149. THE SERUM PROTEINS IN MULTIPLE MYELOMATOSIS

By RALPH AMBROSE KEKWICK

From the Lister Institute, London

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THE serum of patients suffering from multiple myelomatosis has for a considerable time been known to show gross divergences in constitution in comparison with normal human serum. Among these abnormalities may be mentioned a marked hyperproteinemia associated with a change in the albumin-globulin ratio. The condition is also frequently, but not invariably, associated with Bence-Jones proteinuria. An exhaustive review of the literature has recently been given by Bonsdorff *et al.* [1938].

The data on two such sera [McFarlane, 1935] encouraged a further extensive study by the ultracentrifugal and recently improved electrophoretic techniques. This paper is a report of the results obtained in the examination of the sera from five cases by these methods.

Whilst this work was in progress two papers appeared [Jersild & Pedersen, 1938; Longsworth *et al.* 1939], the first giving ultracentrifugal, the second electrophoretic data on the serum of multiple myelomatosis. These will be referred to again in the discussion.

EXPERIMENTAL

The methods used in the treatment and examination of the sera followed those adopted in the examination of a series of normal human sera [Kekwick, 1939].

The sera were dialysed undiluted at constant volume against phosphate buffer *p*H 8·0, $\mu = 0{\cdot}1$, until ionic equilibrium was attained. Any slight precipitate which formed was centrifuged off, and the refraction due to the non-dialysable materials present determined with the dipping refractometer ($\lambda = 546$ mμ). These data are given in Table 2, column 2, headed "*n* Protein", and represent the differences in the refractive indices, n_1, and n_0 of the protein solution and buffer respectively. For examination the dialysed sera were diluted with buffer to $n_1 - n_0 = 0{\cdot}00300$.

The ultracentrifugal examinations were made in a field strength of 270,000 times gravity, with optical observations by the diagonal Schlieren method [Philpot, 1938]. The light source was a high-pressure mercury arc from which monochromatic light, $\lambda = 546$ mμ, was isolated by a suitable filter.

By optical projection of the recorded photographs, with an enlargement of eight diameters on to millimeter graph paper, the boundary positions were measured for sedimentation constant calculations. Tracings were also made, with the same magnification, for the analytical determinations of the quantities of components present in the sera. The sedimentation constants (S_{20}) quoted were reduced to water at 20° according to the usual method [Svedberg & Pedersen, 1940].

All the electrophoretic measurements were made in the Tiselius [1937] apparatus at 0°. For the analytical experiments a potential gradient of 5 V./cm. was applied, higher gradients up to 10 V./cm. being used in mobility experiments.

The Lamm scale method, in conjunction with the Toepler Schlieren method, was used for the electrophoretic analysis. A sodium vapour lamp illuminated the U-tube, and the migration was recorded photographically. Further details of treatment were the same as in a previous paper [Kekwick, 1939].

For electrophoretic mobility measurements on proteins isolated from the sera, the Toepler Schlieren method was used at first, but later the diagonal Schlieren method was substituted. In these experiments a protein concentration corresponding to $n_1 - n_0 = 0{\cdot}00150$ was employed to reduce any concentration effect on mobility as far as possible, whilst still enabling a well-defined boundary to be observed over long periods. After a suitable time interval in the experiments, the current was reversed and the boundaries brought back to the starting position. The mobilities given are mean values calculated from the ascending and descending boundaries, and have been corrected for the viscosity effects of the buffer salts.

As a further means of characterizing the proteins isolated from the sera, carbohydrate-nitrogen ratios were determined. The carbohydrate determinations were made by the method of Sørensen & Haugaard [1933], mannose being used as an arbitrary standard. In earlier determinations a Zeiss step photometer was employed in the measurements of the extinctions. Later a photoelectric photometer was utilized, the blue line (436 mμ) of the mercury arc being found very suitable for the absorption measurements. Nitrogen estimations were made by the micro-Kjeldahl method using selenium oxychloride as a catalyst.

The hydrogen electrode was used for pH measurements, with $N/10$ HCl (pH 1·075) for standardization.

I. Results of ultracentrifugal and electrophoretic examination of the sera

The data obtained from the examination of the sera, which have been arranged in descending order of protein content except for serum 5, are collected in Tables 1 and 2.

In the ultracentrifuge sera 1–4 showed two components corresponding, as an examination of the sedimentation constants in Table 1 shows, to the albumin and globulin of normal human serum. The slight differences in the sedimentation constant values for the pathological and the two normal human sera, are probably due to differences in the amounts of the components in the two types of sera. Serum 5, which is very complex, showed five components, two corresponding in sedimentation constant to the components of normal human serum, and three of higher values not found in normal human serum. Samples of albumin and total globulin were separated electrophoretically from this serum. The albumin sedimented as a single component $S_{20} = 4{\cdot}15$, the globulin showing four components, $S_{20} = 6{\cdot}54$, 9·26, 11·3 and 13·3.

In Fig. 1 are shown sedimentation diagrams of normal human serum and myelomatosis sera 3 and 5, each obtained 45 min. after reaching full speed.

All the myelomatosis sera show an increase in the proportion of globulin in comparison with normal human serum. The analytical data in Table 2, which give the percentages of the total refraction due to each component, refer in each case to the mean value obtained from two ultracentrifuge exposures. It is apparent that the proportion of globulin increases with the total protein content of the serum.

The electrophoretic examination of these sera revealed four components, albumin, α, β, and γ globulins. In most cases the data (Table 2) are mean values

Table 1. *Sedimentation constants S_{20} of human serum components*

Serum	Albumin	Globulin
Myeloma 1	4·29	6·34
Myeloma 2	4·12	6·22
Myeloma 3	4·06	6·54
Myeloma 4	4·10	6·39
Myeloma 5	4·32	6·59, 9·09, 11·2, 14·3
Normal 1	3·86	6·06
Normal 5	3·79	6·01

Phosphate buffer pH 8, $\mu = 0{\cdot}1$. Concentration $n_1 - n_0 = 0{\cdot}00300$. The sedimentation constants, S_{20}, are in units of 10^{-13}.

Table 2. *Ultracentrifugal and electrophoretic analysis of human serum components*

		Ultracentrifugal		Electrophoretic				
					Globulins			
Serum	n Protein	Albumin	Globulin	Albumin	α	β	γ	Albumin
1. (J. B.)	0·02220	31·2	68·8	16·0	2·8	4·2	76·9	0·00355
2. (C. E.)	0·02120	41·2	59·0	22·4	2·9	9·7	65·0	0·00475
3. (L. G.)	0·01941	44·9	55·0	30·4	3·2	11·2	55·3	0·00590
4. (S. H.)	0·01494	67·0	33·2	45·7		14·5	39·8	0·00683
5. (J. J.)	0·02441	30·3	69·7	18·6	15·0	61·7	4·7	0·00454
Normal	0·01293	78·0	22·0	62·5	5·0	11·5	21·0	0·00808
Normal*	—	—	—	64·2	8·5	16·8	10·5	—
Normal†	—	—	—	66·2	7·9	13·9	13·2	—

Phosphate buffer pH 8, $\mu = 0{\cdot}1$. Concentration $n_1 - n_0 = 0{\cdot}00300$.
The amounts of components are percentages of the total refractive increment.
* Svensson [1939]. † Longsworth *et al.* [1939].

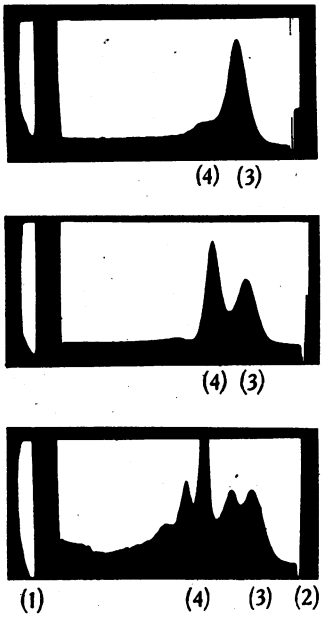

Fig. 1. Sedimentation diagrams. Top: normal human serum. Centre: myelomatosis serum 3. Bottom: myelomatosis serum 5. Phosphate pH 8, $\mu = 0{\cdot}1$, $n_1 - n_0 = 0{\cdot}00300$. Field strength 270,000 × gravity. All photographs taken 45 min. after reaching full speed. (1) Cell index. (2) Meniscus. (3) Albumin. (4) Globulin.

from four exposures, two from the anode and two from the cathode limb. Owing to the quantitative distribution of the components it was possible in these cases to correct for the δ boundary on the anode side, and to make complete analysis of the cathode limb curves, neither of which procedures was possible for normal human serum under the experimental conditions necessarily adopted.

Figs. 2 and 3 are diagrams of sera 3 and 5 respectively, taken 2 hr. after starting the current.

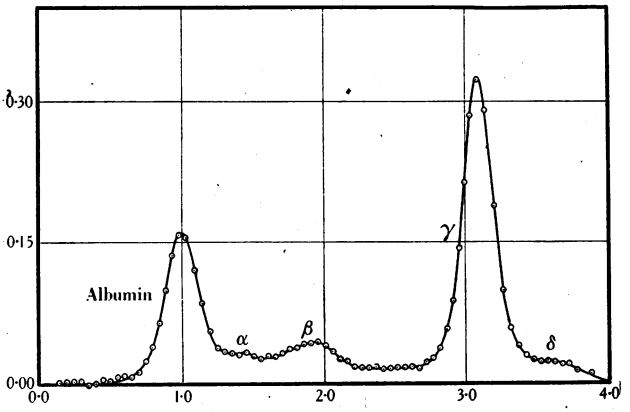

Fig. 2. Electrophoresis diagram. Myelomatosis serum 3. Phosphate *p*H 8, $\mu = 0{\cdot}1$, $n_1 - n_0 = 0{\cdot}00300$, 5 V./cm. Exposure 120 min. after starting current. Abscissa: distance in U-tube in cm. Ordinate: scale line displacement in mm. Anode limbs.

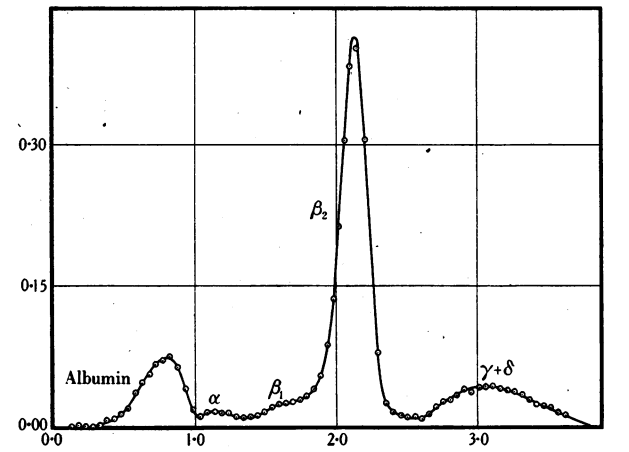

Fig. 3. Electrophoresis diagram. Myelomatosis serum 5. Phosphate *p*H 8, $\mu = 0{\cdot}1$, $n_1 - n_0 = 0{\cdot}00300$, 5 V./cm. Exposure 120 min. after starting. Abscissa: distance in U-tube in cm. Ordinate: scale line displacement in mm. Anode limbs.

The outstanding characteristics of sera 1–4 are the reduced percentage of albumin and the greatly increased percentage of γ globulin, the deviation from the normal in each case increasing with the total protein content of the serum. The percentage of α and β globulins remains fairly close to that of normal human serum. In contrast with these, serum 5 showed a great increase in β globulin, the percentage of γ globulin being below that found in normal human serum.

With improved optical methods, Svensson [1939] has shown that the β component of normal human serum is in effect composed of two substances whose mobilities lie very close to one another. These may be referred to as β_1 and β_2 in decreasing order of mobility. A similar phenomenon has been observed by the writer in normal and immune horse sera. It would appear that in serum 5 it may be the β_2 component which is increased in amount. The high value for the proportion of α globulin found for this serum is due to the inclusion of the area due to globulin β_1 with it, a finer analysis not being possible. It was not possible to analyse the serum 4 diagrams for α and β globulins separately.

In the last column of Table 2 the refractive increment due to the albumin in the serum, obtained from columns 2 and 5, is given. It appears that not only does the percentage of serum albumin decrease as the protein content of the serum rises, but the absolute concentration also decreases. As data on blood volume were not available, calculations of the total circulating serum albumin could not be made. However, it seems improbable that a doubling of the blood volume could occur, which would be necessary in some of the cases, in order that the total circulating serum albumin should remain constant.

An interpolation is necessary here concerning the values quoted for the amounts of the components determined by electrophoretic analysis of normal human serum.

It has been pointed out [Longsworth *et al.* 1939] that analytical values for components determined from descending limb photographs, in this case the cathode, are likely to be the more accurate owing to the absence of the δ boundary, the ϵ boundary effect being very slight. In the study of normal human serum [Kekwick, 1939] only the percentages of albumin and total globulin were estimated from descending limb exposures, as it was considered that the extrapolation of the curves obtained for the individual globulins was too uncertain to be of value. Ascending limb exposures which apart from the γ and δ components were capable of complete analysis, gave a smaller percentage of albumin than those from the descending limb, and the value for γ globulin was too high owing to the inclusion with it of the effect due to the δ boundary.

Accordingly the ascending limb values have been corrected for the δ boundary effect by multiplication by the ratio

$$\frac{\%\text{ albumin (descending limb)}}{\%\text{ albumin (ascending limb)}},$$

and the total excess percentage subtracted from the γ globulin percentage. The corrected values, which are given in Table 2, represent the means from six normal human sera. Some deviation occurs from the data of other workers.

II. The isolation and characterization of some proteins from the sera

It was felt that an important point to establish was whether the γ globulin, present in such excessive amounts in sera 1–4, had the same characteristics as the γ globulin of normal human serum. The measurement of the mobility of the protein in the analytical experiments at a single pH, and the comparison of this value with the corresponding value for normal human γ globulin, the latter determined in the presence of the other normal serum protein components, was not considered sufficient evidence on which to base any conclusions. Experiments showed that the γ globulin from myelomatosis serum could readily be obtained as an homogeneous substance by simple salting out procedures. The β component of serum 5 was isolated in a similar manner.

It has also been found possible to isolate the γ globulin of normal human serum in large quantities by salt fractionation, the product sedimenting and migrating homogeneously.

The various preparations obtained have been characterized by determinations of sedimentation constant, electrophoretic mobility and isoelectric point, and carbohydrate-nitrogen ratio.

Details of preparations of myelomatosis globulins

Preparation 4. As sera 1 and 2 showed a similar constitution, and in view of the small amount of material available, the residues of the sera in phosphate buffer *p*H 8, $\mu=0{\cdot}1$, were combined and precipitated once with half-saturated $(NH_4)_2SO_4$. The precipitate was centrifuged, washed once by suspension in a small amount of half-saturated $(NH_4)_2SO_4$ and recentrifuged.

In the ultracentrifuge a single component only was found, but in phosphate buffer *p*H 7, $\mu=0{\cdot}1$, a small amount of a faster moving component was observed in the electrophoresis apparatus.

Preparation 7. This was obtained from serum 3 in phosphate buffer *p*H 8, $\mu=0{\cdot}1$. The globulin was precipitated by addition of Na_2SO_4 up to 16 g./100 ml. The precipitate was redissolved in phosphate buffer *p*H 8, $\mu=0{\cdot}1$, and reprecipitated by bringing the concentration of Na_2SO_4 up to 20 g./100 ml. The product sedimented and migrated as a single component.

Preparation 6. By precipitating myeloma serum 5 in phosphate buffer *p*H 8, $\mu=0{\cdot}1$, with 20 g./100 ml. of Na_2SO_4, a quite distinctive product was obtained. The material thrown out of solution rose to the surface and had a sticky consistency. Electrophoretically the material was homogeneous, but under all conditions of concentration and of buffer salt composition four components were observed in the ultracentrifuge.

The isolation of normal human γ globulin

For comparison with the proteins isolated from the various myelomatosis sera, it was decided to isolate normal human γ globulin. For this purpose a salt fractionation technique was elaborated, using the electrophoretic method to control the fractionation.

Addition of 18 g./100 ml. of Na_2SO_4 to normal human serum precipitates the globulins almost completely, the precipitate being only slightly contaminated with serum albumin. If this globulin is dissolved in phosphate buffer *p*H 8, $\mu=0{\cdot}1$, to a volume 40 % that of the initial serum, and the Na_2SO_4 concentration brought up to 12 g./100 ml. a characteristic precipitate forms. Very marked streaming occurs in the solution and tactoid bodies can be observed under the microscope. The precipitate settles rapidly and forms a continuous stickly layer over the bottom of the containing vessel. This material consists of γ globulin contaminated with about 5 % of α and β globulins. Resolution of this precipitate in phosphate *p*H 8, $\mu=0{\cdot}1$, to 2 % of the initial serum volume and reprecipitation with 12 g./100 ml. Na_2SO_4 gives pure γ globulin. The yield of thrice precipitated material is about 7 % of the total initial serum protein.

In Tables 3–6 the data obtained from the examination of the various globulin preparations are presented.

The solutions used in the sedimentation constant determinations all contained $0{\cdot}1M$ NaCl in addition to the buffer salts, to inhibit charge effects. The total ionic strength of the solutions varied between $\mu=0{\cdot}12$ and $\mu=0{\cdot}2$, but the protein content was maintained at $n_1-n_0=0{\cdot}00150$.

Table 3. *Sedimentation constants S_{20} of human serum globulin preparations*

Preparation	*p*H ... 5·1	6·0	7·0	8·0	S_{20} Mean
Myeloma globulin 4	—	6·58	6·63	6·60	6·60
Myeloma globulin 4	—	6·68	6·88	6·86	6·81
Myeloma globulin 6	—	—	—	6·54, 9·26, 11·3, 13·3	—
Normal human γ globulin	6·78	6·76	6·93	6·66	6·78
Buffer composition	Acetate	Phosphate	Phosphate	Phosphate	—
μ=0·120–0·200	NaCl	NaCl	NaCl	NaCl	—

The sedimentation constants, S_{20}, are in units of 10^{-13}.

Table 4. *Myeloma globulins. Electrophoretic mobilities*

*p*H	Preparation 4	Preparation 7	Preparation 6
5·11	2·85	2·71	2·10
5·84	—	1·18	−0·05
5·95	1·00	—	—
6·45	—	0·70	—
6·46	—	—	−0·42
6·99	—	—	−0·89
7·00	−0·01	—	—
7·96	—	−0·34	−1·32
7·99	—	—	−1·40*
8·03	−0·73	—	—
I.E.P.	*p*H 6·99		*p*H 5·81

* Electrophoretically separated from myeloma serum 5.

Mobilities are in units of cm.^{2} V.$^{-1}$ sec.$^{-1}$ × 10^{-5}.

Buffers: ionic strength μ=0·1. All buffers were phosphate mixtures except for *p*H 5·11, which was acetate.

Table 5. *Normal human globulin. Electrophoretic mobilities*

*p*H	Mobility
5·12	2·17
5·94	0·52
6·48	0·01
7·03	−0·27
8·01	−0·88
I.E.P.	*p*H 6·58

Mobilities are in units of cm.^{2} V.$^{-1}$ sec.$^{-1}$ × 10^{-5}.

Buffers: ionic strength μ=0·1. All buffers were phosphate mixtures except for *p*H 5·12, which was acetate.

Table 6. *Carbohydrate-nitrogen ratios for globulin preparations*

Preparation	Carbohydrate-N ratio
Myeloma globulin preparation 4	0·100
Myeloma globulin preparation 7	0·083
Myeloma globulin preparation 6	0·167
Normal human γ globulin	0·077

Globulin preparation 6 was unusual in its sedimentation behaviour, showing four components. Preparations 4 and 7 sedimented as homogeneous substances, though for the former the sedimentation constant is rather low in comparison with that found for normal human γ globulin. For an electrophoretically prepared normal human γ globulin, Kabat [1939] gives $S_{20}=7{\cdot}1\times10^{-13}$, somewhat higher than the mean of $6{\cdot}8\times10^{-13}$ found here for the salted out material.

The mobility data of Tables 4 and 5 are plotted in Fig. 4. From this it is immediately apparent that the globulins isolated from the myelomatosis sera differ in some degree from normal human γ globulin. The mobilities of preparations 4 and 7 are in agreement with one another in the more acid part of the range covered, but show some divergence in the more alkaline region.

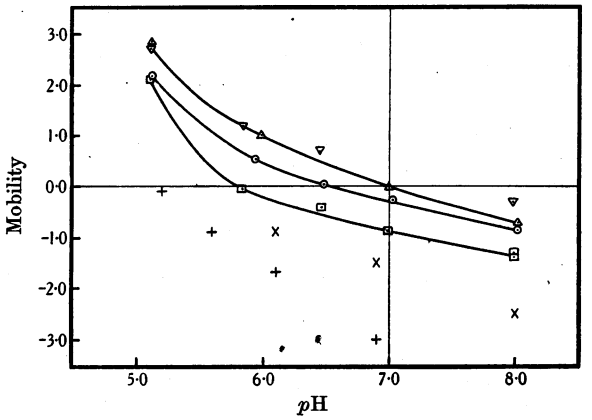

Fig. 4. Mobility-pH curves for human globulin preparations. △ ▽ ⊡ Myeloma globulins 4, 7, 6 respectively. ⊙ Normal human γ globulin. + Normal human β globulin. × Fibrinogen.

Preparation 6, although showing four components in the ultracentrifuge, migrated as a single component at all pH investigated, the mobility curve lying between that of normal human β and γ globulins and differing from that of fibrinogen. The values given for fibrinogen and normal β globulin are those found by Stenhagen [1938] for migration in the presence of all the normal serum components. Some deviations occur in the values for normal human γ globulin determined under his conditions, and those reported here on the pure material. The isoelectric points of the myelomatosis globulin preparations differ markedly from the value found for normal human γ globulin.

A consideration of the carbohydrate-nitrogen ratios of the preparations indicates a similarity between normal human γ globulin and globulins 4 and 7. The fractionation of normal human serum described in the preparation of normal human γ globulin was followed by determinations of carbohydrate-nitrogen ratios as well as by electrophoretic analysis. It became apparent that normal human β globulin had a rather higher carbohydrate-nitrogen ratio of the order 0·14, suggesting that globulin 6 may be more nearly related to normal β than to normal γ globulin. This is in conformity with its electrophoretic behaviour.

Discussion

The myelomatosis sera so far investigated appear to fall into two groups according to their behaviour in the ultracentrifuge. The first group shows the same number of components as normal human serum, the globulin component being quantitatively very much increased. In addition to the sera of this type described in the present communication McFarlane [1935] and Jersild & Pedersen [1938] have each published data on a serum with these characteristics. The second group of sera, of which one is described here, shows several components, in this case five, whilst in one reported on previously by McFarlane [1935] four components were demonstrated.

In the sera of the first group electrophoresis indicated a large increase in γ globulin, whereas in the single second group serum available an excess of β globulin was apparent. It is interesting that of three myelomatosis sera investigated by Longsworth *et al.* [1939] two gave a large increase in β globulin, whilst the third showed no deviation from the normal in appearance.

Those components which appeared in excessive amount in the sera examined here were qualitatively distinguishable from the components of normal human serum, particularly by electrophoresis.

Unfortunately none of the cases whose sera have been examined here showed any proteinuria, neither do Longsworth *et al.* [1939] mention this in connexion with any of the cases they investigated. One case described by McFarlane [1935] had Bence-Jones proteinuria, the serum being of the first type. No further light can, as yet, be thrown on any relationship between the serum proteins and Bence-Jones protein.

The remarkable serum described by Bonsdorff *et al.* [1938] in which spontaneous crystallization occurred was not examined by physical methods, though the crystalline globulin which separated was examined in the centrifuge by Pedersen. The sedimentation constant of this material was $S_{20} = 7{\cdot}1 \times 10^{-13}$ though its molecular weight was 200,000, which is higher than that found for normal human serum γ globulin, viz. 176,000. None of the sera described in this paper revealed any tendency to deposit crystals spontaneously.

Summary

An examination of the sera from five cases of myelomatosis suggests that according to their ultracentrifugal and electrophoretic behaviours the sera are of two types. As far as can be determined, this classification is applicable to those myelomatosis sera which have previously been examined by these methods and reported on in the literature.

In addition to quantitative differences in the constitution of myelomatosis sera as compared with normal human serum, qualitative differences are apparent in some of the protein components.

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APPENDIX

Clinical notes

J. B. Male 27 years. July 1936, knock on forehead followed by swelling. October 1936, swellings in skull and ribs. X-ray: several areas of bone destruction in skull, tumour right lateral forehead, rib destruction with tumour projecting into chest; area of pelvic bone destruction. Palliative treatment to skull and ribs. Died 26. iii. 39. Serum November 1938.

C. E. Male 50 years. September 1937, deformity of upper dorsal spine. X-ray: collapse of L.V. 3 due to myeloma of spine; myelomatous changes in right leg. X-ray therapy to spine. January 1940, other bones showed myelomatous changes. General condition fairly satisfactory. Serum November 1938.

L. G. Female 70 years. May 1938, soft area in scalp behind left ear. X-ray: numerous areas of increased transradiancy throughout skull; right femur and humerus sharply defined defects. Sternal puncture: characteristic of multiple myelomatosis. Serum April 1939.

S. H. Male 61 years. 1936, pain lower sternum. 1937, lump developed on lower sternum. Part of lower sternum removed, "crackled egg shell appearance". 1938, pain in shoulder blades and lower ribs. Deep X-ray treatment. 1939, pain sitting or lying, no spinal tenderness. Sternal puncture: characteristic of multiple myelomatosis. X-ray of skeleton inconclusive. No Bence-Jones proteinuria. Serum May 1939.

J. J. Male 60 years. Upper abdominal pain for 35 years. Sternal puncture: characteristic picture of multiple myelomatosis. X-ray: skull showed numerous small areas of increased translucency. Trace of albumin in urine. No Bence-Jones proteinuria. Died 21. v. 39. P.M., multiple myelomatosis; hypostatic pneumonia. Serum April 1939.