Serum Electrophoretic Pattern and Morphology of Myeloma Cells
An Attempt at Correlation

By WAYNE A. CHADBORN AND HORACE H. ZINNEMAN

FEW ATTEMPTS have been made to correlate the histologic characteristics of myeloma cells with the abnormalities of the serum protein pattern. Publications by Snapper, Turner and Moscovitz,1 Bayrd,2 Lichtenstein and Jaffe,3 and Adams, Alling and Lawrence4 contain references to the degree of maturity of the myeloma cells and the types of serum protein pattern, but correlation was not consistently suggested or noted.

Hertzog and Schleicher5 mentioned that cells of uniform size with deep blue cytoplasm were likely to be found in cases of multiple myeloma with increased serum globulin and absence of Bence Jones protein. Bayrd2 reviewed 51 cases of multiple myeloma and was unable to correlate the cellular differentiation and the presence of Bence Jones protein in the urine or the degree of elevation of the globulin fraction. Wuhrmann, Wunderly and Hugentobler6 showed that an elevation of serum gamma globulin was associated with a prolonged clinical course and a mature type of plasma cell, while elevation of alpha globulin was associated with a short clinical course and immature plasma cells.

Olhagen and coworkers7 studied the nucleic acid distribution and serum protein formation in multiple myeloma and evaluated the morphologic characteristics of the myeloma cells. Their patients with increased gamma or beta globulin fractions had myeloma cells with eccentric nuclei, and a small nuclear-cytoplasmic ratio. Other patients with a normal total protein and minimal globulin changes had myeloma cells with a central nucleus and a large nuclear-cytoplasmic ratio.

Waldenström8 expressed the opinion that an elevated beta globulin fraction in the serum proteins was associated with small myeloma cells in the bone marrow, whereas an elevated gamma globulin fraction was usually associated with large myeloma cells.

Streicher and Sandkühler9 noted that plasma cells in the bone marrow of patients with multiple myeloma may reach four times the normal size, whereas small cells are rare. They state that a given case of multiple myeloma presents cells of similar size and characteristics, but that a reactive plasmocytosis distinguishes itself from multiple myeloma by the polymorphous character of the plasma cells. These authors also describe the cells in myelomas of the "gamma" patterns as small, and those in "beta" patterns as reticular, and believe that patients with "gamma" pattern myelomas have total serum proteins from 9.5

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to 17 Gm. per 100 ml., whereas those with “beta” patterns have total serum proteins below 9 Gm. per 100 ml.

These statements are at variance with those of all other investigators, and it is difficult to evaluate these conclusions, particularly since they were not supported by any data.

The present study is an attempt at a more detailed comparison of the morphologic characteristics of the myeloma cells with the electrophoretic pattern of the serum proteins.

**Materials and Methods**

Twenty-seven cases of multiple myeloma were collected from the Minneapolis Veterans Administration Hospital and the University of Minnesota Hospitals. The total serum proteins were measured by the biuret method and the pattern of protein distribution was determined by paper electrophoresis. All sera were obtained from fasting patients. Paper electrophoresis was performed in a cell with hanging Whatman filter paper strips, 4 cm. wide, using a veronal buffer at pH 8.6 and 0.1 molar ionic strength, with 6 milliamperes current strength for ten hours. Proteins were stained with bromphenol blue after the method of Kunkel and Tiselius. The optical density of the separated proteins was measured with the Photovolt densitometer and quantitation of the protein fractions was obtained by planimetry.

Seventeen of the sera were also examined by free electrophoresis (Tiselius) with the portable model of the American Instrument Company, using veronal buffer at pH 8.6, a current of 10 ma. and a separation time of 2 hours. Planimetric determination of the separated protein fractions was obtained from the photographed electrophoretic curve.

The results obtained by paper electrophoresis differed slightly from those obtained by free electrophoresis in the beta and gamma fractions but never to a degree which would invalidate the basic group pattern.

Bone marrow biopsies were obtained by sternal needle puncture and aspiration. The myeloid-erythroid layer was obtained in all instances by centrifugation of the heparin-treated specimen, according to the method of Schleicher and Sharp. Wright’s stain was used on all slides.

The following cytologic characteristics of the myeloma cells were evaluated in each marrow smear: Diameter of the nucleus, diameter of the cell, eccentricity of the nucleus, clumping of nuclear chromatin, presence and number of nucleoli, mitoses, color of cytoplasm, number of nuclei in the cell, presence of detached masses of cytoplasm in the smear, vacuoles in the cytoplasm, granularity of the cytoplasm, peripheral rim of darkly stained cytoplasm, Russell bodies, Mott cells, presence of a clear area in the cytoplasm (Hof), and rouleaux formation of the red cells.

In addition, the ratio of the diameter of the nucleus to that of the cytoplasm was calculated with the following formula:

\[
\frac{(\text{diameter of nucleus})^2}{(\text{diameter of cytoplasm})^2 - (\text{diameter of nucleus})^2}
\]

The relative area of the cytoplasm was calculated roughly as follows:

\[
\text{Relative area of cytoplasm} = \frac{(\text{diameter of cytoplasm})^2 - (\text{diameter of nucleus})^2}{16}
\]

There seemed to be little purpose in calculating the actual area of the cytoplasm or of the nucleus in smear preparations but the area of the cytoplasm may be obtained by multiplying the figures recorded in table 1 for relative cytoplasm by a factor \(\frac{1}{4}\) microns.

The diameters of the nucleus and of the cell were measured to the nearest micron with a Bausch & Lomb micrometer eyepiece.* In the event of appreciable eccentricity, the meas-

* Calibrated against a Spencer stage micrometer slide.
According to the classification of I. Snapper.

### Table 1.—Electrophoretic Data and Cell Measurements on 27 Patients with Multiple Myeloma

<table>
<thead>
<tr>
<th>Patient</th>
<th>% Plasma Cells in Marrow</th>
<th>% Total Protein</th>
<th>% Alb.</th>
<th>% Alpha Glob.</th>
<th>% Beta Glob.</th>
<th>% Gamma Glob.</th>
<th>% &quot;M&quot; Glob.</th>
<th>Nucleus Diam. μ</th>
<th>Cell Diam. μ</th>
<th>Cytoplasm C(^2)N(^{5})</th>
<th>Ratio N(^2) C(^{-1})</th>
<th>Ratio N(^2) C^{-1}</th>
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<td>246</td>
<td>0.55</td>
<td>0.59</td>
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- x Free and paper electrophoresis
- y Paper electrophoresis only
- z Salt fractionation only
- + Alpha, and alpha2 globulins are combined

Measurements of the long and short axes were averaged. At least 25 cells in each biopsy of bone marrow were measured and their characteristics recorded as averages; 50-100 cells were measured wherever the cellular characteristics were not very uniform. The stated measurements were reviewed independently by three pathologists.

The cases were classified by four types of serum globulin abnormalities: Normal pattern or minimal protein alteration, beta pattern, "M" pattern, and gamma pattern. There were no alpha patterns. The 27 cases were grouped as follows:

1. A "beta pattern" was recognized when the beta globulin exceeded 13 per cent* of the total protein,
2. A "gamma pattern" if the gamma globulin fraction exceeded 15 per cent* of the total protein,
3. "M" pattern if there was a distinct peak between the beta and gamma globulins,
4. "Minimal (0) pattern" if the above globulin fractions were below these limits and there was no "M" peak.

Homogeneity was recognized in beta, gamma, or "M" globulins by abnormal slender peaks with a narrow base.

* According to the classification of I. Snapper.
SERUM PROTEIN PATTERN AND MYELOMA CELLS

Results

Nine of the twenty-seven cases were classified as "beta" pattern, three as "M" pattern, twelve as "gamma" pattern and three as "minimal change." The actual results of protein fractionation are shown in table 1. The proportion of "beta" patterns is higher than the usual incidence; in three of these nine cases the beta globulin was only 14–16 per cent of the total proteins.

These protein patterns were compared with the characteristics recorded for each bone marrow smear. The percentage of plasma cells among nucleated marrow cells varied from 5 per cent to 79 per cent. There was no significant correlation between the relative number of plasma cells and the serum protein pattern. Some of the patients with few plasma cells had the most abnormal and largest total amount of serum protein.

Eccentricity of the nucleus was graded 1+ to 4+. Nine patients of the "gamma" pattern group were 4+, two were 3+, and one was 2+. The remaining three protein groups were scattered through the range of nuclear ecen-

![Diameter of Nucleus](image1)

![Diameter of Cell](image2)

![Relative Area of Cytoplasm](image3)

![Nuclear Cytoplasm Ratio (Diameter)](image4)

![Nucleus/Cytoplasm Ratio (Areas)](image5)

Fig. 1.—Measurements in plasma cells of 27 patients with multiple myeloma. "O" signifies the group of patients with minimal changes in the electrophoretic pattern of their serum, "B" refers to the group of patients with typical beta pattern, "M" signifies the M-pattern and "G" the gamma pattern. Diameters are in microns and areas in square microns.
tricity. Clumping of the chromatin and the presence of nucleoli did not differentiate between groups, however the nuclei of the cells of the patients with borderline and "gamma" pattern showed a more consistent tendency to coarse chromatin clumping than those with "beta" and "M" patterns. Large and frequently multiple nucleoli were seen in all groups. Occasionally the nucleoli were recognizable only as an area of lessened intensity of the nuclear stain. Mitoses were rarely seen and appeared unrelated to globulin patterns.

The quality of the cytoplasm appeared to vary considerably with the staining technic. Nevertheless, the myeloma cells of ten of twelve cases with a "gamma" pattern had deep blue cytoplasm while four of the group with a "beta" pattern stained deep blue and five pale blue.

The incidence of multinucleated plasma cells varied from one to 24 per 100 plasma cells, but most patients, regardless of protein pattern, had a few of these cells. Nuclei in excess of two per cell likewise were unrelated to protein groups.

Cytoplasmic extrusions were few in number, from none to 18 per 100 plasma cells. In one patient with an "M" pattern, however, 160 such cytoplasmic bodies were found for each 100 plasma cells. Some of these extrusions measured up to 18 microns in diameter and some cells were left with only a narrow rim of cytoplasm.

The cytoplasm of the myeloma cells of the "beta" group contained fewer vacuoles than the "gamma" group, but there was marked overlapping. The cytoplasm in the cells of the "beta" group was judged to be homogeneous or finely mottled with equal distribution, while all cells of the "gamma" and "M" group were found to have finely mottled cytoplasm.

The presence of a dark rim of cytoplasm at the periphery appeared to be a function of staining intensity. Some cells, however, did have a more definite border than others. This characteristic was present in 14 of the 27 cases but with no differentiation in the protein patterns.

Russell bodies were found in only a few cases in each group and seemed to be a manifestation of good staining. Mott cells were found in only one patient (L. A.), who had no protein abnormality.

A clear area (Hof) was found near the nucleus in only nine patients and was not confined to any of the four protein groups.

Rouleaux formation was found in the patients with marked elevation of serum globulins. It occurred in ten of twelve in the "gamma" group and five of nine with a "beta" pattern. It was present in all of three patients with an "M" pattern, including one patient with a total serum protein of only 6.8 Gms. ("M" globulin 35 per cent, albumin 29 per cent).

<table>
<thead>
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<th>Table 2.—t-Score Tests Between Beta and Gamma Pattern Groups Where N = 19</th>
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<tbody>
<tr>
<td>Mean of</td>
</tr>
<tr>
<td>betas</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Nucleus Diameter (Micra)</td>
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<td>Cell Diameter (Micra)</td>
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<tr>
<td>Cytoplasm &quot;Area&quot;</td>
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<tr>
<td>Nuclear/Cytoplasm Ratio (Diameters)</td>
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<tr>
<td>Nuclear/Cytoplasm Ratio (&quot;Area&quot;)</td>
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</table>
The patients with “gamma” and “beta” patterns constituted the principal groups in this series. Table 1 and figure 1 compare the results of the electrophoretic serum protein fractionation with the measurements of the myeloma cells, obtained from bone marrow biopsies of these patients.

The data suggest that cells of small diameter were associated with serum proteins of the “beta” pattern whereas large cells indicated serum proteins of the “gamma” pattern. The serum of three patients was characterized by an “M” pattern and these patients also had large plasma cells in the bone marrow.

A statistical test of significance was applied to the difference between the “beta” and “gamma” groups in the mean nuclear diameter, cell diameter, expression of cytoplasmic “area,” ratio of the diameter of the nucleus to the cytoplasm and ratio of the area of the nucleus to that of the cytoplasm. The results

Fig. 2.—Myeloma cells in the bone marrow of a patient (J. W.) whose serum shows a “beta pattern” on electrophoresis. The cells are of small size and have a meager amount of cytoplasm.
Fig. 3.—Myeloma cells in the bone marrow of another patient (A. P.) whose serum shows a "beta pattern" on electrophoresis.

of the test (table 2) indicated probabilities that the data on the nuclear diameter is not significant, the cell diameter is very significant, expression of cytoplasmic area is highly significant, the ratio of the diameter of the nucleus to that of the cytoplasm is very significant, and the ratio of the area of the nucleus to that of the cytoplasm is of doubtful significance.

Photographs of examples of the myeloma cells of the "beta" group are shown in figures 2 and 3, and of the "gamma" group in figures 4 and 5.

Discussion

Distinctive characteristics associating certain types of myeloma cells with one serum protein pattern or another have been sought in the past. The methods of ultraviolet light absorption,7 phase microscopy,19 and determination of anti-
body response have been used in addition to the study of electrophoretic patterns of the serum proteins. None of the data offered more than a suggestion that one or another histologic feature might possibly be correlated with the excess formation of a specific globulin.

It is not possible to state categorically on the basis of our rather small number of cases that any one characteristic was always correlated with a certain type of globulin pattern. However, some earlier impressions by Olhagen and Waldenström concerning a differentiation of serum protein pattern by myeloma cell size were substantiated. Our results showed definite significance with three types of measurements:

1. Cell diameter,
2. The relative area of cytoplasm,
3. Ratio of the diameter of the nucleus to that of the cytoplasm.
Since the nuclear diameters showed little variation, and since the eccentricity of the nuclei was greater when a larger amount of cytoplasm was present, these three types of data are largely expressions of variations in cell diameter.

The differences in total cell size clearly separated the two major groups of "beta" and "gamma" patterns within this series. Small cell size and a small amount of cytoplasm in myeloma cells were associated with the "beta" pattern. Large myeloma cells with a large amount of cytoplasm were associated with the "gamma" and "M" patterns of the blood serum proteins. This is in essence a confirmation of a previous statement by Waldenström. The small cells, associated with a "beta" pattern, had a homogeneous cytoplasm, while the large cells, associated with "gamma" pattern, had a finely mottled cytoplasm.

Some clinical importance may be attached to the correlations since previous reports\(^5,6\) have established that a protracted course often occurs with a "gamma" pattern and a more fulminating course in patients with a "beta" pattern.
One of the cases (E. R. in table 1) with a diagnosis of probable multiple myeloma, based on the bone marrow biopsy and clinical picture, showed a gamma globulin pattern on electrophoresis but the cell size was small, there was little cytoplasm, and little eccentricity of the nucleus. The margins of the cells were ragged with strands of cytoplasm irregularly extending from them and with many detached masses of cytoplasm. The cell measurements were widely separated from the values in other patients in the "gamma" group and were similar to those of patients in the "beta" group.

Many of the patients in this series are recent with too short an interval since diagnosis to allow comparison of the course of the disease in the different protein pattern groups.

**Summary and Conclusions**

The electrophoretic pattern of the serum proteins of 27 patients with multiple myeloma have been compared to the morphologic characteristics of the abnormal cells in the smear of the bone marrow. The cases were classified into four types of serum globulin abnormalities: Normal pattern or minimal protein alteration, "beta" pattern, "M" pattern, and "gamma" pattern. The following morphologic characteristics of the myeloma cells were evaluated: Diameter of the nucleus, diameter of the cell, eccentricity of the nucleus, clumping of nuclear chromatin, presence and number of nucleoli, mitoses, color of the cytoplasm, multiplicity of nuclei, presence of detached masses of cytoplasm in the smear, vacuoles in the cytoplasm, granularity of the cytoplasm, peripheral rim of darkly stained cytoplasm, Russell bodies, Mott cells, presence of a "Hof" and rouleaux formation of the red blood cells.

The relative area of the cytoplasm, derived from the diameter of the myeloma cell, was found to be the most valid among all morphologic characteristics in predicting the type of serum protein pattern. Small size and a small amount of cytoplasm of myeloma cells were related to the beta globulin pattern while large size and abundant cytoplasm were related to the gamma and "M" globulin patterns.

**Summario e Conclusiones in Interlingua**

Le configuration electrophoretic del proteinas seral de 27 patientes con myeloma multiple esseva comparate con le caracteristicas morphologic del cellulas anormal in frottis de medulla osse. Le casos studiate esseva classificate in quatro typos de anormalitates del globulina seral. Isto es: Configuration normal o minimalmente alterate, configuration beta, configuration M, e configuration gamma. Le sequente caracteristicas morphologic del cellulas de myeloma esseva evalutate: Diametro del nucleo, diametro del cellula, eccentricitate del nucleo, agglutination de chromatina nuclear, presentia e numero de nucleolos, mitoses, color del cytoplasma, multiplicitate de nucleos, presentia de distachate massas de cytoplasma in le frottis, vacuolos in le cytoplasma, granularitate del cytoplasma, margin peripherie de cytoplasma a coloration obscur, corpores de Russell, cellulas de Mott, presentia de areas clar circa le nucleos, e formation de rouleaux de erythrocytos.

Il resultava que le area relative del cytoplasma—derivate ab le diametro del
cellula de myeloma—esseeva plus valide que omne le altere caracteristicas morphologic como base de predicer le typo de configuration electrophoretic del proteinas sereal. Parve dimensions e parve quantitates de cytoplasm de cellulas de myeloma correspondeva al configuration beta, durante que grande dimensiones e grande quantitates de cytoplasm a correspondeva al typos gamma e M.

REFERENCES

Serum Electrophoretic Pattern and Morphology of Myeloma Cells: An Attempt at Correlation

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