propionic acid hydrochloride, by Dr S. Wilkinson and Dr T. S. G. Jones, and of L-hydroxylysine hydrochloride, by Dr J. G. Heathcote, are gratefully acknowledged.

REFERENCES

- Barnett, E. de B. (1910). *J. chem. Soc.* **97**, 63.
 Bernheim, F. & Bernheim, M. L. C. (1942). *J. biol. Chem.* **145**, 213.
 Blaschko, H. & Duthie, R. (1945). *Biochem. J.* **39**, 478.
 Blaschko, H. & Hawkins, J. (1950). *Brit. J. Pharmacol.* **5**, 625.
 Boëseken, J. (1936*a*). *Rec. Trav. chim. Pays-Bas*, **55**, 1040.
 Boëseken, J. (1936*b*). *Proc. Acad. Sci. Amst.* **39**, 47.
 Bonnichsen, R. K. (1947). *Arch. Biochem.* **12**, 83.
 Chance, B. & Herbert, D. (1950). *Biochem. J.* **46**, 402.
 Cromwell, B. T. (1943). *Biochem. J.* **37**, 722.
 Hirsch, J. G. (1953). *J. exp. Med.* **97**, 345.
 Kapeller-Adler, R. (1949). *Biochem. J.* **44**, 70.
 Keilin, D. & Hartree, E. F. (1951). *Biochem. J.* **49**, 88.
 Kenten, R. H. (1953). *Biochem. J.* **55**, 350.
 Kenten, R. H. & Mann, P. J. G. (1952). *Biochem. J.* **50**, 360.
 Kenten, R. H. & Mann, P. J. G. (1954). *Biochem. J.* **57**, 347.
 Laskowski, M. (1942). *J. biol. Chem.* **145**, 457.
 Powers, W. H. & Dawson, C. R. (1943). *J. gen. Physiol.* **27**, 181.
 Swedin, B. (1944). *Ark. Kemi Min. Geol.* **17A**, 27.
 Sumner, J. B. & Dounce, A. L. (1937). *J. biol. Chem.* **121**, 417.
 Tabor, H. (1951). *J. biol. Chem.* **188**, 125.
 Tabor, C. W., Tabor, H. & Rosenthal, S. M. (1954). *J. biol. Chem.* **208**, 645.
 Tsuchihashi, M. (1923). *Biochem. Z.* **140**, 63.
 Umbreit, W. W. (1945). In *Manometric Methods and Tissue Metabolism*, p. 9. Ed. by Umbreit, W. W., Burris, R. H. & Stauffer, J. F. Minneapolis: Burgess. Publ. Co.
 Werle, E. & Pechmann, E. (1949). *Liebigs Ann.* **562**, 44.
 Werle, E. & Raub, A. (1948). *Biochem. Z.* **318**, 538.
 Werle, E. & Roewer, F. (1950). *Biochem. Z.* **320**, 298.
 Werle, E. & Roewer, F. (1952). *Biochem. Z.* **322**, 320.
 Willstätter, R. & Stoll, A. (1918). *Liebigs Ann.* **416**, 21.
 Zeller, E. A. (1938). *Helv. chim. acta*, **21**, 880.
 Zeller, E. A. (1940). *Helv. chim. acta*, **23**, 1418.
 Zeller, E. A., Stern, R. & Wenk, M. (1940). *Helv. chim. acta*, **23**, 3.

The Sedimentation Behaviour of Mixtures of Hyaluronic Acid and Albumin in the Ultracentrifuge

By J. P. JOHNSTON*

Department of Biochemistry, University of Oxford, and Department of Physiology, Charing Cross Hospital Medical School

(Received 20 October 1954)

The work described here arose from an investigation of the synovial fluid of human knee joints: its other aspects are described by Johnston (1955*a, b*). In several samples from normal and nearly normal joints, it was observed that the lines representing the variation of the rate of sedimentation of the hyaluronic acid with concentration differed considerably from one sample to another. Since these differences appeared to be correlated with the concentration of protein in the sample, the mutual effects of hyaluronic acid and albumin on the sedimentation of the other were investigated further.

Johnston & Ogston (1946) showed that at a given concentration one protein sediments at a different rate (usually more slowly) in the presence of a second protein than it does alone. If this protein is the more slowly sedimenting of the two, its concentration is increased behind the sedimenting boundary of the faster component; the faster component undergoes an apparent (but unreal) fall of concentration. This increase in concentration of the slower

component would be expected to cause a reduction of its rate of sedimentation. In the systems studied by Johnston & Ogston (1946) the last effect was unimportant, since the sedimentation rates of the proteins studied did not vary rapidly with concentration. In the present case, however, the sedimentation rate of hyaluronic acid varies rapidly with its concentration (Ogston & Stanier, 1950, 1952) and it is shown that the effect of albumin on the sedimentation rate of hyaluronic acid can be explained by the operation of this effect, which increases the concentration of the hyaluronic acid when it is the slower component.

The rate at which hyaluronic acid sediments in the region of the cell in which albumin is present, and the converse case, have also been investigated, and an attempt has been made to explain the results obtained.

METHODS

Materials. Both ox and human synovial fluids were used. Knee-joint fluid from freshly slaughtered cattle was collected and stored as described by Ogston & Stanier (1950). Human synovial fluid obtained at operation at the Wingfield-Morris Orthopaedic Hospital, Oxford, was supplied by

* Present address: Medical Division, Chemical Defence Experimental Establishment, Porton, Wilts.

Mr R. G. Taylor and stored as above but with full sterile precautions. A single human specimen was obtained at post-mortem (12 hr. after death).

Albumin was freeze-dried bovine plasma albumin (Armour Laboratories, Batch 10493) stored at 2°.

Preparation and estimation of hyaluronic acid. All fluids were dialysed against a standard buffer (0.2M-NaCl; 0.0077M-Na₂HPO₄; 0.0023M-KH₂PO₄; pH 7.3). Concentration was estimated by mucin clot formation in undialysed solutions, or after dialysis where a firm clot could

still be obtained. In the former cases possible volume changes on dialysis were allowed for by quantitative transference with washing from the sac after dialysis. Clot preparation, washing and drying were carried out as described by Ogston & Stanier (1952).

Sedimentation. A Svedberg oil-turbine ultracentrifuge with a Philpot (1938) diagonal schlieren optical system was used. Running procedure, sedimentation constant calculation and concentration estimations from boundary areas were carried out as described by Cecil & Ogston (1948). In

Fig. 1. Variation in the reciprocal of the sedimentation constant of hyaluronic acid in different samples of synovial fluid with the logarithm of Ostwald viscosity. Different symbols indicate dilutions of the same synovial fluid. A, as measured; B, after correction for the presence of albumin.

Table 1. Sedimentation constants, concentrations and viscosities of hyaluronic acid and albumin from human synovial fluids

Albumin concentrations were estimated by measurement from the ultracentrifuge diagram. Samples A and B came from normal joints; samples C–F were obtained at operation on cartilages in knee joints (there was no recent history of acute trauma, the joints showed minimal macroscopic abnormality and the fluid present varied from 0.3 to 4 ml.); sample G was obtained in the same way but consisted of four pooled samples; sample H came from a case of acute trauma with a large effusion.

Sample	Sedimentation constants (Svedberg units)		Concentration		Log ₁₀ η _{rel.}
	Hyaluronic acid	Albumin	Hyaluronic acid (mg. mucin/ 100 ml.)	Albumin (g./100 ml.)	
A	2.92	3.96	80	0.384	0.76
	4.22	4.26	40	0.192	0.46
	5.38	4.30	20	0.096	0.26
	6.41	4.56	15	0.072	0.18
B	3.06	3.91	—	0.43	0.69
C	1.66	3.07	725	2.52	1.24
	2.30	3.64	440	1.54	0.78
	2.84	3.75	265	0.922	0.51
	3.33	4.00	220	0.764	0.41
	4.24	4.25	120	0.383	0.22
D	1.52	3.49	680	1.78	1.68
	2.30	3.98	300	0.780	0.82
	3.67	4.11	117	0.306	0.35
	4.65	4.34	74	0.194	0.24
E	2.74	4.01	320	0.632	0.51
F	1.83	3.70	790	1.29	1.25
G	1.84	3.18	680	2.27	0.92
H	1.42	2.89	250	4.51	0.95

graphing results either the sedimentation constant or its reciprocal was used, depending on which gave a plot nearest to a straight line.

Viscosity. Viscosity was measured with an Ostwald viscosimeter (capacity 0.7 ml.; water-flow time 27 sec.).

All dilutions were made by weight.

RESULTS

Human synovial fluids. Eight fluids were investigated over a range of dilutions. The rates of sedimentation of the hyaluronic acid are shown in Fig. 1A, plotted against $\log_{10} \eta_{rel.}$ Table 1 also gives the rates of sedimentation of the albumin and the concentrations of hyaluronic acid (by mucin estimation) and of albumin + globulin. In no case was the albumin/globulin ratio, estimated by measurement of the sedimentation diagram, less than 7; accordingly albumin + globulin has been treated as a single component.

Effect of added albumin. In a series of experiments the albumin concentration was varied by the addition of Armour bovine albumin: the concentrations were adjusted so that three concentrations of hyaluronic acid were used over a range of concentrations of total albumin. The results are shown in Fig. 2 and Table 2.

Serial dilutions. At the concentrations normally found in synovial fluid, hyaluronic acid sediments more slowly than does albumin. However, since its rate of sedimentation increases much more rapidly with dilution than does that of albumin, at extreme dilutions the hyaluronic acid sediments more rapidly. Although observation of the hyaluronic acid boundary becomes difficult, it is possible to obtain fairly accurate values for its sedimentation

constant over a part of this range of concentration. In Fig. 3 the sedimentation rates of hyaluronic acid are shown for two such series of dilutions. Each shows a marked break between the regions where the hyaluronic acid sediments more slowly and more rapidly than the albumin.

Fig. 2. Variation in the sedimentation rate of hyaluronic acid with concentration of albumin. $\square$, human synovial fluid J; $\circ$, ox synovial fluid I. The curves represent different concentrations of hyaluronic acid. The concentrations, expressed as mg. mucin/100 ml., were 400 (curve A), 300 (curve B) and 100 (curve C).

Table 2. *Sedimentation constants, concentrations and viscosities of hyaluronic acid and albumin from normal human and ox synovial fluids with and without added albumin*

Sedimentation constants and concentrations expressed as in Table 1. Sample I was ox fluid; sample J was a human post-mortem sample.

Sample	Sedimentation constants		Concentration		$\log_{10} \eta_{rel.}$
	Hyaluronic acid	Albumin	Hyaluronic acid	Albumin	
I	2.30	3.88	300	1.50	0.99
	2.03	3.42	300	2.68	0.99
	1.90	3.44	300	3.84	1.00
J	1.96	3.78	400	0.90	1.65
	1.67	3.63	400	2.50	1.65
	1.40	2.87	400	5.30	1.65
	2.50	4.16	290	0.66	1.23
	3.12	4.28	190	0.44	0.90
	3.98	4.15	133	0.30	0.64
	4.41	4.40	100	0.23	0.51
	3.34	3.98	100	1.80	—
	1.69	3.17	100	4.80	—
	1.23	1.57	100	14.5	—
	4.76	4.30	80	0.18	0.43
	5.07	4.54	65	0.15	0.35
	6.10	4.70	50	0.11	0.29
	7.40	4.61	40	0.09	0.25

The apparent concentrations of the albumin were also measured in these experiments: in Fig. 4 these are plotted in the form of the apparent concentration $\times 100/\text{expected concentration}$, against the difference between the sedimentation rates of the albumin and hyaluronic acid. Again the results (from three series of dilutions) show a discontinuity between the cases where hyaluronic acid is the slower or faster component.

Fig. 3. Variation of $1/S$ of hyaluronic acid with $\log \eta_{rel.}$, showing a discontinuity where the hyaluronic acid changes from slower to faster component. $\circ$, sample A; $\bullet$, sample J; —, hyaluronic acid forming the trailing boundary; $\cdots$, hyaluronic acid and albumin boundaries superimposed; ---, hyaluronic acid forming the leading boundary.

Fig. 4. The increase in concentration of the albumin boundary in hyaluronic acid-albumin mixtures when, on dilution, albumin becomes the more slowly sedimenting component. Albumin concentration expressed as a percentage of the value predicted from its concentration in the undiluted synovial fluid.

Sharpness of the hyaluronic acid boundary. The boundary of hyaluronic acid is normally too narrow to allow its concentration to be measured in the usual way, from the area of the schlieren peak. However, there are two ways in which qualitative indications of concentrations can be obtained: (i) at the same optical setting and time after reaching full speed, the width of the boundary peak increases with concentration. In a pair of runs, with the same concentration of hyaluronic acid (mucin = 400 mg./100 ml.) and with albumin concentrations 0.9 and 5.3 g./100 ml. respectively, the width of the hyaluronic acid peak was several times greater at the higher concentration of albumin, suggesting that the hyaluronic acid boundary had undergone concentration; (ii) at low concentrations the hyaluronic acid peak becomes noticeably diffuse after sedimentation has continued for some time (cf. Ogston & Stanier, 1950); this occurs at earlier times as the hyaluronic acid becomes more dilute. Addition of albumin was found to lengthen the time before diffuseness became apparent.

DISCUSSION

Correction for the effect of albumin on the sedimentation rate of hyaluronic acid. The results given in Fig. 2 and Table 2 show that, within the range studied, $1/S$ of hyaluronic acid is increased by an amount proportional to the concentration of albumin and independent of that of hyaluronic acid. This provides an empirical basis for correcting for the effect of albumin. Confidence in this is increased by the fact that the $1/S$ intercepts (albumin = 0) of the curves for the different hyaluronic acid concentrations in Fig. 2 agree closely

Fig. 5. Variation in the sedimentation rate of hyaluronic acid with concentration. $\times$, Direct measurement on residue after ultrafiltration; $\circ$, values obtained by calculation from synovial fluid measurement.

with the values determined on a sample of hyaluronic acid freed from protein by ultrafiltration (Ogston & Stanier, 1950, 1952). This ultrafiltered sample was kindly supplied by Dr J. E. Stanier (Fig. 5).

The application of the correction to the results shown in Fig. 1A produces those shown in Fig. 1B. The variation between the different human samples is markedly reduced, though the variation is still outside the experimental error. This method of correction is based directly on the results of experiments and is independent of any theoretical considerations.

Boundary anomalies in solutions of hyaluronic acid and albumin. The type of boundary anomaly discussed by Johnston & Ogston (1946) might be expected to occur in this system, since it is reasonable to suppose that either component, at a given concentration, will sediment more rapidly when present alone than in the presence of the other (i.e. below the faster boundary). This would result in the concentration of the slower component, estimated from the sedimentation diagram, being increased and the apparent concentration of the faster component being correspondingly decreased, in comparison with their known concentrations in the mixed solution used. Unfortunately in the present case direct demonstration of these effects is not wholly possible.

Effect of hyaluronic acid on albumin. When the albumin boundary is the faster, its apparent concentration should be lowered, but this effect will be negligible, because of the small refractive contribution of the hyaluronic acid relative to that of albumin. When albumin forms the slower boundary there should be an appreciable change in its concentration. These predictions are in agreement with the results obtained (Fig. 4).

Effect of albumin on hyaluronic acid. The corresponding effect, when hyaluronic acid is the slower component, cannot be similarly confirmed because the sharpness of the hyaluronic acid boundary prevents direct estimation of its apparent concentration. However, several of the results obtained suggest that the effect is taking place: (i) Since the sedimentation rate of hyaluronic acid varies rapidly with concentration, the marked effect of albumin in lowering the sedimentation rate of hyaluronic acid (when the latter is the slower component) (Fig. 2), is most easily explained by an increase of the concentration of hyaluronic acid behind the albumin boundary. (ii) The curve of $1/S$ against viscosity (Fig. 3) is consistent with this explanation, the hyaluronic acid, as predicted, showing a reduced rate of change of sedimentation constant with dilution of the fluid in the range where the hyaluronic acid is changing from slower to faster component. (iii) The changes produced by

albumin on the width of the hyaluronic acid peak in the sedimentation diagram and on the time required before it becomes diffuse come nearest to a direct demonstration, both suggesting an increase of the concentration of hyaluronic acid sedimenting as the slower component.

The rates of sedimentation of hyaluronic acid and of albumin each in the presence of the other

Hyaluronic acid sedimenting through albumin. The rate of sedimentation of hyaluronic acid through albumin can be observed directly only when hyaluronic acid forms the faster boundary, i.e. at the greatest dilutions; under these conditions, the effect of albumin is very small. At higher concentrations, where hyaluronic acid forms the slower boundary, its rate of sedimentation through albumin (i.e. below the albumin boundary) can be arrived at only indirectly, as follows: Johnston & Ogston (1946) expressed the boundary anomaly by

$$C'/C = (S_1 - S'_2)/(S_1 - S_2),$$

where C is the original concentration of the slower component, C' its concentration behind the faster boundary, S_1 the rate of movement of the faster component (i.e. of the faster boundary), S_2 and S'_2 the rates of movement of the slower component above and below the faster boundary. This relationship is now used to estimate S'_2 . C is known; S_1 and S_2 are directly measured; C' is estimated from the relationship between concentration and $1/S$ in the absence of albumin (Fig. 5). The equation can now be solved for S'_2 . The resulting values (Fig. 6) show

Fig. 6. Variation in the sedimentation constant of hyaluronic acid in albumin with concentrations of albumin and hyaluronic acid. Curves calculated from data obtained in runs in which hyaluronic acid formed the slower boundary. Curve A, albumin concentration 4%; curve B, 2%, and curve C, 0.5%.

that the rate of sedimentation of hyaluronic acid, of given concentration, through albumin, is reduced approximately in proportion to the concentration of albumin. This reduction would be expected from the effect of albumin in increasing both the density and the viscosity of the medium through which the hyaluronic acid is sedimenting.

Albumin sedimenting in the presence of hyaluronic acid. Since albumin is normally the faster component, its sedimentation rate in the presence of hyaluronic acid is directly measured (Table 2). These values are plotted in Fig. 7 to show the effect of constant concentrations of hyaluronic acid on varying concentrations of albumin. Though there is

Fig. 7. Variation in the sedimentation constant of albumin in hyaluronic acid with concentrations of hyaluronic acid and of albumin. Δ , hyaluronic acid concentration = 100 mg. mucin/100 ml.; $\circ$, = 300 mg. mucin/100 ml.; $\square$, = 700 mg. mucin/100 ml.

Fig. 8. Values for the effective (solvated) specific volume of the hyaluronic acid molecule obtained by calculation from the sedimentation of hyaluronic acid-albumin mixtures.

some scatter of the points, the relationship obtained is clearly of quite a different character from that shown in Fig. 6, for the converse case. The striking feature is that any given concentration of hyaluronic acid appears to have no effect on the sedimentation of albumin when the concentration of albumin is zero, although it reduces the rate of sedimentation of albumin at any finite concentration the more so the higher the concentration of hyaluronic acid. This must mean that the sedimentation of separate molecules of albumin is not appreciably interfered with by the presence of hyaluronic acid, and consequently it follows that, in the presence of hyaluronic acid (as in its absence) the fall of sedimentation rate with increasing concentration of albumin must be ascribed to the mutual interference of albumin molecules. The simplest hypothesis of how this effect could be increased by the presence of hyaluronic acid is that the hyaluronic acid denies solution space to the albumin, i.e. that the hyaluronic acid particles are in part impenetrable by the albumin. The albumin would then occupy only a fraction of the total solution, and its effective concentration would be raised. This increase of effective concentration would then account for the lowered rate of sedimentation, in accordance with the behaviour of albumin alone and the theory of Fessler & Ogston (1951).

On the basis of this hypothesis, values of the excluded volume per gram of hyaluronic acid have been calculated from the values in Tables 1 and 2, where albumin forms the faster boundary, and are given in Fig. 8. In view of the model of the hyaluronic acid particle deduced by Ogston & Stanier (1951, 1952), namely a loose and voluminous random coil, this hypothesis is clearly oversimplified, since such a particle is not likely to be uniformly 'impenetrable' over its whole volume. Indeed, both the concept of the 'volume' of such a particle and its impenetrable space must be statistical concepts. However, it is a striking fact that the estimates of both quantities are of the same order of magnitude (Ogston & Stanier, 1952, give a revised estimate of about 1.5×10^3 ml./g. for the effective dynamic volume of ox hyaluronic acid in very dilute solution). The hypothesis itself, at first sight, appears to be unreasonable, since the exclusion of albumin could not possibly be due to steric causes, which would predict an impenetrable volume not more than 5% of the dynamic volume.

SUMMARY

1. Experiments have been done on the ultracentrifugal sedimentation of hyaluronic acid and protein (albumin) in human synovial fluids, and in human and ox synovial fluid with added albumin.

2. An empirical correction for the effect of albumin on the sedimentation of hyaluronic acid is obtained, and applied to the results obtained for human synovial fluids.

3. The effect of albumin on the sedimentation of hyaluronic acid, when the latter is the slower component, is shown to be consistent with the 'McFarlane' (1935*a, b*) boundary anomaly.

4. The effect of albumin on the sedimentation of hyaluronic acid and the reverse effect, in regions of the cell where both are present, have been investigated. The former is shown to be consistent in general with current theory. The latter effect requires special explanation, and the hypothesis is put forward that a large fraction of the hyaluronic acid particle is not penetrable by albumin.

The author is most grateful to Dr A. G. Ogston for encouragement and for very helpful discussions; to Mr R. G.

Taylor of the Wingfield-Morris Orthopaedic Hospital, Oxford and to Dr Robb-Smith of the Radcliffe Infirmary, Oxford, for supplies of human synovial fluid, and to the Oxford Co-operative Society Ltd., for facilities at their Botley Road slaughterhouse.

REFERENCES

- Cecil, R. & Ogston, A. G. (1948). *Biochem. J.* **43**, 592.
 Fessler, J. H. & Ogston, A. G. (1951). *Trans. Faraday Soc.* **47**, 667.
 Johnston, J. P. (1955*a*). *Biochem. J.* **59**, 626.
 Johnston, J. P. (1955*b*). *Biochem. J.* **59**, 633.
 Johnston, J. P. & Ogston, A. G. (1946). *Trans. Faraday Soc.* **42**, 789.
 McFarlane, A. S. (1935*a, b*). *Biochem. J.* **29**, 407, 660.
 Ogston, A. G. & Stanier, J. E. (1950). *Biochem. J.* **46**, 364.
 Ogston, A. G. & Stanier, J. E. (1951). *Biochem. J.* **49**, 585.
 Ogston, A. G. & Stanier, J. E. (1952). *Biochem. J.* **52**, 149.
 Philpot, J. St L. (1938). *Nature, Lond.*, **141**, 283.

A Comparison of the Properties of Hyaluronic Acid from Normal and Pathological Human Synovial Fluids

By J. P. JOHNSTON*

Department of Biochemistry, University of Oxford, and Department of Physiology, Charing Cross Hospital Medical School

(Received 20 October 1954)

A number of studies have previously been carried out on synovial fluid from both human and animal sources. The present work was undertaken primarily to compare the hyaluronic acid in fluids from normal and pathological human knee joints. Because of the small volume of fluid available from a normal joint, investigation by electrophoresis or by preparation of ultrafilter-residue (Ogston & Stanier, 1950) was precluded and these methods were accordingly not used on pathological effusions. The methods used included ultracentrifugal and viscosity measurements, the formation of mucin, and the study of its composition. It was hoped that any difference between these properties in normal and pathological fluids might throw light on any changes in the hyaluronic acid in trauma and disease. Furthermore, an assessment of the results from these techniques might reveal a reliable and sensitive test for abnormality in joint fluids.

EXPERIMENTAL

Synovial fluids, sources and collection

Knee-joint fluid from freshly slaughtered cattle was collected and stored as described by Ogston & Stanier (1950). Human synovial fluid from knee joints was obtained with

full sterile precautions. The human samples, may be divided into three groups: (i) fluids from normal joints, (ii) fluids from joints as near normal as possible, and (iii) frankly pathological fluids. Those in the intermediate group were obtained only from joints which appeared at operation to be nearly normal; all injuries were past the acute phase and the joints had been previously rested. Brief data on the samples are given below.

Normal group. *A* and *B*: normal joint fluid obtained at operation, 1 ml. *J*: post-mortem sample obtained 12 hr. after death, 1 ml.

Intermediate group. *C*: internal derangement of the knee, approx. 15 ml. *D*: pooled sample from four cases of cartilage operation, approx. 12 ml. *E*: cartilage operation. Slightly blood-stained, 8 ml. *F*: cartilage operation, 3 ml. *G*: cartilage operation, 8 ml.

Pathological group. *H*: acute trauma with large effusion, approx. 40 ml. *K* and *L*: untreated case of rheumatoid arthritis. Fluids from right and left joints respectively.

M and *N*: from the same joints as *K* and *L* after treatment with cortisone. Samples *K-N* were obtained by partial aspiration of large effusions.

Storage. All fluids were cooled to 4° as soon as possible. Human fluids retained their viscosity at this temperature for from 3 weeks to several months, despite brief removal to room temperature and contamination on withdrawal of part of the sample. After 12 hr. at room temperature a fluid fell in viscosity and therefore during dialysis or prolonged experiments all fluids were kept at 4°, but no sterile precautions were taken for brief experiments. Fluids were

* Present address: Medical Division, Chemical Defence Experimental Establishment, Porton, Wilts.