12·5 for 10 mins., returned to pH 7, allowed to stand for 96 hrs., pptd., and dried. Made up to 0·5% and run.	11·3	Heterogeneous component accompanied by gel-forming component (see Fig. 3 for S_{20}).

The diffusion constant was measured over a range of concentration at 20° in 0·2N-sodium chloride by the method of Philpot, Coulson, Cox, and Ogston (*Proc. Roy. Soc.*, 1948, *A*, **192**, 382). The results are given in Table I*b* and Fig. 2*b*. The extrapolated value is $0{\cdot}81 \times 10^{-7}$ cm.²/sec.

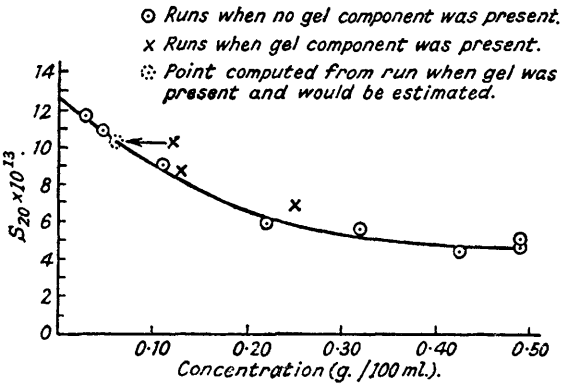
There were suggestions that this component forms only a part of the material :

(*a*) In some runs a second boundary was seen. This first appeared near the bottom of the cell; in the only measurements which could be obtained on it, its rate of movement fell with

time. This behaviour suggested that this boundary was due to a highly heterogeneous material which forms a gel when it becomes concentrated at the bottom of the cell (Fig. 1*b*).

(*b*) Integration of the schlieren boundary of the main component was possible in a few cases, and values of only about 50% of total refracting solute (excluding salt) were obtained. In one case an approximate estimate by integration of the amount of the gel-component was possible; the total integration was then 90% to which the two components contributed about equally.

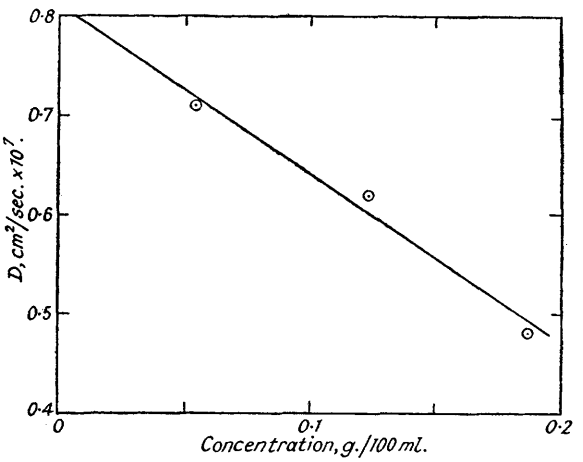
FIG. 2*a*.



Plot of S_{20} *of sodium thymonucleate against concentration.*

On the other hand, in most runs the second component was not seen; it did not develop in a solution of 0·5 g./100 ml. which was left for 13 days at 2°. There was some suggestion that the presence of the second component was dependent on mechanical disturbance of the TNA in the course of solution, appearing particularly when it was continuously stirred. The integration of the schlieren boundaries is not very trustworthy, since so sharp a boundary moves a considerable fraction of its width during an exposure, and this tends to give a low value for its

FIG. 2*b*.



area; moreover, diffraction introduces errors with very sharp boundaries whose magnitude is unknown.

Consideration of Fig. 2*a* makes it seem likely that in most cases there was no significant quantity of the second component. The values of $S_{20\,(corr.)}$, obtained in those experiments in which the second component was seen, lie above the average curve; this would be expected, since the formation of the second component reduced the concentration of the first. In order to lie truly on the curve, the value of $S_{20\,(corr.)}$ should be plotted against the actual concentration of the first component. Where the amounts of both components could be estimated, the value of

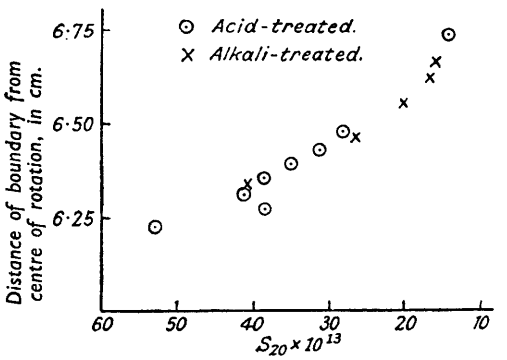
$S_{20\,(corr.)}$ for the first component, plotted against its concentration, does in fact lie well on the average curve (see Fig. 2*a*).

It is therefore likely that the sample of TNA investigated consists of a single homogeneous component with probably not more than 10% of heterogeneous material, provided that the solution is not excessively stirred.

In view of the rapid change of the relative viscosity of solutions of TNA at low concentrations of sodium chloride found by Creeth, Gulland, and Jordan (*loc. cit.*), sedimentation of a solution 0·05 g./100 ml. in 0·01M-sodium chloride was observed. The form of the boundary was the same as in 0·2M-sodium chloride, but it sedimented abnormally slowly, $S_{20\,(corr.)} = 8{\cdot}0 \times 10^{-13}$, as compared with $10{\cdot}8 \times 10^{-13}$ in 0·2M-sodium chloride. Calculation of the " salt effect " (Svedberg and Pedersen, " The Ultracentrifuge ", Oxford, 1940), using a value for the mobility of TNA of $1{\cdot}92 \times 10^{-3}$ cm.²/volt. sec. (Creeth, Gulland, and Jordan, private communication), showed that correction for it is negligible.

2. *TNA treated at* pH 3·5 (*Table* II).—All experiments were done with solutions containing 0·5 g./100 ml. in 0·2M-sodium chloride.

FIG. 3.



Plot of S_{20} *against distance of boundary from centre of rotation for gel components of acid- and alkali-treated thymonucleate.*

(*a*) A neutral solution of TNA was brought to pH 3·5; the time from the addition of acid to the beginning of observations was about 40 minutes. The diagrams (Fig. 1*c*) show two components, one giving a sharp and the other a broad boundary. It is likely that the former is homogeneous and asymmetric : the latter is certainly rather heterogeneous, but it is possible that some of its breadth is due to a greater rate of diffusion, as a result of disaggregation into less asymmetric particles.

(*b*) A neutral solution was brought to pH 3·5 and neutralised again after varying periods (Figs. 1*d*, *e*, *f*). The diagrams showed behaviour closely similar to that at pH 3·5; there was some variation of the sedimentation constants, but there seemed to be no important progressive change.

(*c*) A sample of TNA, which had been brought to pH 3·5 for 10 minutes, neutralised, precipitated immediately, and dried (supplied by Gulland, Jordan, and Taylor, *loc. cit.*) was dissolved and run at pH 7. The diagrams (Fig. 1*g* and *h*) show a quite different composition from the above; there was a heterogeneous component of S_{20} $10{\cdot}9 \times 10^{-13}$. In addition, there was a considerable amount of a component which gave a sharp boundary; its sedimentation constant was initially very high but fell rapidly as sedimentation proceeded (Fig. 3); it had reached the bottom of the cell before the boundary of the slower component had left the meniscus.

3. *TNA at* pH 12·5 (*Table* III).—(*a*) A neutral solution of TNA 0·5 g./100 ml. was brought to pH 12·5 and run immediately (40 minutes to the first photograph). The solution contained a single component of sedimentation constant $3{\cdot}2 \times 10^{-13}$ (Fig. 1*i*) which gave a fairly sharp boundary, though less so than that of untreated TNA. The molecules of this material are certainly asymmetric and probably homogenous. A sample of the same solution was run 24 hours later : signs of a boundary were observed, but it had not separated from the meniscus after 2 hours at 1010 revolutions/sec. This indicates that at pH 12·5 a progressive disaggregation occurs into small particles.

(*b*) A neutral solution (0·5 g./100 ml.) was brought to pH 12·5 for 10 minutes, neutralised again to pH 7, and run at once. The material appeared to be very heterogeneous (Fig. 1*j*),

with recognisable components; the sedimentation constant of the faster of these could not be measured. A sample of the same solution, run 96 hours later, gave a similar diagram, showing in addition the presence of a small amount of a faster material (Fig. 1*k*).

(*c*) A sample of TNA, which had been brought to pH 12·5 for 10 minutes, neutralised, precipitated, and dried (supplied by Gulland, Jordan, and Taylor, *loc. cit.*) was dissolved at 0·37 g./100 ml. and run at pH 7. It showed a single heterogeneous component (Fig. 1*l*). A second sample, which had stood for 96 hours after neutralisation before being precipitated showed, by contrast, a heterogeneous component and a considerable amount of gel-forming material (Fig. 1*m*); this showed the same sedimentation properties as that obtained following acidification [Fig. 3; see para. 2 (*c*)].

Discussion.

The values found for $S_{20\,(corr.)}$ and for D_{20} (in 0·2M-sodium chloride) for TNA, extrapolated to zero concentration, are $12{\cdot}6 \times 10^{-13}$ and $8{\cdot}1 \times 10^{-7}$. The corresponding value of S_{20} (in 0·2M-sodium chloride) is $12{\cdot}3 \times 10^{-13}$. From these values, and the value of 0·55 for the partial specific volume, we obtain $M = 8{\cdot}2 \times 10^{5}$, $f/f_0 = 4{\cdot}7$ and $a/b = 120$ for an unsolvated prolate spheroid, by the equation of Perrin (*J. Phys. Radium*, 1936, **7**, 1).

These results are in general agreement with those of Schmidt, Pickels, and Levene (*J. Biol. Chem.*, 1939, **127**, 251), Svedberg and Pedersen (*loc. cit.*), and Tennent and Vilbrandt (*J. Amer. Chem. Soc.*, 1934, **65**, 424). The former measured only the sedimentation constant and their samples were stated to have been polydisperse. One of the samples of Tennent and Vilbrandt appeared to be monodisperse, and its sedimentation constant was studied over a range of concentration from 0·29 to 0·025 g./100 ml. Their values for the sedimentation constant agree fairly well with ours at concentrations greater than 0·06 g./100 ml., but we failed to confirm the rapid increase of sedimentation constant which they reported at lower concentrations. They measured the diffusion constant at 0·29 g./100 ml. and obtained a value of $0{\cdot}61 \times 10^{-7}$: our results at lower concentration indicate a value of D lower than theirs. Evidently the preparation of TNA on which we have worked differs somewhat from theirs (they do not give its history), though the molecular dimensions of the two are broadly similar. It is interesting to note that lower values of M are obtained by calculating from our values for S and D at finite concentrations.

Treatments at both pH 3·5 and 12·5 cause profound, but different, changes. Treatment with acid leads to the formation of two fractions, one of which is relatively homogeneous and resembles the original material except that it is altered by reprecipitation. The formation of the heterogeneous component is probably the result of disaggregation. The changes brought about at pH 3·5 proceed to a limit, and no further important change follows subsequent neutralisation. Reprecipitation of the neutralised solution causes a further large change, the homogeneous component disappearing and a gel-forming material appearing in its place: this change is likely to be due to a re-aggregation.

The effect of alkali is progressive and more profound. The large change of sedimentation constant indicates disaggregation. Neutralisation produces a further change which is likely to be the result of partial re-aggregations. This seems to be progressive: a fast component appears after 96 hours at pH 7, and precipitation of this solution yields a product which contains a considerable fraction of a gel-forming material.

The similarity of the sedimentation constants of various fractions might suggest that the changes caused by acid and alkali result in essentially the same products; the evidence of the sedimentation constants alone, however, is insufficient to make this at all certain. Changes of sedimentation constant can be due to changes of molecular size, shape, and hydration. It is also impossible to distinguish clearly between the effects of heterogeneity and of change of diffusion constant on the sharpness of a boundary.

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