

# An Electrophoretic Study on the Origin of the Abnormal Plasma Proteins in Multiple Myeloma\*

GAIL LORENZ MILLER,† CLARK E. BROWN, ELIZABETH ESHELMAN MILLER,  
AND EDWARD S. EITELMAN

(The Institute for Cancer Research, the Lankenau Hospital Research Institute,  
and the Lankenau Hospital, Philadelphia, Pa.)

Multiple myeloma is usually accompanied by a high level of globulins in the plasma (3). A possible site of origin of the high level of plasma globulins is the rather abundant cytoplasm of the myeloma cells themselves. Direct evidence for this is provided by the report of Martin (5) that the sedimentation properties of globulins present in extracts of a tumor from a multiple myeloma subject were similar to those of the abnormal globulins obtained from the plasma of the same subject. Also suggestive is the finding of Ågren (2) that the amino acid content of a preparation of Bence-Jones protein obtained from tumor tissue was similar to that of the Bence-Jones protein obtained from the urine of a multiple myeloma subject. More indirect evidence comes from observations of Rundles, Dillon, and Dillon (11) that treatment with urethan of subjects with multiple myeloma produced a simultaneous inhibition of the growth of myeloma cells and decrease in the level of abnormal plasma proteins. Finally, it appears likely, although it has not been proved, that the crystalline cytoplasmic inclusions (6, 12) found in myeloma cells may represent this same, or related, abnormal protein.

To obtain additional and more conclusive evidence on this question, comparisons between multiple myeloma plasma and extracts of multiple myeloma tumor tissue were made in the present investigation by means of electrophoresis. Tumor tissue from vertebrae, ileum, and lymph nodes was obtained from a typical multiple myeloma subject at autopsy. The material from the marrow and the lymph nodes was composed very largely of myeloma cells, while that from the ileum was al-

most entirely made up of the myeloma cells. Control experiments were carried out with normal vertebral marrow, normal lymph nodes, and also with tumor tissue from a bone malignancy other than multiple myeloma, namely, myelogenous leukemia. In special studies, an effort was made to exclude blood or lymph as possible contaminants in the extracts. Vertebral marrow obtained at autopsy from a subject with multiple myeloma that had undergone treatment with urethan also was investigated.

## MATERIALS AND METHODS

*Sources of material.*—Subject MM, a 55-year-old female, showed on admission to Lankenau Hospital an anemia, a marked hyperglobulinemia, the presence of Bence-Jones protein in the urine, and the presence of abnormally high proportions of myeloma cells in the sternal marrow, but no skeletal irregularities as revealed by x-ray. Electrophoretic examination of her blood plasma demonstrated the presence of a large, sharp peak migrating somewhat slower than normal  $\gamma_2$ -globulins. Skeletal changes gradually became prominent, and the patient died 12 months after her original symptoms of chest oppression. She had been given palliative treatment with blood transfusions, but no urethan. At autopsy, affected vertebrae, ileum, and lymph nodes were removed and stored temporarily at dry ice temperature.

Subject MF, a 48-year-old female, showed a clinical picture similar to that of subject MM, except that the abnormal electrophoretic component of her plasma possessed a mobility much closer to that of normal  $\gamma_2$ -globulins. This patient was given urethan by mouth at the rate of 4 gm/day for a period of 2 weeks, but died 8 weeks after termination of the treatment. Affected vertebrae were obtained at autopsy.

Subject MM, a 60-year-old female, died of acute myelogenous leukemia. Affected vertebrae were obtained at autopsy.

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† Present address: Pioneering Research Laboratories, U.S. Army Quartermaster Corps, Philadelphia.

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Subject EH, an 82-year-old male, died of coronary sclerosis. Vertebrae obtained at autopsy appeared entirely normal and were used as such.

Subject MT, a 35-year-old female, died of rheumatic myocarditis. Samples from numerous hyperplastic lymph nodes obtained at autopsy appeared otherwise normal and were used as such.

**Extraction technics.**—For extracting the vertebral marrow, the marrow was first cut with the aid of a coping saw into strips about 2 mm. in thickness. The strips were then broken into small pieces, mixed with 4 volumes of 0.1 N buffered NaCl (7) at pH 7, and homogenized for 2 minutes in a Waring Blender at 4° C. Approximately 9 gm. of marrow was used for a given extraction. The homogenates were finally clarified by centrifugation. These extracts were quite red in color, however, owing to contamination with hemoglobin from blood in the marrow.

For extracting the solid tumor masses from the ileum, and also for extracting the lymph node tissues, these tissues were first carefully rinsed in isotonic saline to remove most of the adhering blood. After this treatment, the tissues were only slightly pink in color. The weights of samples of these tissues were 1.4–5.8 gm. They were then extracted in the Waring Blender, and the resulting extracts were clarified by centrifugation.

Even though precautions were thus made to remove blood as a contaminant in the extracts, it still appeared possible that lymph fluid between the individual cells of the tissues might contribute contaminating protein. To obviate this final source of contamination, free myeloma cells were prepared. To obtain the cells, 47 gm. of affected vertebral tissue were sliced and broken and were then suspended in 2 volumes of isotonic saline and shaken gently for 5 minutes at room temperature. Microscopic examination of the fresh suspension, stained with Wright's stain, showed many free, very few clumped, myeloma cells. The suspension was decanted from the larger pieces of tissues and was poured through a funnel having a loose plug of glass wool. The filtrate was centrifuged briefly to pack the cells, and the supernatant fluid was discarded. The original pieces of vertebral tissue were resuspended in saline, the entire process was repeated several additional times, and the different batches of cells were combined. The preparation thus obtained was then homogenized with 2 volumes of buffered NaCl, and the resulting extract was finally clarified by centrifugation.

**Electrophoretic experiments.**—The electrophoretic measurements were carried out in the Longworth modification of the Tiselius apparatus. The boundary patterns were recorded with the aid of the Philpot-Svensson cylindrical lens-knife edge optical system. The angle of the knife edge was varied in different experiments, depending upon the protein concentration. Photographs of the boundary patterns were taken with the aid of infrared light and infrared sensitive plates, since the extracts were usually opalescent and often pigmented with hemoglobin. The buffer used in all the experiments was 0.1 N diethyl barbiturate at pH 8.6, against which the test samples were equilibrated before electrophoresis.

Protein concentrations of the plasmas and extracts were based on nitrogen contents determined by a micro-Kjeldahl-Nessler method (8) and the use of a factor of 6.25.

## RESULTS

The electrophoretic pattern for the plasma of multiple myeloma subject MM is shown opposite *a* at the top of Chart 1. The large, sharp peak of

the abnormal<sup>1</sup> plasma component, labeled  $\gamma$ , may clearly be seen. This component was found to have a mobility in the ascending limb of 0.9, which was significantly lower than the corresponding value of 1.4 units found for normal  $\gamma_2$ -globulins.<sup>2</sup> Opposite

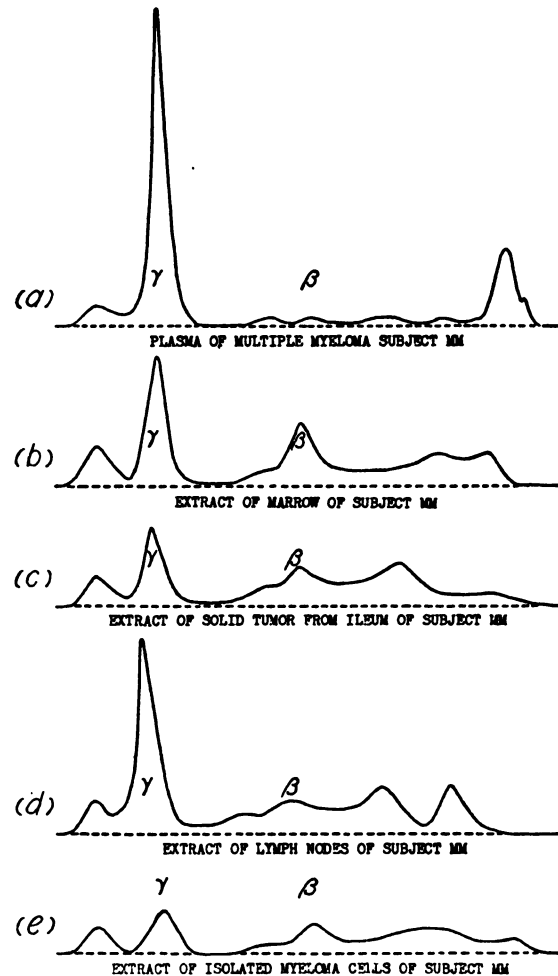


CHART 1.—Electrophoretic patterns of ascending boundaries of plasma of multiple myeloma subject MM and of extracts of tumor of the same subject. The direction of migration is from left to right. The peak at the far left in each pattern represents the salt boundary. Time of electrophoresis: approximately 140 minutes at 23 milliamperes. Protein concentrations: (a) 1.0 per cent after dilution to  $\frac{1}{2}$ ; (b) 0.9 per cent after dilution to  $\frac{1}{2}$ ; (c) 0.5 per cent; (d) 0.6 per cent; (e) 0.3 per cent.

*b* on the figure is presented the electrophoretic pattern of the extract of the vertebral marrow of this

<sup>1</sup> Although the  $\gamma$  component observed in the pattern of the myeloma plasma is not detectable in patterns of normal plasma, one cannot say with certainty that it is completely absent in the latter. It appears reasonable, however, to describe the component as "abnormal," since even if it were not abnormal from a qualitative point of view, it still would be abnormal quantitatively.

<sup>2</sup> G. L. Miller and E. E. Miller, unpublished data.

same subject. Opposite *c* is the corresponding pattern for the rinsed solid myeloma tissue from the ileum; opposite *d* is the pattern for the rinsed lymph node tissue; and opposite *e* is the pattern for the isolated myeloma cells from the marrow, all still from this same subject. The presence of the characteristic abnormal protein is evident in each

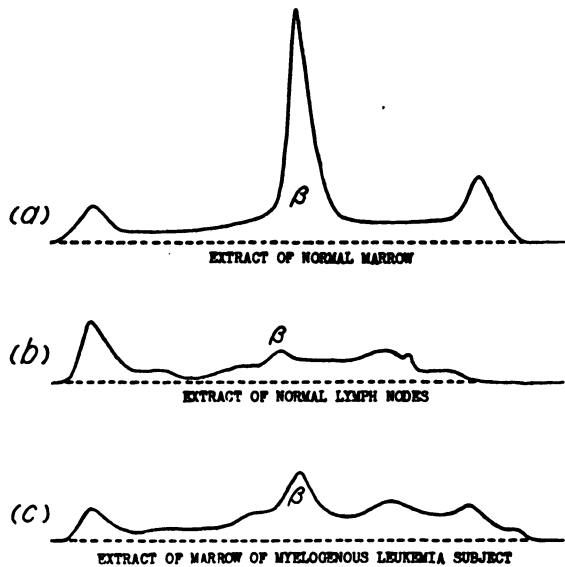


CHART 2.—Electrophoretic patterns of ascending boundaries of extracts of normal marrow, of normal lymph nodes, and of myelogenous leukemia marrow. Protein concentrations: (a) 0.9 per cent after dilution to  $\frac{1}{3}$ ; (b) not determined; (c) 0.9 per cent after dilution of  $\frac{1}{3}$ .

of these diagrams, as well as in the diagram of the plasma. Its presence in the extracts of the washed solid myeloma and lymph node tissues minimizes the possibility that it resulted from contamination by blood, while its presence in the extract of the free myeloma cells minimizes the possibility of contamination by either blood or lymph.

In Chart 2 are shown the electrophoretic patterns for the control experiments. As may be seen, the extracts of the normal marrow, the normal lymph tissue, and the marrow of the myelogenous leukemia subject all failed to reveal significant quantities of the distinctive myeloma component. These results indicate that the occurrence of the myeloma type of protein is peculiar to tissues containing myeloma cells.

In Chart 3, patterns for the plasma of myeloma subject MF, before treatment with urethan, and for extracts of her bone marrow obtained at autopsy after treatment are shown. The absence in the marrow extract of significant amounts of the abnormal protein which characterized the plasma may be explained on the basis of the effect of urethan in inhibiting the growth of myeloma cells

and, as a consequence, in inhibiting the production of the myeloma protein. Other evidence for the effect of the urethan in this subject was provided by microscopic examination of the marrow, which revealed the development of an aplastic condition, and by determinations of plasma protein level, which revealed a marked drop in the globulin fraction from 11.5 gm/100 cc to 5.0 gm/100 cc during the course of the treatment.

#### DISCUSSION

The demonstration that the plasma proteins and the myeloma cells of a case of multiple myeloma contained the same abnormal protein favors the interpretation that the abnormal component was manufactured by the cells in the first place. It does not seem likely that the protein could have been manufactured elsewhere in the body and subsequently accumulated by the myeloma cells. The data are too few, however, to permit the conclusion that the abnormal plasma proteins will always be found to occur within myeloma cells. Since the

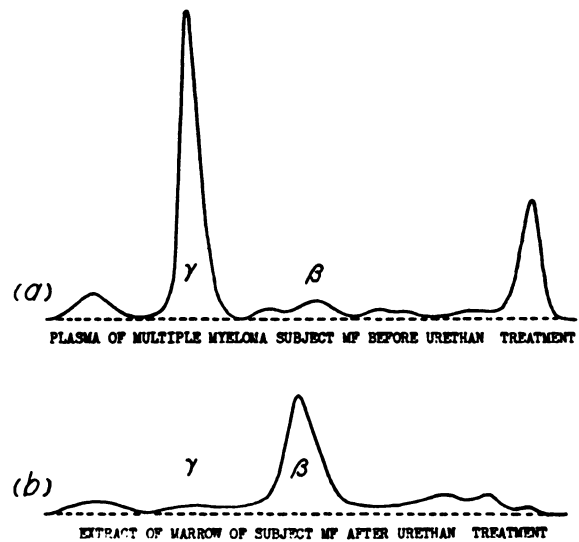


CHART 3.—Electrophoretic patterns of ascending boundaries of plasma of multiple myeloma subject MF before treatment with urethan and of extract of marrow of the same subject after treatment. Protein concentrations: (a) 1.5 per cent after dilution to  $\frac{1}{3}$ ; (b) not determined.

proteins distinctive for multiple myeloma have been previously shown by other investigators to have a variety of electrophoretic mobilities (11) and molecular sizes (10), it is conceivable that differences in their physical-chemical properties might be related to their rate of manufacture by the cells, their rate of secretion or shedding by the cells, or their rate of excretion into the urine in the form of Bence-Jones protein. Further experiments with multiple myeloma tissues from subjects

whose plasmas show other types of abnormal myeloma proteins would, therefore, be of considerable interest.

The present findings are of additional interest in connection with the hypothesis of Ehrich (4) that antibody is made by plasma cells. Since the myeloma cells of multiple myeloma are thought to be related cytologically to plasma cells, and since antibodies of blood occur in the  $\gamma$ -globulin fraction, the presence within myeloma cells of large amounts of a protein having a mobility approximating that of normal  $\gamma$ -globulins might be taken as support for the hypothesis. The occurrence in the plasmas of different multiple myeloma subjects of a variety of types of multiple myeloma proteins, as just mentioned, might then be explained by the fact that different multiple myeloma tumors are composed of plasma cells which are in varying stages of differentiation and which might reasonably be expected to manufacture different myeloma proteins.

Other significant peaks were seen in the electrophoretic diagrams of the various tissue extracts. Most consistent among these was the peak labeled  $\beta$ , which had the same mobility as  $\beta$ -globulins of blood plasma. This peak, particularly where it was most conspicuous, was due largely to hemoglobin (9). Further study will be required for a more certain identification or characterization of the other components of the extracts.

#### SUMMARY

An electrophoretic study has been made of the plasma and extracts of tumor of a typical multiple myeloma subject. Tumor tissue was obtained at autopsy from the vertebrae, ileum, and lymph nodes. Control tests were made with corresponding normal tissues and with tumor tissue from a patient with myelogenous leukemia. Vertebral marrow obtained from a multiple myeloma subject who had undergone treatment with urethan was also investigated.

A large, sharp peak characterizing the electrophoretic pattern of the plasma of the test subject also characterized the patterns of extracts of tu-

mor-containing tissues of the same subject. This component was not observed in any of the control experiments. It was also absent in the extract of the marrow of the urethan-treated multiple myeloma subject, due, presumably, to the effect of the urethan in inhibiting or destroying the myeloma cells. The results appear to support the view that myeloma cells are the site of formation of the abnormal plasma proteins of multiple myeloma.

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