CXLVI. THE BEHAVIOUR OF PATHOLOGICAL SERA IN THE ULTRACENTRIFUGE.

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(Received April 1st, 1935.)

IN a previous paper [McFarlane, 1935, 1] we have described the results of the examination of horse serum albumin and globulin in the ultracentrifuge, both separately and in the form of mixtures of the two. It was shown that these two proteins behave ideally in dilute mixtures of the two, but in concentrated mixtures their general behaviour is abnormal. In particular, each molecular fraction is present in abnormal concentration, there being always more of the lighter ("albumin") fraction present than corresponds to the albumin added and correspondingly less of the heavier ("globulin") fraction. It is believed that there exists in mixtures of serum albumin and globulin a form of molecular dimensional equilibrium the exact nature of which has still to be discovered. In the course of this work a modified optical system for the ultracentrifuge has been developed which enables protein solutions as highly concentrated as untreated serum to be examined.

In a second paper [McFarlane, 1935, 2] we have described the application of this system in the analysis of the serum proteins of the normal cow, horse and man. Since it is clearly a matter of great interest to know the molecular dimensional distribution of the protein in untreated serum it was our aim to examine these sera as far as possible without dilution. In the case of human serum, however, it is necessary to dilute slightly in order to avoid a form of distortion in the photographs, the nature and cause of which is discussed. It has been shown that this slight dilution of a normal human serum does not affect the results, whereas dilution of all sera with more than 2-3 vols. of 1 % sodium chloride causes an alteration in the concentration of the fractions which is in exact agreement with the facts relating to artificial mixtures of albumin and globulin. We have found that the sera of all three species are constituted in a similar manner of three molecular fractions, two of which correspond approximately in sedimentation constants (with due allowance for the effect of protein concentration) to purified horse serum albumin and globulin. The chemical nature of the third fraction, of molecular weight slightly greater than albumin, is obscure and it is not proved that the substance is capable of an independent existence. It appears from the examination of sera from a series of normal healthy persons of various ages and of both sexes that the concentration ratios of the three fractions vary only within narrow limits in the presence of large variations in the concentration of total protein. The concentration of the globulin fraction in the series is (10 ± 5) % of the total protein.

In the present paper there are presented in detail the results of the examination of a series of pathological sera, chosen more or less at random, and obtained

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mostly from the general hospital in Upsala¹. We wish to emphasise the general nature of the investigation. There has been repeatedly in the course of these experiments a strong temptation to interrupt the series in order to pursue in greater detail the investigation of one or two particularly interesting sera. We resisted this because it was believed that a more useful purpose could be served at the moment by collecting a series of results which would give an impression—however superficial—of the field as a whole. This is our justification for omitting to proceed with several more detailed investigations which logical consideration will at once suggest to the reader might have been done on certain sera. We need hardly say that it is planned to investigate many of these individual problems in the immediate future.

In the two previous papers [1935, 1, 2] there will be found a complete description of the general technique employed in this work. We will only draw attention here to one important fact, *viz.* that all calculations of protein concentration are based on the apparent baseline of the sedimentation diagram. As a result the only method of detecting the presence of any polydisperse, and presumably denatured, protein is by comparing the protein refraction increment of the serum, as measured with the refractometer, with the total refraction increment from the area of the sedimentation curve. We have, therefore, in most cases, dialysed a portion of the serum at constant volume against 1 % sodium chloride and measured the refractive indices of solution and dialysate using the difference prism of the Pulfrich refractometer and the sodium D line. In certain cases of nephritis the serum was characteristically turbid owing to suspended lipid matter and considerable difficulty was experienced in detecting any transmitted beam in the refractometer. Centrifuging for an hour or more at 3000 r.p.m. in a standard laboratory centrifuge failed to produce any detectable reduction in the degree of turbidity. The effect of this turbidity in the centrifuge-cell as seen from the camera is to give rise to a green coloration due to the selective scattering of the lower wave-lengths in the mercury spectrum. It is interesting to record in these cases that as the ultracentrifuge accelerated a band of clear serum appeared at the bottom of the cell and the green coloration invariably disappeared into the region of the meniscus within 10–15 minutes from starting, and before the rotor had attained a speed of 25,000 r.p.m.

The conditions of the various experiments have been made more comparable by employing as far as possible centrifuge-cells 2.0 mm. in thickness and by using centrifugal forces uniformly in the region of 250,000 times gravity.

Each experiment is recorded under the initials of the donor of the serum and the clinical diagnosis of the condition. In the form of an appendix there is given a series of notes on each case, those features in the history being stressed which might have a connection with the state of the serum proteins.

In accordance with previous practice we shall refer to the fractions in a serum as *A* and *G* which correspond in sedimentation constants to purified horse serum albumin and globulin, and to other fractions as *X*, *Y*, *Z*, in order of increasing sedimentation constant.

In order to determine whether two fractions correspond in sedimentation constants it is necessary to take into consideration the concentration in which the fractions are present. This should be possible in the case of the lightest fractions with considerable accuracy since the boundary is sedimenting in a

¹ I am greatly indebted for facilities to obtain pathological sera to Profs. G. Bergmark, G. Nyström, and Axel Westman of Kungl. Akademiska Sjukhuset, and to Dr O. Bratt of Epidemisjukhuset, Upsala.

medium which is free of other proteins. In the case of the heavier fractions however, this is not so, and it is not possible with the data at present available to effect more than an approximate comparison of the sedimentation constants of corresponding *X* or *G* fractions. In order to facilitate the identification of *A* fractions we have constructed the graph in Fig. 1 by plotting the values already

Fig. 1. Showing the relationship between the refraction increments and sedimentation constants of the *A* fractions of normal human sera. Data taken from Table XVII of earlier paper [1935, 2].

found for the *A* boundary sedimentation constants of normal human sera, and shown in Table XVII of a previous paper [1935, 2], against the refraction increments ($n_1 - n_0$) of the same fractions. We shall have occasion to refer to it in the description of the following experiments.

In the preceding paper [1935, 2] we have calculated the concentrations of the protein fractions in g./100 ml. from the specific refraction increment (α) values of the albumins and globulins described in the literature. In this work we shall calculate only the total protein concentrations using the values α (total horse serum) = 0.00184, and α (total human serum) = 0.00202, both for the sodium *D* line.

Before describing the experiments with pathological human sera there will be given some results obtained with two samples of antidiphtheritic horse serum kindly supplied by Messrs Burroughs and Wellcome, Beckenham, Kent.

Exp. 1. Antidiphtheritic horse serum.

Serum I, containing 200 units of antitoxin, was examined undiluted in a cell 2.00 mm. in thickness, and excellent scale photographs were obtained with the scale 1.0 cm. from the centre of the cell. When allowance is made for the refractive index of the quartz cell plate this distance corresponds to an optical distance of 0.78 cm. The experimental conditions and results are shown in Table I, and Fig. 2 *a* shows the curve of an exposure taken 83 minutes after reaching full speed. The distance separating the two boundaries is small, but owing to the small amount of boundary spreading in this concentrated mixture, it is sufficient to give a symmetrical graphical delineation of both boundary curves. The sedimentation constant of the *G* fraction is unusually low. Owing to the high concentration of this fraction, however, and to the fact that the *G* molecules are sedimenting in a dense and viscous medium of *A* molecules, the effect of which is not taken into account in the $S_{W,20}$ figure, the value of the

constant is not inconsistent with the fraction having a molecular weight the same as that of horse serum globulin. We can find no evidence of an *X* fraction such as is present in normal horse serum, but this may be present in low concentration and not detectable because of the overlapping of the two boundaries.

Fig. 2a

Fig. 2b.

Table I. Results obtained in the examination of two antidiphtheritic horse sera.

	Serum 1. Undiluted	Serum 2. Undiluted	Serum 2. Diluted with ½ vol. 1 % NaCl
Cell thickness (mm.)	2.00	1.50	2.00
Optical scale distance (cm.)	0.78	1.12	1.69
Temperature inside cell at time of first exposure	36.3°	33.9°	33.4°
Temperature inside cell at time of last exposure	38.6°	36.1°	35.0°
Approximate duration of run (hours)	2½	2½	2
Mean centrifugal force used (times gravity)	245,000	255,000	250,000
Total $n_1 - n_0$, by refractometric measurement on the serum and its dialysate; $\lambda = 589 m\mu$	0.0204	0.0177	0.0118
Corresponding total protein concentration (g./100 ml.)	11.1	9.62	6.41
Total $n_1 - n_0$, corrected to $\lambda = 366 m\mu$	0.0220	0.0191	0.0127
Average $S_{w, 20} \cdot 10^{13}$ A fraction	2.97	3.00	3.86
G fraction	3.71	3.86	5.11
Average $n_1 - n_0$, calculated from sedi- mentation diagram, $\lambda = 366 m\mu$ A fraction	0.0102	0.0090	0.0052
G fraction	0.0109	0.0089	0.0073
Total	0.0211	0.0179	0.0125
Difference between measured and calculated total $n_1 - n_0$ expressed as a percentage of the former	-4 %	-6 %	-2 %
Proportion of total calculated $n_1 - n_0$ due to G fraction	52 %	50 %	58 %
Spreading coefficient of A boundary (cm. ² /sec. $\times 10^7$)	0.4	0.3	3.8

From our experiences with normal sera we are of opinion that only very small amounts of *X* fraction, if any, can be present. The value of the spreading coefficient of the A boundary is in favour of this fraction being homogeneous.

Serum 2, containing 1200 units of antitoxin, was also examined without treatment of any kind and gave rise to sedimentation diagrams very similar to those of serum 1. The results of this experiment are shown in Table I and it will

be seen that they are generally similar to the results of the previous experiment. In particular, it is noteworthy that there is no increase in the proportion of *G* fraction which might correspond to the increased antitoxic value of serum 2. We have also examined the same serum after slight dilution with 1 % sodium chloride. Fig. 2 *b* shows the curve of an exposure from this experiment taken 81 minutes after reaching full speed. It will be seen from this and from the results in Table I that there has occurred a considerable increase in the concentration of the *G* fraction, and a proportionate decrease in that of the *A* fraction. The extent of separation of the two boundaries has not been greatly affected by the dilution although the sedimentation constants of both the *A* and *G* fractions are quite markedly increased. There is also a substantial increase in the degree of spreading at the *A* boundary.

In all three experiments there is very close agreement between the measured and calculated total protein refraction increment values. This is probably accidental since with very short scale distances the experimental error is increased considerably.

Exp. 2. Serum from A.J. (ovarian carcinoma).

This serum from an advanced case of malignancy was examined first without any treatment and then after dilution. Large quantities of pleural exudate were also available which resembled the serum closely in colour and general appearance. A sample of this was examined also without treatment of any kind. The results of all three experiments are collected in Table II, and Fig. 3 *a* shows the curve of an exposure in the first experiment taken 112½ minutes after reaching full speed. Comparison of this curve with that on Fig. 14 of the previous paper [1935, 2] showing a typical normal human serum, shows that gross changes have taken place.

In this malignant serum three fractions are present which we have labelled *A*, *G* and *X*. The sedimentation constant of the lightest fraction, *viz.* 2.32, is considerably lower than the corresponding value for the *A* fraction of a normal human serum obtained from Fig. 1, *viz.* 3.4. The difference between these values is much greater than could be explained by any experimental error. The value for the *A* fraction in Table II is indeed lower than any value for an *A* fraction recorded in Table XVII of the previous paper irrespective of concentration. The evidence that we are dealing with a substance of lower molecular weight than serum albumin seems therefore to be quite definite. It is to be noted however from Table II that the sedimentation constants of the *X* and *G* fractions in this serum are unusually low, and also that the sedimentation constants of the three fractions bear approximately the same ratios to each other as do those of the *A*, *G* and *X* fractions in normal human sera. These facts suggest either that all the fractions have abnormally low molecular weights or else that some common factor is acting on normal *A*, *G* and *X* fractions to make them sediment abnormally slowly.

There is no evidence in the large number of experiments carried out by Svedberg and collaborators on purified proteins of the existence of any such obscure influence. We have noted, however, that in their experiments a better degree of agreement is to be found between the values for the sedimentation constant of a particular protein than we have obtained between values for the *A* fraction of normal human sera (*cf.* Fig. 1). In whole serum it is possible that some factor, perhaps electrochemical in nature, or some substance, possibly lipid, by influencing the molecular shape or charge, or the viscosity at the

surface of the particles, may cause a change in the sedimentation constant without altering the molecular weight. These possibilities cannot be excluded so long as we are examining a solution of relatively undefined composition. So long as they exist an element of doubt attaches to our method of identifying the serum fractions, particularly in cases where all the fractions have abnormal sedimentation constants. It cannot therefore be said without further investigation whether any or all of the fractions in the serum of A.J. differ in molecular weight from the *A*, *G* and *X* fractions of normal sera. As a matter of convenience we have labelled these fractions meantime as *A*, *G* and *X*.

The proportions of both *X* and *G* fractions in this serum are abnormally high. This is perhaps best brought out by comparing the average proportion of *A* fractions in normal human serum, 66 %, with the proportion in this case,

Fig. 3a.

Fig. 3b.

Table II. Results obtained in the examination of serum and pleural exudate from A.J. (ovarian carcinoma).

	Serum A.J. Undiluted	Serum A.J. Diluted with 4 vols. 1 % NaCl	Pleural exudate A.J.
Cell thickness (mm.)	2.00	4.00	2.00
Optical scale distance (cm.)	3.64	9.64	5.14
Cell temperature (first exposure)	31.9°	30.6°	31.6°
Cell temperature (last exposure)	34.5°	32.9°	34.1°
Approximate duration of run (hours)	3½	2	2½
Mean centrifugal force used (times gravity)	245,000	240,000	245,000
Total $n_1 - n_0$, by refractometer $\lambda = 589 m\mu$	0.0128	0.00256	0.00745
Corresponding total protein concentration (g./100 ml.)	6.34	1.27	3.69
Total $n_1 - n_0$, corrected to $\lambda = 366 m\mu$	0.0138	0.00276	0.00805
Average $S_{w, 20} \cdot 10^{13}$			
<i>A</i> fraction	2.32	4.66	3.51
<i>G</i> fraction	4.20	6.93	5.73
<i>X</i> fraction	2.73	—	—
Average $n_1 - n_0$, from sedimentation diagram, $\lambda = 366 m\mu$			
<i>A</i> fraction	0.0050	0.00146	0.0056
<i>G</i> fraction	0.0031	0.00093	0.0021
<i>X</i> fraction	0.0036	—	—
Total	0.0117	0.00239	0.0077
"% difference" total $n_1 - n_0$	-14 %	-13 %	-4 %
Proportion of total $n_1 - n_0$ due to <i>G</i> fraction	26.5 %	39 %	27 %
Proportion of total $n_1 - n_0$ due to <i>X</i> fraction	31 %	—	—
Spreading coefficient of <i>A</i> boundary (cm. ² /sec. 10 ⁷)	—	21.3	16.1

42.5 %. It is not possible to effect a symmetrical graphical separation of the overlapping *A* and *X* curves in this serum for reasons discussed in the previous paper, so that the *A* and *X* concentration values given are not exact. The combined *A* + *X* value is exact, and in our graphical estimation of the *A* and *X* concentrations in the present case we have given the benefit of the doubt to the *A* fraction. The value for the *A* fraction can therefore be regarded as an upper limit to the true value. Similarly, the true *X* value is not less than the one given. Also, owing to the extent of overlapping of the *A* and *X* boundaries we cannot estimate the spreading coefficient of the *A* boundary.

In the second experiment the serum was diluted with 4 volumes of 1 % sodium chloride. The sedimentation rates of both boundaries are markedly increased. The degree of *A* boundary spreading is also much increased and definite signs of a separation of the lighter fraction into *A* and *X* fractions cannot consequently be seen. The curves of the more slowly sedimenting boundary are however all unsymmetrical in a manner suggesting the presence of two fractions in concentrations not differing greatly from each other. The heterogeneity of the fraction is confirmed by the general dimensions of the curve and the extremely high value of the spreading coefficient, *viz.* 21×10^{-7} cm.²/sec. In Table IV of a previous paper [1935, 1, p. 423] we have shown the spreading coefficient values for a boundary of recrystallised horse serum albumin (sedimenting under standardised centrifuge conditions) plotted against the protein refraction increment of the solution. From this curve we find that a sedimenting protein boundary, assumed to be homogeneous, of the same protein concentration as the *A* boundary in this experiment has a spreading coefficient of approximately 6.0×10^{-7} cm.²/sec.

Table II also shows that there has been a definite increase in the proportion of the total protein refractivity due to the *G* fraction on diluting the serum.

In the third experiment we examined the untreated pleural exudate. Fig 3 *b* shows the curve of an exposure taken 83 minutes after reaching full speed. The results of this experiment are also shown in Table II. It happened that the total protein concentration in the pleural exudate was almost an exact mean between the corresponding values in the two preceding experiments. Table II shows that the sedimentation constants of the two fractions in the pleural exudate are also roughly a mean between the values in the former experiments. From this we deduce that the protein fractions in the pleural exudate have approximately the same mean molecular dimensions as the corresponding protein fractions in the serum.

From Fig. 3 *b* it will be seen that the *A* curve does not show any obvious signs of dividing into two components, but it does not have the perfect symmetry which has characterised the homogeneous proteins in our earlier experiments [1935, 1]. Once again, the view that we are concerned with a mixed fraction, the heterogeneous nature of which is obscured by the increased spreading of the boundaries, is confirmed by the general dimensions of the curve and by the high value of the spreading coefficient, *viz.* 16×10^{-7} cm.²/sec. The value for a purified albumin boundary of corresponding protein concentration is 1.6×10^{-7} cm.²/sec. The proportion of *G* fraction in the pleural exudate is in agreement with the other results and all conform with the view that the fluid is a passive transudate of whole serum which has undergone simple dilution in passing from the blood into the pleural cavity.

Exp. 3. Oxalate plasma from K.H.B. (acute phthisis).

In this case we obtained oxalate plasma and no preliminary dialysis was carried out. The plasma did not keep well over the period of the experiments but gave rise to a small deposit of fibrin. The amount of protein thus removed from the solution was not sufficient to affect materially the results obtained. In the first experiment we examined the undiluted plasma and obtained curves showing marked distortion of the *A* and *X* curves. The *G* curve was symmetrical, however, and from the area of this curve we find:

Calculated $n_1 - n_0$ of *G* fraction in undiluted plasma = 0.00318.

The plasma was then examined after dilution with one-third its volume of 1 % sodium chloride. Fig. 4*a* shows the curve of an exposure taken 98½ minutes after reaching full speed, and the general results of the experiment are recorded in Table III.

Fig. 4*a*. Fig. 4*b*.

Table III. *Showing the results obtained in the examination of oxalate plasma from K.H.B. (pulmonary tuberculosis).*

	Plasma diluted with ½ vol. 1 % NaCl	Plasma diluted with 1 vol. 1 % NaCl	Plasma diluted with 2 vols. 1 % NaCl
Cell thickness (mm.)	2.00	2.00	2.00
Optical scale distance (cm.)	2.74	6.14	6.14
Cell temperature (first exposure)	34.4°	32.8°	33.3°
Cell temperature (last exposure)	35.2°	34.1°	35.3°
Approximate duration of run (hours)	3½	2½	2½
Mean centrifugal force used (times gravity)	250,000	245,000	245,000
Average $S_{w,20} \cdot 10^{13}$			
<i>A</i> fraction	2.79	3.23	4.17
<i>G</i> fraction	4.72	5.40	6.22
<i>X</i> fraction	3.12	4.42	—
Average $n_1 - n_0$, from sedimentation diagram, $\lambda = 366 m\mu$			
<i>A</i> fraction	0.00454	0.00461	0.00274
<i>G</i> fraction	0.00324	0.00260	0.00219
<i>X</i> fraction	0.00338	—	—
Total	0.01116	0.00721	0.00493
Calculated total $n_1 - n_0$ of original undiluted serum	0.0149	0.0142	0.0148
Proportion of total $n_1 - n_0$ due to <i>G</i> fraction	29 %	36 %	44 %
Proportion of total $n_1 - n_0$ due to <i>X</i> fraction	30 %	—	—

A comparison of the curve with that of Fig. 3 *a* shows a close general similarity to exist between the two. The sedimentation constant of the lightest fraction, 2.79, is considerably lower than the value 3.5 for a normal *A* fraction in corresponding concentration (*cf.* Fig. 1). As in the previous experiment however the $S_{W,20}$ values for all the fractions are unusually low and bear approximately the same ratios to each other as do the values for the fractions of normal sera. We conclude that this plasma also represents a case in which it cannot be said without further investigation whether the fractions are identical with the fractions of normal sera.

The calculated value of $n_1 - n_0$ for the *G* fraction is interesting. If this value is corrected for the dilution we find:

Calculated $n_1 - n_0$ of <i>G</i> fraction in original plasma based on				
proportion found after dilution	...	...	...	0.00432

We conclude that unlike the state of affairs in a normal human serum the effect of even slight dilution of a pathological serum is to produce a change in the concentration ratio of the fractions which is apparent as an increase in the absolute concentration of the *G* fraction. We have studied the effect of dilution further in this case by examining the plasma after dilution with an equal volume, and with twice its volume, of 1 % sodium chloride. The results of these experiments are collected in Table III and call for little comment. It is apparent that the proportion of the *G* fraction increases steadily on dilution. We cannot say whether there is at the same time any change in the ratio of the *A* and *X* fractions because the two do not separate sufficiently in the more dilute solutions. Fig. 4*b* shows the curve of an exposure taken $97\frac{1}{2}$ minutes after reaching full speed in the experiment with plasma diluted with an equal volume of 1 % sodium chloride. The dual nature of the lighter boundary is rather obvious but we cannot resolve the curve graphically. In the final experiment the *A* curve has only one peak but it is unsymmetrical, with a squat appearance (*i.e.* a high ratio of breadth at the base to height) and an abnormally high spreading coefficient, *viz.* 9×10^{-7} cm.²/sec.

Exp. 4. Sera from B.I.L. and J.O.D. (scarlatina).

The object of this experiment was to examine the serum protein changes associated with the development of immunity. Sera from two patients in the early stages of scarlet fever were obtained and examined in the centrifuge. It was planned to obtain serum again from both patients in the later stages of the disease but owing to unforeseen circumstances this was only possible in one of the cases. This particular case (B.I.L.) was a child of four years and the first specimen of serum had a rather low total protein concentration. This fact induced us to examine the serum in the untreated state and curves were obtained which showed only slight distortion of the *A* and *X* curves. Fig. 5 *a* shows one of these curves obtained from a photograph taken 73 minutes after reaching full speed and the results are shown in Table IV.

The serum from the second case had a normal protein concentration and was therefore examined after slight dilution with 1 % sodium chloride. The results are also shown in Table IV. The sedimentation constant of the *A* fraction in the case of B.I.L.'s serum, 2.28, is again lower than the corresponding value for a normal *A* fraction obtained from Fig. 1, 2.9. Unlike the results in the earlier experiments the $S_{W,20}$ value for the *G* fraction in this case does not appear to be abnormally low. It is possible in this serum that the fraction we have labelled *X* is really an *A* fraction and that our *A* fraction is a new molecular type of lower molecular weight than serum albumin. The evidence however is inconclusive. The

fractions of J.O.D.'s serum correspond satisfactorily in sedimentation constants with the fractions of normal human sera. The proportions of *G* fraction in both sera fall within the limits of the formula $(10 \pm 5) \%$ which we have found to hold for normal sera.

Fig. 5a.

Fig. 5b.

Table IV. *Results obtained in the investigation of sera from two cases of scarlatina.*

	Serum B.I.L. (6th day of disease).	Serum B.I.L. (29th day of disease). Diluted 5 to 2 vols. 1 % NaCl	Serum J.O.D. (8th day of disease). Diluted 5 to 2 vols. 1 % NaCl
Cell thickness (mm.)	2.00	2.00	2.00
Optical scale distance (cm.)	0.785	2.28	1.685
Cell temperature (first exposure)	33.6°	35.7°	34.2°
Cell temperature (last exposure)	36.8°	36.2°	35.6°
Approximate duration of run (hours)	3½	3½	3
Mean centrifugal force used (times gravity)	255,000	250,000	255,000
Average $S_{w, 20} \cdot 10^{13}$			
<i>A</i> fraction	2.28	2.70	2.75
<i>G</i> fraction	5.08	4.95	4.90
<i>X</i> fraction	2.60	3.31	3.30
Average $n_1 - n_0$, from sedimentation diagram, $\lambda = 366 m\mu$			
<i>A</i> fraction	0.0070	0.0060	0.0067
<i>G</i> fraction	0.00102	0.0026	0.0014
<i>X</i> fraction	0.0046	0.0021	0.0024
Total	0.0126	0.0107	0.0105
Calculated total $n_1 - n_0$ of original serum	0.0126	0.0150	0.0147
Proportion of total $n_1 - n_0$ due to <i>G</i> fraction	8.1 %	24 %	13 %
Proportion of total $n_1 - n_0$ due to <i>X</i> fraction	36.5 %	19.5 %	23 %
Spreading coefficient of <i>A</i> boundary (cm. ² /sec. 10^7)	0.4	1.6	0.3

The serum of J.O.D. was dialysed at constant volume and the total protein refraction increment from refractometric measurements on serum and dialysate was found to be 0.0157 ($\lambda = 589 m\mu$), giving 0.0169 ($\lambda = 366 m\mu$). The percentage discrepancy between measured and calculated $n_1 - n_0$ is therefore in this experiment -13 %.

When the undiluted serum from B.I.L. was examined 23 days later we obtained curves showing marked distortion at the *A* and *X* boundaries. These

curves however showed symmetrical G boundary curves which gave the following value:

Calculated $n_1 - n_0$ of G fraction in undiluted serum (B.I.L.) 0.00336

The serum was re-examined after diluting 5 vols. of serum with 2 vols. of 1 % sodium chloride. Fig. 5 *b* shows the curve from an exposure taken 86½ minutes after reaching full speed and the general results are shown in Table IV. The distortion of the A and X boundaries has disappeared and there is obviously an increased proportion of G fraction present. In the period of 23 days during which immunity to scarlatina developed the total serum protein concentration has increased by 19 % of its original value.

The proportion of G fraction in the undiluted serum is $\frac{0.00336 \times 100}{0.0150} = 22.4 \%$.

The effect of slight dilution on the proportion of G fraction is not therefore very significant in this case. The large increase in the proportion of G fraction—from 8.1 to 22.4 %—would appear to be associated with the development of immunity to the disease.

Exp. 5. Serum from A.B. (myeloma).

This patient first came to our notice some months before the scale projection system for examining undiluted sera had been worked out. The patient's urine contained typical Bence Jones protein and we attempted an ultracentrifugal analysis of the serum in the hope of finding some evidence of the presence in it of a substance of lower molecular weight than albumin. An investigation of the urinary protein is reported in a subsequent paper. For the serum investigation we had no choice at the time but to dilute the serum, and a dilution of 2 vols. of serum with 3 vols. of Ringer's solution was found satisfactory. The curves show no evidence of the presence of an unusual molecule, but the proportion of G fraction is high, even for a diluted serum. The patient was lost sight of for eight months when she returned to hospital with a spontaneous fracture of the femur and obviously in the terminal stages of the disease. During this interval there had been continuous excretion of Bence Jones protein in the

Table V. *Results obtained in the examination of two specimens of serum from A.B. (myeloma)—one in the early and one in the late stages of the disease.*

		Serum A.B. Diluted 1 vol. serum to 1.5 vols. Ringer	Serum A.B. (8 months later). Diluted 5 vols. serum to 2 vols. 1 % NaCl
Cell thickness (mm.)		2.00	2.00
Optical scale distance (cm.)		3.64	2.58
Cell temperature (first exposure)		34.5°	34.3°
Cell temperature (last exposure)		34.6°	36.3°
Approximate duration of run (hours)		2	2½
Mean centrifugal force used (times gravity)		250,000	245,000
Average $S_{w,20} \cdot 10^{13}$		3.71	2.98
	A fraction	5.44	5.11
	G fraction	—	3.38
	X fraction	—	—
Average $n_1 - n_0$, from sedimentation diagram, $\lambda = 366 m\mu$	A fraction	0.0039	0.0070
	G fraction	0.0012	0.0018
	X fraction	—	0.0014
	Total	0.0051	0.0102
Calculated total $n_1 - n_0$ of original serum		0.0127	0.0143
Proportion of total $n_1 - n_0$ due to G fraction		23 %	18 %
Proportion of total $n_1 - n_0$ due to X fraction		—	14 %
Spreading coefficient of A boundary (cm. ² /sec. 10 ²)		5.3	2.4

urine. We were able to obtain another specimen of serum and this was examined by the scale projection method after diluting 5 vols. of the serum with 2 vols. of 1 % sodium chloride. The diagrams again show no signs of a new molecule, the $S_{W,20}$ values for the fractions corresponding well with the values for the fractions of normal sera. The results of the two experiments are shown in Table V. The lower proportion of G fraction in the second experiment is probably due in part to the higher total protein concentration in which the serum was examined. In both experiments the degree of spreading at the A boundary appears to be unusually high. This feature will be discussed more fully later.

Exp. 6. Serum from G.L.E. (myeloma).

The results of this experiment form an interesting comparison with those of the previous one. Almost at the same time as we obtained the first specimen of serum from A.B. another clinically characteristic case of myeloma (ordinarily a comparatively rare disease) was discovered, but this case had very little protein in the urine and that present did not give the characteristic reactions of Bence Jones protein. We found the total protein concentration of the serum to be unusually high (10.9 g./100 ml.) and could only obtain satisfactory sedimentation diagrams at the time by diluting the serum with twice its volume of Ringer solution. The disease terminated fatally shortly afterwards and the *post mortem* examination confirmed the diagnosis of myeloma. The presence of Bence Jones protein in the urine however was never definitely established. We preserved a specimen of the serum in the refrigerator for some months until it was possible to undertake an examination with the scale projection system. It was then found that dilution with at least an equal volume of 1 % sodium chloride was still required to avoid completely distortion of the A and X curves.

We show in Table VI the results of the two experiments. Fig. 6 *a* shows the curve of an exposure taken 43½ minutes after reaching full speed in the experiment with the serum diluted with an equal volume of 1 % sodium chloride, and Fig. 6 *b* is from an exposure taken 26½ minutes later in the same experiment. The results show that there are present four molecular fractions in the serum, two of which correspond in sedimentation constants to the A and G fractions of normal sera. The heaviest fraction, which we have labelled Z , is not of much significance since it represents a very small proportion of the total protein. The remaining fraction, which we have called Y , is of great interest since it is clearly quite different from the X fraction of normal sera and of the pathological sera already described. The sedimentation constant is much higher than that of any X fraction in comparable circumstances which we have found and is also unusual in lying nearer to the value for the G fraction in the same experiment. This is also seen in Figs. 6 *a* and 6 *b* in which the separation of the A and Y peaks is greater than of the Y and G peaks and also greater than that of the A and X peaks in any of the curves previously obtained. It is also interesting to note that the A curve is quite symmetrical, indicating the absence of any X fraction. We regard this experiment as definite evidence, to be supported by data from later experiments, that there may exist in pathological sera fractions of molecular weight not ordinarily present in normal sera.

Increasing the dilution of the serum from 1 : 1 to 1 : 2 appears to have little, if any, effect on the proportion of the G fraction. It appears, however, that the Y and A fractions exist in a form of concentration equilibrium just as we have shown the G and A fractions to exist in normal sera. It is not possible to examine the serum satisfactorily at a greater dilution than 1 : 2 because it will be

appreciated that with three major diffusion boundaries to be accommodated in a short depth of centrifuge-cell marked overlapping must occur between them. At dilutions greater than 1 : 2 this overlapping makes graphical resolution of the component curves impossible.

Fig. 6a.

Fig. 6b.

Table VI. Results obtained in the investigation of serum from G.L.E. (myeloma).

	Serum diluted with an equal volume of 1 % NaCl	Serum diluted with 2 vols. of Ringer solution
Cell thickness (mm.)	2.00	1.50
Optical scale distance (cm.)	2.14	3.80
Cell temperature (first exposure)	33.8°	32.3°
Cell temperature (last exposure)	34.6°	34.8°
Approximate duration or run (hours)	2½	1½
Mean centrifugal force used (times gravity)	260,000	250,000
Total $n_1 - n_0$, by refractometer $\lambda = 589 m\mu$	0.0110	0.0073
Total $n_1 - n_0$, corrected to $\lambda = 366 m\mu$	0.0119	0.0079
Average $S_{W, 20} \cdot 10^{13}$		
A fraction	3.09	3.64
G fraction	5.21	6.32
Y fraction	4.30	5.35
Z fraction	6.43	8.46
Average $n_1 - n_0$, from sedimentation diagram, $\lambda = 366 m\mu$		
A fraction	0.00694	0.00344
G fraction	0.00178	0.00111
Y fraction	0.00250	0.00194
Z fraction	0.00025	0.00024
Total	0.01147	0.00673
"% difference" total $n_1 - n_0$	-3.4 %	-14.8 %
Proportion of total $n_1 - n_0$ due to G fraction	15.5 %	16.5 %
Proportion of total $n_1 - n_0$ due to Y fraction	21.8 %	28.8 %
Proportion of total $n_1 - n_0$ due to Z fraction	2.2 %	3.6 %

Exp. 7. Sera from K.M., C.P. and J.A.F. (malignant disease).

Although we have classed these three sera together under the heading of malignant disease the clinical conditions of the patients from whom they were obtained differed considerably. The first patient (K.M.) was in an extreme state

of jaundice due to obstruction of the bile duct, and the serum was highly coloured with bile pigment. The second (C.P.) was emaciated and had an advanced ulcerative condition in the rectum. The third patient had a tumour in the epigastrium, but the liver was not palpable, and the general condition was not so advanced as in the other two. Further clinical details of these cases will be found in the appendix.

Fig. 7a.

Fig. 7b.

Table VII. *Results obtained in the investigation of sera from three cases of malignancy.*

	Serum K.M. diluted 5 vols. serum to 2 vols. 1 % NaCl	Serum C.P. diluted 5 vols. serum to 2 vols. 1 % NaCl	Serum J.A.F. diluted 3 vols. serum to 1 vol. 1 % NaCl
Cell thickness (mm.)	2.00	2.00	2.00
Optical scale distance (cm.)	2.58	2.58	1.84
Cell temperature (first exposure)	33.2°	34.2°	33.2°
Cell temperature (last exposure)	36.3°	36.4°	34.6°
Approximate duration of run (hours)	3	2½	3½
Mean centrifugal force used (times gravity)	255,000	255,000	245,000
Total $n_1 - n_0$ of undiluted serum, by refractometer, $\lambda = 589 m\mu$	0.0118	0.0147	0.0142
Corresponding total protein concentration (g./100 ml.)	5.84	7.28	7.03
Total $n_1 - n_0$ of diluted serum, by refractometer, and corrected to $\lambda = 366 m\mu$	0.00910	0.0113	0.0115
Average $S_{w,20} \cdot 10^{13}$			
A_1 fraction	2.57	—	—
A fraction	3.42	2.94	2.91
G fraction	5.58	4.96	4.99
X fraction	3.57	3.32	3.21
Average $n_1 - n_0$, from sedimentation diagram, $\lambda = 366 m\mu$			
A_1 fraction	0.00290	—	—
A fraction	0.00239	0.00525	0.00646
G fraction	0.00127	0.00302	0.00188
X fraction	0.00108	0.00163	0.00194
Total	0.00764	0.00990	0.01028
"% difference" total $n_1 - n_0$	-16 %	-12.4 %	-10.4 %
Proportion of total $n_1 - n_0$ due to A_1 fraction	38 %	—	—
Proportion of total $n_1 - n_0$ due to G fraction	16.5 %	30.5 %	18 %
Proportion of total $n_1 - n_0$ due to X fraction	14 %	16.5 %	19 %
Boundary spreading coefficient of lightest fraction ($cm.^2/sec. \times 10^7$)	4.3	3.5	0.7

All three sera were diluted slightly with 1 % sodium chloride before examination in the centrifuge. The results are collected in Table VII. Fig. 7 *a* shows the curve of an exposure taken 79 minutes after reaching full speed in the first experiment (K.M.), and the curve of Fig. 7 *b* is from an exposure taken 81 minutes after reaching full speed in the second experiment (C.P.). If we consider first the results for sera C.P. and J.A.F. it will be seen that both contain three molecular fractions which correspond in sedimentation constants with the *A*, *G* and *X* fractions of normal sera. The proportion of *G* fraction in the case of J.A.F. is just above our limit for a normal serum, and in the case of C.P. there is definitely an abnormally high proportion of this fraction. The other results call for no comment.

In the serum of K.M. there are present four definite molecular types. The lightest and heaviest fractions separate well and give rise to symmetrical curves. The two intermediate fractions do not separate completely, resembling the *A* and *X* fractions of normal sera in this respect. Also, if the curve of the lightest fraction is temporarily disregarded the diagram which remains resembles very closely the diagram which we have obtained with some diluted normal sera, with the exception that the proportion of *G* fraction is greater than usual.

The $S_{W,20}$ value for the lightest fraction (which we have called A_1), 2.57, is much lower than the value for a normal *A* fraction in corresponding concentration, 3.9 (*cf.* Fig. 1). It is not possible to compare the $S_{W,20}$ value for the *A* fraction in Table VII with the value for a normal *A* fraction in corresponding concentration because the *A* protein is sedimenting in a medium of A_1 molecules. The effect of the A_1 molecules will be to reduce the sedimentation velocity of the *A* molecules. The $S_{W,20}$ value for a normal *A* fraction in a concentration equal to the sum of the *A* and A_1 fractions in this serum is 3.3 (*cf.* Fig. 1). Earlier results obtained for albumin and globulin mixtures [1935, 1] show that globulin sediments more slowly in pure solution than in a solution of the same total protein concentration but containing quantities of albumin. The value of 3.42 for the *A* fraction of this serum therefore appears to be in keeping with the view that the fraction is a normal *A* fraction sedimenting in the presence of lighter A_1 molecules. The $S_{W,20}$ values for the *X* and *G* fractions also support the view that the serum of K.M. has the qualitative constitution of a normal serum with the addition of a new molecular species sedimenting more slowly than the others. The spreading coefficient of the A_1 boundary, 4.3×10^{-7} cm.²/sec., is consistent with the view that the fraction represents a homogeneous protein of lower molecular weight than serum albumin.

Exp. 8. Sera from G.N. and K.L. (pneumonia).

In this experiment also we are comparing the sera from patients showing marked differences in their clinical conditions. The first (G.N.) was a young man who showed all the signs of a fulminating type of pneumonia and died shortly after the serum was taken. The *post mortem* examination revealed septic infarcts in both lungs secondary to thrombo-phlebitis of the iliac vein. The other (K.L.) was from a woman of 78 years with a long history of pulmonary trouble, who was dying from croupous pneumonia with empyema.

The serum from G.N. was first dialysed at constant volume and the value of $n_1 - n_0$ obtained from refractometric measurements.

Total $n_1 - n_0$ of undiluted serum (G.N.), by refractometer ($\lambda = 589 m\mu$)	...	0.0122
Total $n_1 - n_0$ of undiluted serum corrected to $\lambda = 366 m\mu$	...	0.0132
Corresponding total protein concentration	...	6.04 g./100 ml.

Owing to the low concentration of total protein the serum was centrifuged without dilution. The results are shown in Table VIII and Fig. 8 shows the curve of an exposure taken 96 minutes after reaching full speed. It will be seen that three fractions are present, each of the two heavier fractions in greater concentration than the lightest one. This very severe alteration in the composition of the normal serum must have taken place inside three weeks, between the onset of the disease and the taking of the blood and was probably produced in a much shorter time.

A consideration of the $S_{W,20}$ values for the fractions shows that these are all unusually low but bear approximately the same ratio to each other as do the corresponding values for normal serum protein fractions. Once again we cannot be certain whether the fractions in this serum have abnormal molecular weights

Fig. 8.

Fig. 9.

Table VIII. *Results obtained in the investigation of sera from two cases of pneumonia.*

	Serum G.N. undiluted	Serum K.L. diluted 3 vols. serum to 1 vol. 1 % NaCl	Serum K.L. diluted with an equal vol. 1 % NaCl
Cell thickness (mm.)	2.00	2.00	2.00
Optical scale distance (cm.)	3.34	1.68	3.64
Cell temperature (first exposure)	31.0°	32.7°	33.4°
Cell temperature (last exposure)	35.1°	34.4°	34.9°
Approximate duration of run (hours)	3	2½	3
Mean centrifugal force used (times gravity)	245,000	250,000	245,000
Average $S_{W,20} \cdot 10^{13}$			
<i>A</i> fraction	2.07	2.67	3.15
<i>G</i> fraction	4.02	5.40	5.55
<i>X</i> fraction	2.49	3.32	—
Average $n_1 - n_0$, from sedimentation diagram, $\lambda = 366 m\mu$			
<i>A</i> fraction	0.00361	0.00548	*0.00568
<i>G</i> fraction	0.00406	0.00153	0.00112
<i>X</i> fraction	0.00432	0.00272	—
Total	0.01199	0.00973	0.00680
Calculated total $n_1 - n_0$ of original undiluted serum	—	0.0130	0.0136
Proportion of total $n_1 - n_0$ due to <i>G</i> fraction	34 %	16 %	16.5 %
Proportion of total $n_1 - n_0$ due to <i>X</i> fraction	36 %	28 %	—
Spreading coefficient of <i>A</i> boundary (cm. ² /sec. $\times 10^7$)	—	0.8	—

* Value for combined *A* and *X* fractions.

or whether a common influence is causing normal *A*, *G* and *X* fractions to sediment unusually slowly. The discrepancy (" % difference ") between measured and calculated total $n_1 - n_0$ in this experiment is -9.1 %.

The serum of K.L. was first examined undiluted and the curves showed distortion at the *A* and *X* boundaries. The serum was then diluted with one-third its volume and with an equal volume of 1 % sodium chloride, and the results of the two experiments are reported in Table VIII. No curves are reproduced since these show no significant points of difference from the curves of a normal serum. The results in Table VIII show that the *A* fraction of this serum sediments rather more slowly than a normal *A* fraction, while the *G* and *X* fractions correspond approximately in $S_{W,20}$ values with the *G* and *X* fractions of normal sera. It is interesting to find that the serum changes should be so small in this case of low-grade pulmonary and pleural infection, while on the other hand in the acute and rapidly fatal septic pneumonia the changes should be so radical.

Exp. 9. Sera from A.K. and E.K.L. (phthisis).

Both sera in this experiment were from elderly men with histories of pulmonary tuberculosis. A.K. was probably in a more advanced stage of the disease than E.K.L. and had been treated by induced pneumothorax. The results in Table IX show that both sera have approximately the same qualitative

Table IX. *Results obtained in the investigation of sera from two cases of phthisis.*

	Serum A.K. diluted 5 vols. serum to 2 vols. 1 % NaCl	Serum E.K.L. diluted 3 vols. serum to 1 vol. 1 % NaCl
Cell thickness (mm.)	2.00	2.00
Optical scale distance (cm.)	2.28	2.14
Cell temperature (first exposure)	33.2°	32.4°
Cell temperature (last exposure)	36.1°	34.3°
Approximate duration of run (hours)	2½	2½
Mean centrifugal force used (times gravity)	255,000	250,000
Total $n_1 - n_0$ of undiluted serum, by refractometer, $\lambda = 589 m\mu$	0.0145	—
Corresponding total protein concentration (g./100 ml.)	7.18	—
Total $n_1 - n_0$ of diluted serum, by refractometer, and corrected to $\lambda = 366 m\mu$	0.0112	—
Average $S_{W,20} \cdot 10^{13}$		
<i>A</i> fraction	2.88	2.92
<i>G</i> fraction	4.98	4.96
<i>X</i> fraction	3.29	3.21
Average $n_1 - n_0$, from sedimentation diagram, $\lambda = 366 m\mu$		
<i>A</i> fraction	0.00546	0.00619
<i>G</i> fraction	0.00246	0.00164
<i>X</i> fraction	0.00165	0.00112
Total	0.00957	0.00895
" % difference " total $n_1 - n_0$	- 14.5 %	—
Proportion of total $n_1 - n_0$ due to <i>G</i> fraction	26 %	18.5 %
Proportion of total $n_1 - n_0$ due to <i>X</i> fraction	17 %	12.5 %
Spreading coefficient of <i>A</i> boundary (cm. ² /sec. $\times 10^7$)	1.7	0.4

protein constitution as normal sera, but contain an abnormally high proportion of *G* fraction. This is especially marked in the case of A.K. A curve from this serum is shown in Fig. 9. It is from an exposure taken 85 minutes after reaching full speed. Many observers [quoted by Sato, 1933] have found a high proportion of globulin in the serum in tuberculosis using precipitation methods of analysis.

Exp. 10. Sera from P.P. and M.L. (nephritis).

Both the patients P.P. and M.L. had histories of chronic albuminuria. In the case of M.L. the undiluted serum gave distorted *A* and *X* curves. From the area of the symmetrical *G* curve we obtain the values

$n_1 - n_0$ of <i>G</i> fraction in undiluted serum (M.L.)	...	...	...	...	0.00094
Proportion of total $n_1 - n_0$ due to <i>G</i> fraction in undiluted serum	...	...	...	...	7.1 %

The sera were then examined after dilution with sodium chloride and the results are shown in Table X. No curves are reproduced since they show no unusual features. From Table X it will be seen that the $S_{W,20}$ value for the *A* fraction of P.P.'s serum, 2.98, is lower than the value for a normal *A* fraction in corresponding concentration, 3.5 (cf. Fig. 1). The other fractions of both sera appear to correspond in sedimentation constants to the fractions of normal sera. Serum M.L. appears to be normal in every respect. In serum P.P. the proportion of *G* fraction is higher than we have found in any normal sera. The other features are normal.

Table X. *Results obtained in the investigation of sera from two cases of nephritis.*

		Serum P.P. diluted 3 vols. serum to 1 vol. 1 % NaCl	Serum M.L. diluted 2 vols. serum to 1 vol. 1 % NaCl
Cell thickness (mm.)		2.00	2.00
Optical scale distance (cm.)		2.58	2.44
Cell temperature (first exposure)		33.8°	32.1°
Cell temperature (last exposure)		35.3°	33.8°
Approximate duration of run (hours)		2½	3¼
Mean centrifugal force used (times gravity)		250,000	250,000
Total $n_1 - n_0$ of undiluted serum, by refractometer, $\lambda = 589 m\mu$		0.0122	0.0147
Corresponding total protein concentration (g./100 ml.)		6.04	7.31
Total $n_1 - n_0$ of diluted serum, by refractometer, and corrected to $\lambda = 366 m\mu$		0.00988	0.0106
Average $S_{W,20} \cdot 10^{13}$			
	<i>A</i> fraction	2.98	2.83
	<i>G</i> fraction	5.76	5.03
	<i>X</i> fraction	3.77	3.14
Average $n_1 - n_0$, from sedimentation diagram, $\lambda = 366 m\mu$	<i>A</i> fraction	0.00451	0.00704
	<i>G</i> fraction	0.00138	0.00085
	<i>X</i> fraction	0.00207	0.00160
	Total	0.00796	0.00949
"% difference" total $n_1 - n_0$		-20 %	-10.5 %
Proportion of total $n_1 - n_0$ due to <i>G</i> fraction		17 %	9 %
Proportion of total $n_1 - n_0$ due to <i>X</i> fraction		26 %	17 %
Spreading coefficient of <i>A</i> boundary (cm. ² /sec. $\times 10^7$)		0.3	0.6

Exp. 11. Sera from K.A.B. (congenital syphilis), H.A.A. (metastatic tumour of bone), and A.S.L. (pneumonia).

These sera are grouped together arbitrarily. The patients had no symptoms in common with exception that both H.A.A. and A.S.L. were excreting protein in the urine. The results for the serum of K.A.B. show that fractions corresponding in sedimentation constants to the *A*, *G* and *X* fractions of normal sera are present, but the proportion of *G* fraction is abnormally high. The results for the sera from H.A.A. and A.S.L. show an unusual feature. The curve in Fig. 10 *a* was obtained from an exposure in the first experiment (H.A.A.) taken 93½ minutes after reaching full speed, and that in Fig. 10 *b* is from an exposure in the second experiment (A.S.L.) taken 76½ minutes after reaching full speed. In Fig. 10 *a* the

general appearance of the $A + X$ curve is the same as is ordinarily obtained from a normal serum diluted with at least an equal volume of 1 % sodium chloride [cf. McFarlane, 1935, 2, Fig. 15]. There is no definite appearance of an X peak in the curve, the presence of an X fraction being inferred from the asymmetrical shape. The squat and rounded appearance of the curve in this case suggests that the A and X fractions have a power of boundary spreading which is greater than corresponds to the protein concentration represented by the area of the curve.

Fig. 10a.

Fig. 10b.

Table XI. Results obtained in the investigation of sera from K.A.B. (congenital syphilis), H.A.A. (metastatic tumour of bone), and A.S.L. (pneumonia).

	Serum K.A.B. diluted 3 vols. serum to 1 vol. 1 % NaCl	Serum H.A.A. diluted 5 vols. serum to 2 vols. 1 % NaCl	Serum A.S.L. diluted 3 vols. serum to 1 vol. 1 % NaCl
Cell thickness (mm.)	2.00	2.00	2.00
Optical scale distance (cm.)	1.98	2.88	2.58
Cell temperature (first exposure)	34.5°	35.0°	34.2°
Cell temperature (last exposure)	36.6°	36.1°	35.5°
Approximate duration of run (hours)	3	2½	2½
Mean centrifugal force used (times gravity)	255,000	255,000	250,000
Total $n_1 - n_0$ of undiluted serum, by refractometer, $\lambda = 589 m\mu$	0.0165	0.0147	—
Corresponding total protein concentration (g./100 ml.)	8.17	7.28	—
Total $n_1 - n_0$ of diluted serum, by refractometer, and corrected to $\lambda = 366 m\mu$	0.0134	0.0113	—
Average $S_{w,20} \cdot 10^{13}$			
A fraction	2.85	3.10	3.30
G fraction	4.62	5.19	5.37
X fraction	3.28	—	—
Average $n_1 - n_0$, from sedimentation diagram, $\lambda = 366 m\mu$			
A fraction	0.00783	0.00827	0.00648
G fraction	0.00186	0.00254	0.00060
X fraction	0.00125	—	—
Total	0.01094	0.01081	0.00708
"% difference" total $n_1 - n_0$	-18.6 %	-4.4 %	—
Proportion of total $n_1 - n_0$ due to G fraction	17 %	23.5 %	8.5 %
Proportion of total $n_1 - n_0$ due to X fraction	11.5 %	—	—
Spreading coefficient of A boundary (cm. ² /sec. $\times 10^7$)	1.2	5.03	3.3

This suggestion is confirmed by the unusually high value of the boundary spreading coefficient, 5.03×10^{-7} cm.²/sec. We cannot resolve the combined curves graphically. For this reason the $n_1 - n_0$ value given for the *A* fraction in Table XI represents the value for the combined *A* and *X* fractions. A similar phenomenon appears in the serum of A.S.L. The curve (Fig. 10 *b*) is practically symmetrical and no evidence of an *X* peak is seen. On the other hand the general dimensions of the curve are not in keeping with a homogeneous boundary of the same protein concentration but closely resemble those for a strongly diluted human serum [cf. 1935, 2, Fig. 16]. The boundary spreading coefficient is again high. The impression which we have from this curve is that *A* and *X* fractions are present in the serum in concentrations which do not differ greatly from each other. We may also note the abnormally high proportion of *G* fraction in H.A.A.'s serum.

Exp. 12. Sera from K.J.K. (chronic nephritis) and L.E. (albuminuria of pregnancy).

The patient K.J.K. had been passing large amounts of protein in the urine for at least a year. In the patient L.E. proteinuria commenced suddenly about a month before parturition, when twins were born. During this period excessive amounts of protein were passed in the urine. Both specimens of serum were turbid in appearance and behaved in the centrifuge cell as we have described earlier in this paper for sera containing quantities of suspended lipoids. An examination of the urinary proteins will be described in a subsequent paper.

Fig. 11 *a* shows the curve of an exposure taken 65 minutes after reaching full speed in the experiment with serum, slightly diluted, from K.J.K. The complete absence of any signs of an *X* fraction and the marked dissymmetry of the *A* curve are to be noted. It occurred to us at this point that some of the unusual features of this serum might be due to the presence of quantities of urea or other waste substances in the serum. We therefore dialysed the serum (with slight incidental dilution) against changes of 1 % sodium chloride until equilibrium was obtained and re-examined the diluted serum in the centrifuge. The results of both experiments are shown in Table XII and demonstrate that no significant change has resulted from the dialysis. The general shapes of the curves for the dialysed serum are the same as those for the undialysed serum. If urea, or other dialysable substances, in serum have any effect on the serum proteins we conclude that this is irreversible.

Fig. 11 *b* shows the curve from a photograph taken 61 minutes after reaching full speed in the experiment on the serum of L.E. Once again, there are no obvious signs of an *X* fraction, but the general dimensions of the *A* curve resemble those of the boundary curve of a homogeneous protein in considerably more dilute solution. The boundary spreading coefficients in all three experiments (cf. Table XII) are much higher than correspond with the concentrations of the protein fractions. The large percentage discrepancies between measured and calculated total protein refraction increments also support the view that significant amounts of grossly polydisperse protein are present in both sera. It is to be noted however that the refractometric measurements of $n_1 - n_0$ include the refraction effects of the suspended lipoids which are not included in the values of $n_1 - n_0$ obtained from the centrifugal diagrams. This may introduce a significant error. The above facts, in conjunction with the dissymmetry of the *A* curve in Fig. 11 *a*, suggest that at least for the serum of K.J.K. the most probable explanation of the absence of any *X* fraction is the one suggested above—that all or a large part of the protein in the lighter fraction is polydisperse.

Fig. 11a.

Fig. 11b.

Table XII. Results obtained in the investigation of sera from K.J.K. (nephritis), and L.E. (albuminuria of pregnancy).

	Serum K.J.K. diluted 3 vols. serum to 1 vol. 1 % NaCl	Serum K.J.K. dialysed and slightly diluted	Serum L.E. diluted 5 vols. serum to 2 vols. 1 % NaCl
Cell thickness (mm.)	2.00	2.00	2.00
Optical scale distance (cm.)	2.88	3.48	3.78
Cell temperature (first exposure)	34.6°	32.3°	33.6°
Cell temperature (last exposure)	35.7°	35.3°	35.8°
Approximate duration of run (hours)	2½	2½	2½
Mean centrifugal force used (times gravity)	250,000	250,000	250,000
Total $n_1 - n_0$ of undiluted serum, by refractometer, $\lambda = 589 m\mu$	—	—	0.0126
Corresponding total protein concentration (g./100 ml.)	—	—	6.24
Total $n_1 - n_0$ of diluted serum, by refractometer, and corrected to $\lambda = 366 m\mu$	—	0.00790	0.00972
Average $S_{w,20} \cdot 10^{13}$ A fraction	3.24	3.90*	3.08*
G fraction	5.61	6.65*	5.43*
Average $n_1 - n_0$, from sedimentation diagram, $\lambda = 366 m\mu$			
A fraction	0.00544	0.00414	0.00528
G fraction	0.00098	0.00075	0.00086
Total	0.00642	0.00489	0.00614
"% difference" total $n_1 - n_0$	—	-38 %	-37 %
Proportion of total $n_1 - n_0$ due to G fraction	15 %	15 %	14 %
Spreading coefficient of A boundary (cm. ² /sec. $\times 10^7$)	6.2	10.5	8.2

* Owing to the dissymmetry of the curves these values are only approximate.

DISCUSSION.

The more important results from the foregoing experiments are collected together in Table XIII. In a comparison of these results with the results characteristic of normal human sera the following points from the data in Table XVII of the previous paper [1935, 2] are important:

Maximum proportion of G fraction recorded in a normal serum (total $n_1 - n_0$ of sera greater than 0.00600) = 13 %.

Maximum spreading coefficient recorded for the A boundary of a normal serum ($n_1 - n_0$ of A fractions greater than 0.00500) = $1.9 \cdot 10^{-7}$ cm.²/sec.

Table XIII. *Collected results for pathological human sera.*

Serum	Diagnosis	Dilution	Calculated $n_1 - n_0$ of A fraction	$S_{W,20} \cdot 10^{13}$ of A fraction	$S_{W,20} \cdot 10^{13}$ of normal A fraction	A boundary spreading coefficient	Proportion of X fraction, %	Proportion of G fraction %
A.J.	Malignant disease	Undiluted	0.00500	2.32	3.4	—	31	26.5
B.I.L.	Scarlatina (6th day)	Undiluted	0.00700	2.28	2.9	0.4	36.5	8.1
B.I.L.	Scarlatina (29th day)	5 : 2	0.00600	2.70	3.1	1.6	19.5	24
J.O.D.	Scarlatina (8th day)	5 : 2	0.00670	2.75	3.0	0.3	23	13
A.B.	Myeloma (early)	1 : 1.5	0.00390*	3.71	3.7	5.3	—	23
A.B.	Myeloma (late)	5 : 2	0.00700	2.98	2.9	2.4	14	18
G.L.E.	Myeloma	1 : 1	0.00694	3.09	2.9	—	{ No X mol. 122 % new (Y) mol. 14 % X mol. 138 % new (A_1) mol.	15.5
K.M.	Malignant disease	5 : 2	0.00290†	2.57†	3.9	4.3†	16.5	16.5
C.P.	Malignant disease	5 : 2	0.00525	2.94	3.3	3.5	19	30.5
J.A.F.	Malignant disease	3 : 1	0.00646	2.91	3.0	0.7	36	18
G.N.	Septic pneumonia	Undiluted	0.00361	2.07	3.7	—	28	34
K.L.	Empyema	3 : 1	0.00548	2.67	3.3	0.8	17	16
A.K.	Phthisis	5 : 2	0.00546	2.88	3.3	1.7	12.5	26
E.K.L.	Phthisis	3 : 1	0.00619	2.92	3.1	0.4	26	18.5
P.P.	Nephritis	3 : 1	0.00451	2.98	3.5	0.3	17	17
M.L.	Nephritis	2 : 1	0.00704	2.83	2.9	0.6	17	9
K.A.B.	Congenital syphilis	3 : 1	0.00783	2.85	2.7	1.2	11.5	17
H.A.A.	Malignant disease	5 : 2	0.00827*	3.10	2.6	5.0	—	23.5
A.S.L.	Pneumonia	3 : 1	0.00648	3.30	3.0	3.3	0	8.5
K.J.K.	Chronic nephritis	3 : 1	0.00544	3.24	3.3	6.2	0	15
L.E.	Albuminuria of pregnancy	5 : 2	0.00528	3.08	3.3	8.2	0	14

* Combined values for A and X fractions.† Value for lightest (A_1) fraction.

There is also shown in Table XIII for each serum the value for the sedimentation constant of a normal A fraction of the same concentration as the A fraction in the serum (*cf.* Fig. 1). It will be seen that the agreement between the $S_{W,20}$ values for normal and pathological A fractions is good in some cases and very poor in others. The agreement is particularly bad in the cases of A.J., B.I.L., K.M. (A_1 fraction) and G.N. In one of these sera (K.M.) the evidence for the presence of a new molecular fraction is conclusive since four fractions are present altogether. In the other three it is possible that the fractions which we have labelled A are really of lower molecular weight than the A fractions of normal sera. It is to be noted that in these three cases also we have found abnormally high proportions of X fraction. It is possible in these cases that the fractions which we have labelled X are really A fractions and the fractions labelled A are substances of lower molecular weight. It is seen in Table XIII that the general agreement of the $S_{W,20}$ values for the lightest fractions among themselves is not as good as the agreement in the case of the A fractions of normal sera. The latter in turn do not agree to within the full experimental accuracy of the method. These facts suggest that in pathological sera, and to a lesser extent in normal sera, a variable factor of which we have no precise knowledge influences the sedimentation of the proteins. This factor does not affect the sedimentation of purified proteins in solutions of defined inorganic composition. Alternatively, there may exist in normal and pathological sera a great many A , X and G fractions of different molecular weights. To decide between these views it is necessary to carry out further investigations of the effect of the physical and chemical conditions which hold in normal and pathological sera on the sedimentation constants of the fractions. We have obtained however in the results for two sera (G.L.E. and K.M.) conclusive evidence that fractions of molecular weight not ordinarily found in normal sera may be present in pathological sera.

When we consider the concentration relationships of the fractions in pathological sera it is seen in Table XIII that with the exception of the early scarlatinal

sera and those from M.L. (nephritis) and A.S.L. (pneumonia) all the sera have higher proportions of *G* fraction than normal. On the one hand this is not surprising in view of the severity of most of the conditions; on the other, it is interesting to find these increased proportions of *G* fractions in diseases such as malignancy to which immunity is not ordinarily developed. It does not seem possible to associate the increased proportions of the *G* fractions with a purely protective mechanism. The increases occur in a wide variety of conditions. There seems to be nothing specific therefore about the occurrence of a high proportion of *G* fraction in pathological sera. Various observers have come to the same conclusion with regard to the proportion of globulin in pathological sera as estimated by precipitation with neutral salts (see reviews by Peters and Van Slyke [1931] and Boyland [1932]).

In the various experiments of this series we have recorded a wide variety of values for the proportions of the *X* fractions, many of them lower and many higher than the extreme values obtained in our series of normal sera. We hesitate however to base any important conclusions on our individual figures because in many cases an element of uncertainty attaches to the process of graphical separation of the *X* curves from overlapping *A* curves. The general deduction is drawn from the values obtained that there exist in pathological sera variations in the proportions of *X* fractions which are wider than the variations found in normal sera.

A voluminous literature has been compiled in recent years by various observers [Schade and Clausen, 1924; Govaerts, 1925; Farkas, 1927; Krogh and Nagazawa, 1931; Kylin and von Pein, 1931; Iversen, 1932; to mention only a few contributions] on the colloid osmotic pressure of normal and pathological sera. A few of these observers have calculated the osmotic pressure per gram of serum protein and have deduced from this figure the presence of an abnormal concentration of the albumin fraction or of the globulin fraction as the case may be. It cannot be said that the application of this method has yielded consistent results and the centrifugal data furnish an explanation of this. So long as there are more than two variables, *i.e.* more than two molecular fractions in the serum, a measurement of the osmotic pressure (presuming this to be reliably corrected for the effects of the diffusible ions) can give no idea of the relative concentrations of the fractions. The method cannot be applied to normal sera because of the presence of *A*, *G* and *X* fractions, and may be applied still less to pathological sera in which additional fractions may be present. It appears to us that the recorded measurements of colloid osmotic (oncotic) pressure in pathological sera can have little precise significance in relation to the protein composition of the sera.

Consideration of the spreading coefficients in Table XIII shows that the following sera, all with *A* fraction $n_1 - n_0$ values greater than 0.00500, have abnormally high *A* boundary spreading coefficients; A.B. (myeloma), C.P. (malignancy), H.A.A. (malignancy), A.S.L. (pneumonia), K.J.K. (nephritis) and L.E. (albuminuria of pregnancy). The variety of the pathological conditions indicates that this physico-chemical index appears to have no specific disease relationship. The striking fact however is that with the exception of C.P., of whom unfortunately we have no accurate urinary details, all the patients are passing protein in the urine. It appears probable that the poly- or hetero-disperse protein of the *A* fractions in these sera is capable of passing through the kidneys. On the other hand we cannot say that poly- or hetero-dispersity of serum protein is an essential condition for proteinuria because in our series there are two cases of chronic albuminuria (P.P. and M.L.) in which the spreading coefficients of the

A boundaries are normal. We give some further important evidence on this subject in a later paper dealing with the analysis of urinary proteins.

We believe that the existing methods of serum protein analysis by precipitation with neutral electrolytes do not give rise to fractions of even approximately uniform composition and therefore the results have a doubtful significance. Our evidence for this will be given in a later paper. The question arises whether the results of ultracentrifugal analysis are likely to have a greater significance. In the first place we may expect the results to be consistent since they represent measurements of a single well-defined property of serum, *viz.* the molecular dimensional distribution of the protein. Secondly, they have the merit of being obtained in circumstances which involve little, if any, change in the physico-chemical properties of the environment of the protein. This helps to eliminate arbitrary assumptions. It is true that the chemical natures of the fractions of concentrated sera which separate in the centrifugal field are not determined. This constitutes an important limitation in the interpretation of the results which we hope will be eliminated in the future. On the other hand, it is conceivable that the concentrations of molecules of a few particular sizes—no matter of what chemical constitution—may be of great significance. When one considers the relationship of protein molecular size to the viscosity and colloid osmotic pressure of serum, and the vital functions which those two serum properties have in the organism, it is realised that the results of the purely molecular dimensional analysis of serum protein may be of considerable importance. Finally, it is clear that in the consistent measurement of a single property of serum—no matter if the true significance of the property is obscure—there is a higher probability of finding an empirical relationship with some general or local condition in the organism, and this is frequently the object of serum protein analysis. For these reasons we believe that the ultracentrifugal method of analysis is at present a more rational procedure to use in the investigation of pathological sera than the precipitation method of analysis.

We have shown that the concentration values of the protein fractions obtained in the ultracentrifuge are dependent on the protein concentration in which the serum is examined. This dependence of the results on protein concentration means that it is necessary to use one of three procedures in order to give the results for various sera a comparative significance. We must determine the molecular dimensional distribution of the sera, by direct or indirect methods: (1) in the undiluted state; (2) in a state of dilution such that no change in the concentration of the fractions occurs on further dilution; or (3) at a constant concentration of protein. The second procedure is a difficult one in practice as the graphical analysis of the serum diagrams is often unsatisfactory, or impossible, in the case of highly diluted sera. Frequently also, there occur irregularities in the diagrams which are of doubtful significance. The third is not always ideally applicable because it is impossible by mere dilution of two sera each containing several fractions to obtain comparable conditions for all the fractions. The method is nevertheless frequently capable of giving valuable comparative information, and it is proposed in the continuation of these studies in the future to depend on a combination of this method and of the first procedure mentioned above.

SUMMARY.

A series of pathological sera from cases representing a variety of diseases has been examined in the ultracentrifuge.

1. In the cases of two antidiphtheritic horse sera examined without treatment of any kind it was found that the protein is approximately equally distri-

buted between an albumin and a globulin fraction. Dilution of the sera causes a marked increase in the proportion of the globulin fraction.

2. The majority of the pathological human sera examined contain three molecular fractions. In some of these sera the fractions have the sedimentation constants characteristic of the *A*, *G* and *X* fractions of normal human sera. In others the sedimentation constants are different indicating either that the fractions have different molecular weights or that some unknown and variable factor is present in pathological serum which affects the sedimentation rates of the fractions.

3. In two sera definite evidence has been found of the presence of fractions of molecular weight not normally found in human sera. In one case of malignant disease 30 % of a substance of substantially lower molecular weight than serum albumin was present in addition to the *A*, *G*, and *X* fractions. In another case of myeloma 22 % of a substance of molecular weight intermediate between the *X* and *G* fractions of normal sera was present. In this case *A* and *G* fractions were present but no *X* fraction.

4. In a considerable majority of these pathological sera the proportions of the total proteins in the untreated or slightly diluted sera represented by the *G* fractions are greater than in normal sera. The increase in the proportion of *G* fraction appears to have no special relationship to one or more diseases but to represent a general form of reaction to a number of unrelated pathological states.

5. A wide variety of values for the proportions of *X* fractions are recorded and it is evident that in a number of diseases the proportion of this fraction does not fall within the limits for a normal serum.

6. Some of the sera examined react more readily to simple dilution than do normal sera. The nature of this reaction consists as in normal sera in an increase in the proportion of *G* fraction at the expense of the lighter fractions. Fractions not normally present in serum appear to exist also in equilibrium with the other fractions.

7. Untreated pleural exudate from a case of malignancy behaved in the centrifuge like the serum of the same patient diluted to the same concentration of total protein.

8. Certain sera show an unusual phenomenon which takes the form of an abnormal spreading of the overlapping *A* and *X* boundaries. There is evidence that this is partly or wholly due to the presence of polydisperse protein molecules of mean molecular weight approximately the same as that of the *A* fraction. In four out of five such cases protein was present in the urine.

APPENDIX OF CLINICAL NOTES.

A.J. Female. 60 years. Ascites since December, 1933, with loss of weight. 21. ii. 34: large tumour palpable on right side of abdomen, exudate in left pleura, no signs of pulmonary metastases on X-ray examination, trace of protein in urine but this was not consistently present. 17. iv. 34: many large abdominal tumours palpable suggesting peritoneal metastases. Died 29. iv. 34. Permission for P.M. refused. Diagnosis: ovarian tumour with secondary metastases. Blood taken for centrifugal examination of serum on 18. iii. 34.

K.H.B. Male. 23 years. Very tired in summer of 1933 and unfit for work, later became hoarse. December, 1933: blood in sputum and attacks of shivering. 19. ii. 34: temperature 104° F., pulse 100–120, sputum T.B. + +, X-ray showed advanced T.B. in both lungs. Died 27. iii. 34. P.M.: cavities in both lungs, tuberculous ulceration of larynx, trachea and intestines, inflammation of spleen. Blood taken on 16. iii. 34.

B.I.L. Male. 4 years. 16. v. 34: headaches and throat pains. 17. v. 34: rash on chest and back, temperature 103° F., pulse 120. Diagnosis: typical scarlatina. Blood taken on 22. v. 34 and again on 14. vi. 34.

J.O.D. Male. 11 years. 10. v. 34: throat pains, difficulty in swallowing. 11. v. 34: typical rash all over body, swollen submaxillary glands, inflamed throat and tonsils. 18. v. 34: desquamation, temperature 100–102° F., pulse 90–120. Diagnosis: typical scarlatina. Blood taken on 18. v. 34.

A.B. Female. 47 years. Pains in lumbar region and chest for one year until 10. xi. 33. Urine gave typical Bence Jones precipitate at 50°, clear at 100°, 6–12‰. Esbach, non-protein nitrogen 35 mg./100 ml., B.P. 170/140. X-ray on 20. xi. 33 showed enlargements on 1st and 2nd ribs on right side and several tumours of pelvic bones accompanied by much destruction. 17. iii. 34: spontaneous fracture of right femur, X-ray showed neck and trochanter destroyed. Patient still alive on 27. vi. 34. Diagnosis: myeloma. Blood taken on 18. xi. 33 and on 27. vi. 34.

G.L.E. Male. 68 years. January, 1933: pain in lumbar region and both shoulders, girdle pain at 11th and 12th thoracic vertebrae, later also at 8th and 9th thoracic and 2nd and 3rd lumbar, poor appetite but no emaciation, dyspnoea, coughing and hoarseness but no X-ray evidence of pulmonary metastases, liver slightly enlarged, prostate normal, symmetrical atrophy of both legs, marked osteoporosis of vertebrae with compression of several in lumbar and upper thoracic regions, several ribs quite soft, fracture of 8th and 9th, old fracture of 4th (right side), only a trace of protein in the urine (giving slight opalescence at 67°, definite at 70°, and no change at 100°), Esbach 0.5‰. Died 8. x. 33. P.M.: medullary tumour (myeloma). Blood taken on 9. ix. 33.

K.M. Male. 78 years. Increasing tiredness and emaciation for a month. 13. v. 34: intense jaundice, urine dark brown and frothy, clay-coloured stools, plasma colour equivalent to 71 Meulengrecht, mobile tumour in epigastrium (finger-thick, cord-shaped, and very hard). Diagnosis: malignant tumour of bile duct. Blood taken on 15. v. 34.

C.P. Female. 67 years. October, 1933: pains at stool, constipation and diarrhoea alternately, mucus and blood in faeces. 15. v. 34: tired, pale and emaciated, 5 inches inside rectum hard stenotic tumour with finger-wide central opening and ulcerated. Diagnosis: rectal carcinoma. Blood taken on 15. v. 34.

J.A.F. Male. 62 years. 10. xii. 33: complained of weakness. 19. iii. 34: emaciated and pale, blood in faeces, palpable tumour in epigastrium, liver not palpable, temperature 100° F., treated with cobra venom. Diagnosis: gastric carcinoma. Blood taken on 6. iv. 34.

G.N. Male. 28 years. 20. ii. 34: temperature 100–104° F., pulse 110–140, a trace of protein in urine. 6. iii. 34: two pleural punctures without result, B.P. 115/70. Died 19. iii. 34. P.M.: thrombophlebitis of iliac vein, bilateral emboli of pulmonary artery and large infarcts, organised pneumonia on right side and acute on left secondary to infection of infarct, acute nephritis. Blood taken on 13. iii. 34.

K.L. Female. 78 years. 10. iii. 34: rigors, temperature 102° F., 12. iii. 34: 270 ml. purulent fluid obtained from right pleural cavity. Died 20. iii. 34. P.M.: cardio-arterio-sclerosis, croupous pneumonia, mucopurulent bronchitis, emphysema, pleural empyema, uterine fibromyomata, ovarian cyst. Blood taken on 18. iii. 34.

A.K. Male. 59 years. Dry pleurisy in 1930. Feb. 1934: much coughing, phthisis diagnosed, sputum T.B. + + +, patient treated with pneumo-thorax. 27. vi. 34: still in hospital. Blood taken on 11. v. 34.

E.K.L. Male. 69 years. In sanatorium in April 1922. 14. iii. 34: temperature 97–98° F. in morning, 100–101° F. in evening, pulse 72–120, X-ray showed T.B. in upper third of both lungs with large cavities, sputum T.B. +. Discharged rather better 1. v. 34. Diagnosis: chronic pulmonary tuberculosis. Blood taken on 21. iv. 34.

P.P. Male. 65 years. Dyspnoea for 10 years with intermittent oedema and palpitations. 11. iv. 34: oedema, ascites, enlargement of liver, pleural effusion, Esbach 3–7‰, non-protein nitrogen 120 mg./100 ml., W.R. negative. Went home rather better on 12. v. 34. Diagnosis: chronic glomerulo-nephritis. Blood taken 20. iv. 34.

M.L. Female. 45 years. Protein in urine and high blood pressure since 1931. 16. iii. 34: slight oedema of legs, no eye symptoms, Esbach 1‰, blood and casts in urine, non-protein nitrogen 37.5 mg./100 ml., B.P. 165-100. Went home rather better on 3. v. 34. Diagnosis: recurrent nephritis (? acute). Blood taken on 18. iii. 34.

K.A.B. Male. 17 years. Partially deaf in both ears, becoming quite deaf in right ear in 1927. 1933: accident to knee, swollen and painless, yellow fluid in joint (Esbach 2.5‰), C.S.F. gave negative protein reactions, W.R. +, Kahn + +. Treated with malaria on 24. iii. 34. Diagnosis: congenital syphilis. Blood taken on 23. iii. 34.

H.A.A. Male. 59 years. Pain in right leg in January, 1934, with later pain in lumbar region. 6. iii. 34: movements impaired, emaciated, X-ray showed collapse of vertebrae with atrophy and sclerosis of surrounding tissue. No protein in urine until 3. v. 34 and thereafter only present intermittently in small amounts. Patient was treated with snake venom but died on 1. vi. 34. P.M.: sympathico-gonioma in region of superior ganglion with metastases in ribs and vertebrae, cardio-arterio-sclerosis. Blood taken on 9. v. 34.

A.S.L. Male. 27 years. Treated five times for croupous pneumonia. 1. iii. 34: acute attack of shivering and pains in chest, haemorrhagic sputum, X-ray showed pneumonia in upper lobe of right lung, typical pneumonia temperature chart, temperature normal on 8. iii. 34, urinary protein up to 16‰ Esbach, non-protein nitrogen 33-40 mg./100 ml. Went home better on 11. v. 34. Diagnosis: pneumonia. Blood taken on 26. iv. 34.

K.J.K. Male. 42 years. October, 1933: oedema of legs, eye symptoms, swollen abdomen, tiredness, dyspnoea. 16. i. 34: oedema of penis, scrotum, and legs, urine 600-1700 ml. per day, protein always present up to 3-15‰ Esbach, casts and a few red cells, B.P. 145/90-180/110. Diagnosis: chronic nephritis. Blood taken on 24. iv. 34.

L.E. Primipara. 21 years. No miscarriages. Protein first found in urine on 20. iv. 34, thereafter present every day in large amounts (Esbach 10-12‰), no eclampsia, B.P. 165/90. Twins were born on 16. v. 34. Diagnosis: albuminuria of pregnancy. Blood taken on 12. v. 34.

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