Nagoya J. med. Sci. 31: 325-338, 1968.

IMMUNOELECTROPHORESIS *VERSUS* ELECTROPHORESIS IN STUDY OF DYSGAMMAGLOBULINEMIA

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ABSTRACT

The total 476 sera were examined by a routine electrophoresis and further by the use of immunoelectrophoresis. 1) Among the 42 broad banded hypergammaglobulinemics, neither M-components nor a lack of any class of immunoglobulin was detected. 2) Out of the 23 hypogammaglobulinemics, 3 cases of dysgammaglobulinemia were found only after the immunoelectrophoresis. 3) All of the 51 cases with a spike on the electrophoresis was proved to be a monoclonal gammopathy due to M-IgG in 36, M-IgA in 10, M-IgM in 3, and Bence-Jones type in 2 cases. 4) Out of the 360 cases without any detectable abnormality of electrophoretic globulin fractions, one case with recurrent infections was found to lack IgA in the serum by the use of immunoelectrophoresis.

From these data, "merits and demerits" of the two methods, *i.e.*, routine electrophoresis and immunoelectrophoresis were discussed. Some of the interesting cases were presented and compared with reports by others.

INTRODUCTION

Development of the serum electrophoresis by Tiselius[1] has much contributed to a more understanding of many diseases.

Recent technical modifications[2] of the original method by the use of filter papers or cellulose acetate strips have facilitated its wider clinical application and led us to renew our understanding of the electrophoresis not only in analysis of a patient's serum but also in study of various isoenzymes.

The development of Tiselius' electrophoresis was soon followed by the rather accidental discovery by Tiselius and Kabat[3] that the antibody activity is present in the γ-globulin fraction on electrophoresis. Their important observation has further broadened applications of the electrophoresis to the field of immunobiology.

The splendid idea of Graber and Williams[4], that is, a combination of the principle of Tiselius' electrophoresis and agar-gel immunodiffusion techniques developed by Oudin[5] and Ouchterlony[6] has brought a classical immunoglobulin under a new light. Since then, numerous studies have been made on analysis

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Received for publication, December 18, 1967.

of immunoglobulins by the use of Grabar's immunoelectrophoresis and, to date, have firmly established the presence of at least four classes of immunoglobulin, thus having deepened our understanding of particular diseases.

Clinical application of the immunoelectrophoresis to unveil dysgammaglobulinemia is, however, presently limited only in large and well-equipped laboratories and is not as a routine as the electrophoresis in many of clinical laboratories.

In the present study, sera of patients with various diseases were first analysed by the routine electrophoresis and then further studied by the immunoelectrophoresis. Two results on each of the sera were compared and interrelated in an attempt to find a clue as to whether a given serum needs to be further analysed by the immunoelectrophoresis and "merits and demerits" of the two methods were discussed.

Some of the interesting cases encountered were also presented and compared with reports by others.

MATERIALS AND METHODS

Serum samples: The sera studied immunoelectrophoretically in the present work comprised four groups of total 476 sera.

1) The first group of 42 sera had electrophoretically showed a broad gamma-peak resulting in hypergammaglobulinemia of more than 1,820 mg/dl*.

2) The second group of 23 sera had showed a flattened gamma-peak resulting in hypogammaglobulinemia of less than 900 mg/dl*.

3) The third group of 51 sera had showed a spike with a varying mobility ranging from α_2 to γ region.

4) The fourth group comprised 360 sera which were randomly chosen from total 633 sera without any detectable abnormality on electrophoresis.

Serum total protein was measured with a hand protein refractometer (Hitachi Co.).

Electrophoresis of the sera was carried out on cellulose acetate strips (Oxoid, Oxo Ltd., London, England) in veronal buffer, pH 8.6 and ionic strength 0.05, for 40 minutes at 1 mAmp./cm. Strips were stained with Ponceau 3 R and scanned with a recording densitometer (Kayagaki Co.).

Immunoelectrophoresis in agar was done by a modification of the Scheidegger's method[7].

Preparation of polyvalent and specific antisera: Rabbits were immunized by the 4 weekly subcutaneous injections into the footpads and the back with each

* In the present study, 900-1,820 mg/dl was taken for a normal range of gammaglobulin quantity based on the data obtained from twenty healthy adults.

of the following antigens incorporated in an equal volume of Freund's complete adjuvant (Difco Lab.): (a) pooled normal human whole sera, (b) IgG-M-components obtained through chromatography on DEAE-cellulose with phosphate buffer of pH 6.5, (c) IgM-M-components isolated through successive chromatographies, first on Sephadex G-200 with saline, then on DEAE cellulose using a gradient phosphate buffer elution, starting from 0.01 M to 0.30 M, (d) IgA-M-components prepared by zone electrophoresis on starch block, and (e) protein-ammonium sulfate-precipitates obtained from the urine containing Bence-Jones protein of kappa or lambda type.

After completion of immunizing injections, rabbits were bled to death, and sera were individually tested for a specific antibody on Ouchterlony diffusion.

The antiserum against IgG showed a single line on Ouchterlony diffusion without any purification.

However, the antiserum expected to be specific for IgM, IgA, kappa and lambda chain, respectively, gave several precipitin lines when tested on Ouchterlony diffusion, and, therefore, needed to be purified.

Purification of each antiserum was performed by absorption techniques. The specific antiserum for IgM was obtained by absorption with the hypogammaglobulinemic serum lacking IgM, and similarly the specific antiserum for IgA with cord serum. The anti-K and anti-L were also obtained by absorption with Bence-Jones protein of lambda type for anti-K, and with that of kappa type for anti-L. Each of these specific antisera, thus obtained, gave a single precipitin line when tested on Ouchterlony diffusion.

The anti-IgD serum used in the present study was kindly supplied from Dr. John L. Fahey*.

Analytical ultracentrifugation was carried out using a Spinco Model E ultracentrifuge (Beckman Instruments Corp., Spinco Division, U.S.A.).

RESULTS

1) Among the 42 cases with hypergammaglobulinemia associated with a broad gamma-peak, neither M-component nor a lack of any class of immunoglobulin was detected on immunoelectrophoresis.

In this category were 11 cases of liver cirrhosis, 9 systemic lupus erythematodes (SLE), 4 hepatomegaly clinically undefined, 3 vasculitis, 2 sarcoidosis, 2 acute rheumatic fever, and one each of chronic rheumatoid arthritis, Banti's syndrome, infectious mononucleosis, diabetes mellitus, aplastic anemia, erythroleukemia, Hodgkin's disease, giant follicular lymphoma, ulcerative colitis, pulmonary tuberculosis, and lymphocytosis of unknown etiology included.

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2) In the hypogammaglobulinemic group of 23 cases, one case of ataxia teleangiectasia was found to be lacking both IgG and IgA, and two cases of primary acquired hypogammaglobulinemia were proved to lack IgM.

The remaining 20 cases which did not show M-component nor lack any class of immunoglobulin included 4 cases of Hodgkin's disease, 4 reticulum cell sarcoma, 3 nephrotic syndrome, 2 lymphoadenopathy, 2 chronic lymphatic leukemia (CLL), 2 apparently healthy individuals, and one each of SLE with ascites, gastric cancer, and neuroblastoma.

Case presentation

a) Ataxia teleangiectasia (Table 1): An 8-year-old girl with a history of a premature birth at 7th month of gestation was admitted to our hospital because of her gait disturbance and recurrent fabrile episodes.

Physical examination revealed a poorly nourished, somewhat stunted girl with teleangiectasia of the cconjuntiva bulbi and with spastic movements associated with a marked dysmetry. She was diagnosed as having ataxia teleangiectasia with a complication of bronchiectasis. Laboratory findings are summarized in Table 1 and an immunoelectrophoresis of her serum is shown in Fig. 1.

b) Primary acquired hypogammaglobulinemia (Table 1): An 18-year-old girl complaining of recurrent febrile episodes and splenomegaly was admitted to the hospital. The first febrile episode occurred at the age of 6, and her splenomegaly was first detected at the age of 7. Her stunted growth had been noted since the age of 10.

Physical examination, on admission, revealed a poorly nourished and stunted Korean girl with a moderate hepatosplenomegaly. Her past and family histories were not contributed.

Only after the electrophoretic and immunoelectrophoretic analyses of her

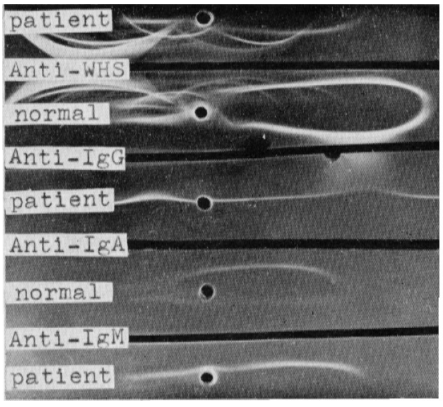

FIG. 1

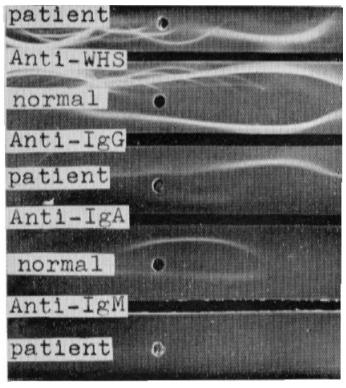

FIG. 2

TABLE 1

	Ataxia teleangi-ectasia	Primary acquired hypogamma-globulinemia	Ataxia teleangi-ectasia
age (years)	8	18	10
sex	female	female	female
past history	measles (2 y.) gait disturbance (3 y.) otitis media (4 y.) pneumonia (4 y.) bronchiectasia (6 y.)	measles (3 y.) fever (6 y.) splenomegaly (7 y.) erythema (12 y.)	measles (3 y.) wheeping cough (5 y.) gait disturbance (5 y.)
inter-marriage	−	−	+
body length (cm)	107	142	116
body weight (kg)	14.0	34.5	16.0
speech distrubance	−	−	+
ataxic gait	+	−	+
intelligence	normal	normal	low
mental state	depressive	normal	depressive
hepatomegaly	−	1 finger breadth	−
splenomegaly	−	4 finger breadth	−
teleangiectasia	+	−	+
serum total protein (g/dl)	6.4	6.1	7.9
gammaglobulin (mg/dl)	415	338	1280
immunoelectro-phoresis IgA IgG IgM	absent absent +	+ + absent	absent + +
R.B.C. ($\times 10^4$/cmm)	446	435	340
Hb (%)	60	72	67
W.B.C./cmm	5700-13200	2000-5800	5800-8000
lymphocyte/cmm	1310- 1840	1200-2262	910- 986
Widal R.	−	−	−
CRP	++	−	−
RA	−	−	−
ASLO	−	−	−
Cold hemagglutinin	×64	−	×256
tuberculin R.	−	−	−
lymphnode biopsy	atrophic and fibrotic	absence of germi-nal center and plasma cell	atrophic, cellular depletion

TABLE 2

Case	Age	Sex[1]	Total protein (g/dl)	M-component (g/dl)	Electrophoretic mobility	Type of immunoglobulin	Type of light chain	Bence-Jones proteinuria	Marrow plasma cell (%)	Bone involvement	Diagnosis[2]
1	44	M	10.6	7.4	α_2	G	L	+	26.6	+	m.m.
2	60	M	13.2	7.4	〃	G	L	+	58.6	+	m.m.
3	71	M	9.6	4.8	$\alpha_2-\beta$	A	K	−	68.6	+	m.m.
4	62	F	8.8	0.5	〃	A	K	−	15.6	−	m.m.
5	58	F	11.4	6.3	〃	A	L	−	62.8	+	m.m.
6	70	M	9.8	4.7	〃	G	L	+	n.d.	+	m.m.?
7	63	M	9.9	5.3	β	A	K	+	7.0	+	m.m.
8	62	M	12.8	8.7	〃	A	K	−	62.0	+	m.m.
9	56	F	10.5	6.2	〃	A	K	+	54.3	+	m.m.
10	73	M	7.2	0.5	〃	A	K	−	1.0	−	pulmonary tuberulosis
11	77	M	9.4	7.4	〃	A	L	−	56.0	+	m.m.
12	60	M	6.0	0.7	〃	G	K	−	35.1	+	m.m.
13	54	M	10.8	2.4	〃	G	L	−	22.6	+	m.m.
14	61	M	9.2	1.3	〃	BJ	K	+	41.5	+	m.m.
15	54	M	6.6	0.7	〃	BJ	L	+	45.0	+	m.m.
16	69	M	8.6	2.6	$\beta-\gamma$	A	K	−	10.0	−	Rhinitis necroticans
17	50	M	8.4	2.2	〃	G	L	−	0.4	−	solitary myeloma
18	38	M	8.9	4.1	γ	A	L	−	42.2	+	m.m.
19	63	F	8.0	2.6	〃	M	K	−	89.2*	−	chronic lymphatic leukemia
20	43	M	9.0	4.3	〃	M	K	−	20.8*	−	malignant lymphoma
21	72	M	10.6	5.8	〃	M	L	−	22.8*	−	macroglobulinemia
22	57	M	7.2	1.9	〃	G	K	−	3.0	−	arteriosclerosis
23	53	M	7.6	2.6	〃	G	K	−	n.d.	−	normal
24	61	M	14.4	8.3	〃	G	K	−	24.0	+	m.m.
25	40	M	18.0	12.3	〃	G	K	−	80.8	+	m.m.
26	53	F	12.0	3.7	〃	G	K	−	28.0	+	m.m.
27	68	M	12.3	7.2	〃	G	K	+	58.2	+	m.m.
28	62	M	16.0	10.9	〃	G	K	+	55.0	+	m.m.
29	68	M	11.3	5.9	〃	G	K	−	38.6	+	m.m.
30	58	M	12.2	3.6	〃	G	K	−	24.4	+	m.m.
31	73	F	11.0	6.3	〃	G	K	−	49.4	+	m.m.
32	70	F	10.4	8.0	〃	G	K	−	64.6	+	m.m.
33	54	M	9.6	3.6	〃	G	K	−	17.4	+	m.m.
34	58	F	11.4	4.0	〃	G	K	−	81.6	+	m.m.
35	69	M	8.2	3.0	〃	G	K	−	25.2	+	m.m.
36	34	M	12.6	8.1	〃	G	K	−	6.4	+	m.m.
37	42	F	10.4	3.2	〃	G	K	+	n.d.	+	m.m.
38	67	M	9.4	2.8	〃	G	K	−	28.5	+	m.m.
39	56	M	10.4	5.9	〃	G	L	−	3.8	−	nephrolithiasis
40	51	M	9.5	1.6	〃	G	L	−	36.0	−	m.m.?
41	75	M	8.0	3.4	〃	G	L	−	n.d.	−	cerebral softening
42	52	M	8.6	1.7	〃	G	L	−	1.5	−	extramedullary plasmacytoma
43	61	M	9.4	3.8	〃	G	L	−	22.4	+	m.m.
44	67	M	10.4	6.2	〃	G	L	+	27.8	+	m.m.
45	60	M	11.8	4.9	〃	G	L	+	52.5	+	m.m.
46	62	M	13.4	7.8	〃	G	L	−	78.0	+	m.m.
47	53	M	5.8	1.4	〃	G	L	+	88.2	+	m.m.
48	76	F	12.0	4.3	〃	G	L	−	49.8	+	m.m.
49	78	M	11.0	7.7	〃	G	L	−	32.4	+	m.m.
50	58	M	10.8	4.8	〃	G	L	−	55.6	+	m.m.
51	59	M	12.0	8.5	〃	G	L	−	42.0	+	m.m.

[1] M=male; F=female [2] m.m.=multiple myeloma * plasmocytoid lymphocyte.

serum was a diagnosis of primary acquired hypogammaglobulinemia made. Laboratory findings are summarized in Table 1, and a serum immunoelectrophoresis is shown in Fig. 2.

3) The hypergammaglobulinemic group associated with a spike on electrophoresis comprised 50 patients and an apparently healthy adult. In 41 out of the 50 patients, a diagnosis of multiple or solitary myeloma was established by a sternal bone marrow puncture. A clinical diagnosis on each of the remaining 9 patients was macroglobulinemia (Waldenström) malignant lymphoma, C.L.L., nephrolithiasis, cerebral softening, rhinitis necroticans, suspected extramedullary plasmacytoma, pulmonary tuberculosis and arteriosclerosis, respectively. Apparently healthy adult included in this group was a 53-year-old man. Serum immunoelectrophoresis on these 51 cases disclosed an M-component of a various type in 49 cases and Bence-Jones protein in 2 cases. Data on these 51 cases with a spike were presented in Table 2, grouped according to its electrophoretic mobility.

i) Group having a spike with a mobility ranging from α_2- to β-region (Table 2):

A spike with an α_2-mobility observed in 2 cases was proved to consist of monoclonal IgG.

A spike with an intermediate mobility between α_2- and β-region seen in 4 cases was found to be due to a presence of monoclonal IgA in 3 cases and monoclonal IgG in one case.

A spike with a β-mobility occuring in 9 cases consisted of monoclonal IgA in 5, monoclonal IgG in 2 and Bence-Jones protein in 2 cases.

A spike with an intermediate mobility between β- and γ-region observed in 2 cases was found to be composed of monoclonal IgG and monoclonal IgA in each case.

Thus, of the total 17 cases with a spike in the other than γ-region, 9 cases had monoclonal IgA, 6 monoclonal IgG and 2 Bence-Jones protein.

A light chain type of a monoclonal IgA was kappa in 7 and lambda in 2 cases; that of a monoclonal IgG was kappa in one and lambda in 4cases; and that of a Bence-Jones protein was kappa and lambda in each case.

ii) Group having a spike with γ-mobility (Table 2):

A spike with a γ-mobility found in the total 34 cases was proved to consist of a monoclonal IgA in one, monoclonal

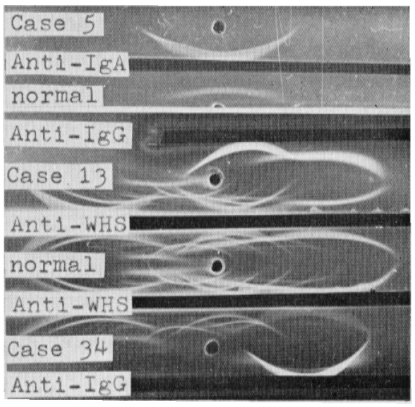

FIG. 3

IgM in 3, and monoclonal IgG in the remaining 30 cases (83.3%).

A light chain type of the monoclonal IgA was lambda; that of a monoclonal IgM was kappa in 2 and lambda in one case; and that of a monoclonal IgG was kappa in 17 and lambda in 13 cases.

Three examples of serum immunoelectrophoresis of multiple myeloma (Case

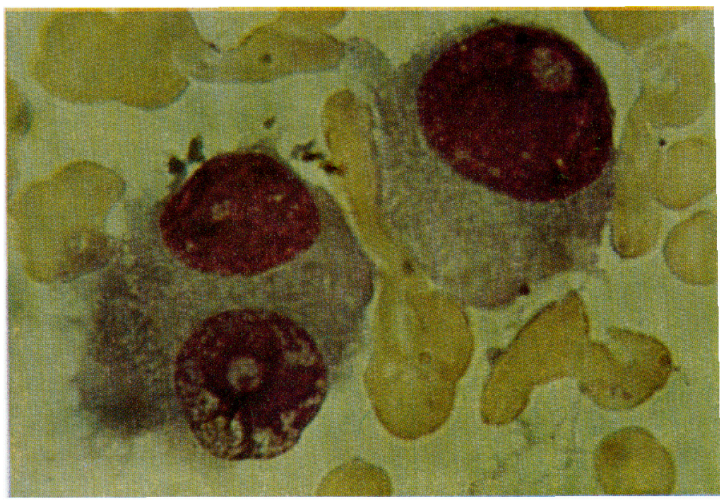

FIG. 4

FIG. 5

No. 5, 13, and 34) are demonstrated in Fig. 3, and an appearance of myeloma cells in Case No. 5 and 13 was shown in Fig. 4, and 5, respectively.

Case presentation

a) Solitary myeloma (Case No. 17) : A 50-year-old man visited our hospital complaining of an increasing chest pain for the past three months. On physical examination, he was found to have a tender hard lump on the sternum. By Histopathological Diagnosis of the lesion, Plasmacytoma was revealed (Fig. 6). Repeated bone marrow punctures at the other parts of the sternum and pelvis did not show myeloma cells, nor excess proliferation of plasma cells, therefore a

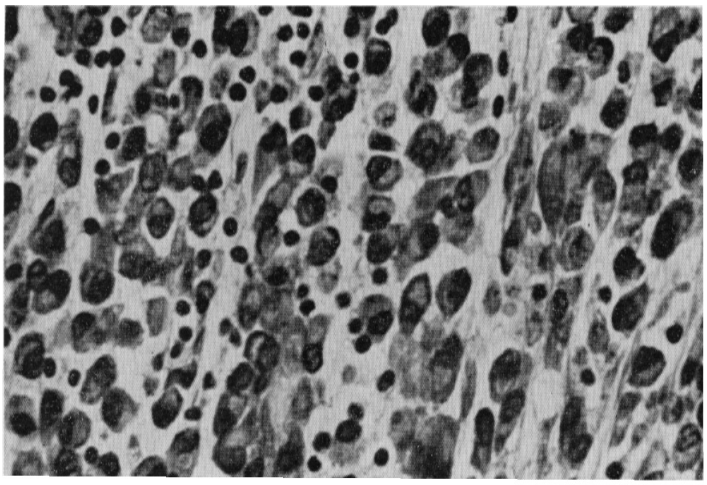

FIG. 6

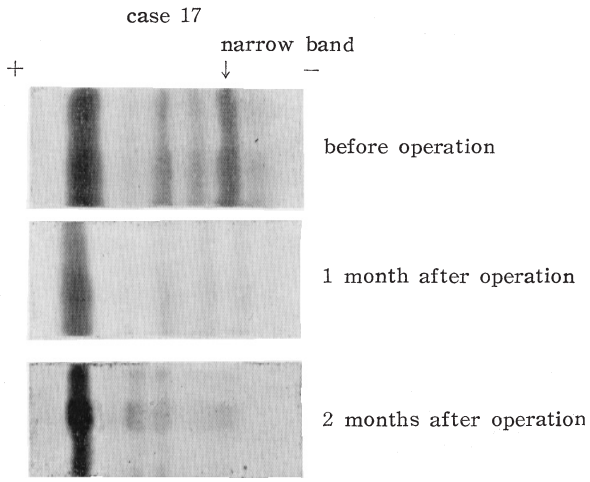

FIG. 7

diagnosis of solitary myeloma was made. Serum electrophoreses performed on three occasions during the hospital course are shown in Fig. 7, demonstrating a fading and disappearance of the narrow band within about two months after surgical resection of the tumor.

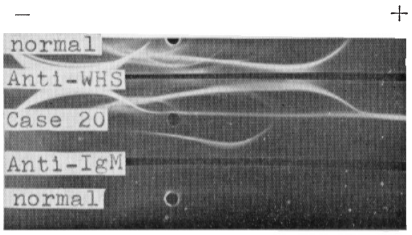

FIG. 8

b) Macroglobulinemia (Waldenström) (Case No. 20): A 72-year-old man visited the hospital because of facial flushing. On laboratory examinations, an elevated ESR and hyperproteinemia were noted, and an abnormal narrow band was detected on serum electrophoresis.

Immunoelectrophoresis of his serum revealed a monoclonal IgM (Fig. 8). A study of his serum by ultracentrifugation disclosed the presence of abnormal quantities of macroglobulins (Fig. 9). Plasmocytoid lymphocytes occupied 22.8% of the marrow cells in the sternal bone marrow aspirate. He was diagnosed as having macroglobulinemia (Waldenström).

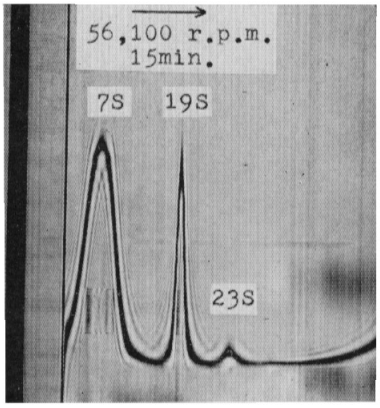

FIG. 9

c) Arteriosclerosis (Case No. 22): A 57-year-old man visited the hospital because of coughing. He was diagnosed as having arteriosclerosis and common cold. Electrophoresis done on his serum as one of randomly chosen samples revealed a spike with γ-mobility and the spike was found to be due to a presence of a monoclonal IgG on immunoelectrophoresis (Fig. 10). He had been well for the following 2 years since then.

4) In those 360 cases with any detectable abnormality on electrophoresis which were further immunoelectrophoretically studied, an absence of IgA was detected in one case of ataxia teleangiectasia and Bence-Jones proteinemia in 3 cases of multiple myeloma. Neither M-component nor a lack of any class of immunoglobulin was found in the remaining 356 cases.

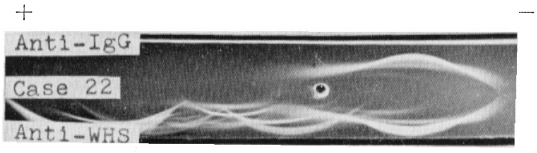

FIG. 10

Case presentation

a) Ataxia teleangiectasia: A 10-year-old girl with a history of recurrent infections was admitted to our hospital.

Physical examinations revealed a poorly nourished somewhat stunted girl with teleangiectasia of the conjunctiva bulbi (Fig. 11) and with ataxic gait associated with dysmetry and dysarthria. She was diagnosed as having ataxia teleangiectasia. Laboratory findings are summarized in the Table 1, and immunoelectrophoresis of her serum is shown in Fig. 12.

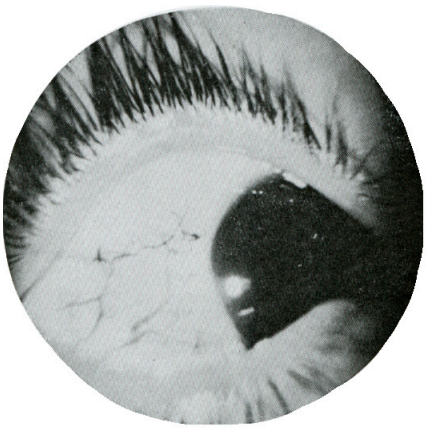

FIG. 11

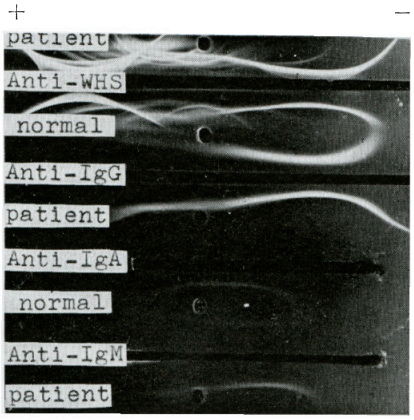

FIG. 12

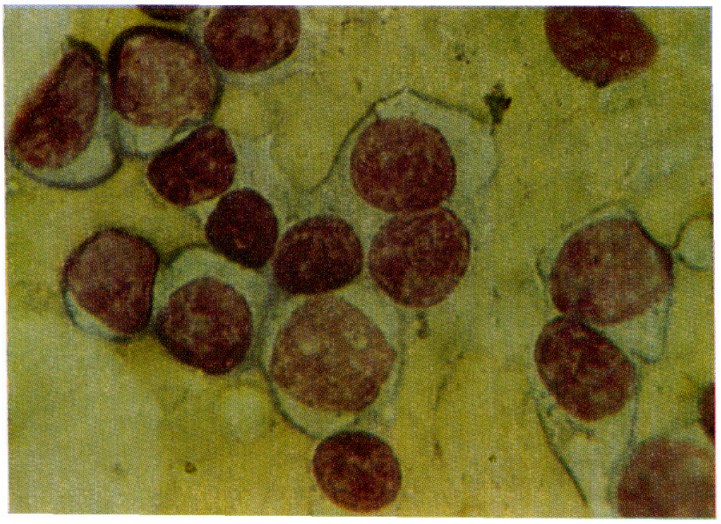

FIG. 13

b) Multiple myeloma with Bence-Jones proteinemia unaccompanied with other M-components: A 52-year-old man was hospitalized because of a persisting proteinuria. The proteinuria was found to be due to Bence-Jones protein and a diagnosis of multiple myeloma was established by a sternal bone marrow puncture (Fig. 13) and characteristic radiological findings. An immunoelectrophoresis of his serum disclosed a faint Bance-Jones protein precipitation line near the trough (Fig. 14). Another example of a similar case was shown together in the Fig. 14.

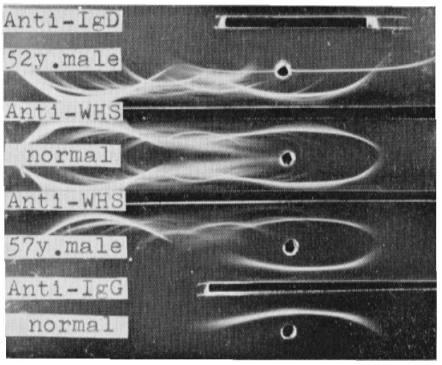

FIG. 14

DISCUSSION

As already stated above, the serum electrophoresis is now one of the routine procedures in most clinical laboratories whereas the immunoelectrophoresis of a patient's serum is feasible only in a small number of laboratories. The former is simple and quantitative and a result is easily reproducible, as wanted, with a drop of the stored original serum even years after the serum was taken. Moreover, results obtained in different laboratories are comparable as they are. On the other hand, the latter is rather complicated in its procedures and not quantitative though, in a sense, semiquantitative to some extent. It requires preparations of various types of specific antisera in each individual laboratory until they become commercially available as standard sera, and results obtained in different laboratories may not be comparable without considering many complicating factors. Nevertheless, the immunoelectrophoresis is essential for analysis of immunoglobulins and their constituents in a given serum. Yet, for the moment, it is too laborious and almost unpractical to subject every patient's serum to the immunoelectrophoresis.

The present study gives a certain clue as to whether a given serum has to be further analysed by the immunoelectrophoresis for a possible presence of a particular type of M-component and/or for a lack of an immunoglobulin.

The results suggest that all the sera showing a spike on routine electrophoresis should be subjected to immunoelectrophoresis because such sera invariably have an abnormality of an immunoglobulin and/or its constituent, and that the sera of hypogammaglobulinemics with a history of recurrent febrile episodes had better also to be examined for absence of certain immunoglobulins.

The present study also indicates that the sera of broad banded hypergamma-

globulinemics and of patients without any detectable abnormality on electrophoresis may not necessarily be studied by immunoelectrophoresis unless other circumstantial evidences suggest the presence of M-components or lack of immunoglobulins.

In the present study, monoclonal gammopathy was encountered in 7 cases of a variety of diseases without any evidence of plasmacytoma. Such a condition was termed "plasma cell dyscrasia of unknown significance" by Osserman[8] and assumed as a premyelomatous condition by Others[9]. However, in a view that the development of a certain type of leukemia or lymphoma is preceded by a bone marrow aplasia or an atrophy of lymphoid tissue, a possible premyelomatous condition is more likely to be associated with a decrease of a certain type of immunoglobulin, although it is uncertain if plasma cells behave like blood cells. On the contrary, plasma cells appear to behave like secreting cells which may undergo a malignant transformation in step-wise manners through a benign hyperplasia. In the latter view, therefore, a premyelomatous condition could be a functional hyperplasia of a certain plasma cell clone. This sort of problem may be answered by the results of a long term follow-up study on such monoclonal gammopathy.

In relation to a possible premyelomatous condition, the broad banded hypergammaglobulinemia, particularly persisting one, associated with a chronic inflammation needs to be followed up for a possible progression towards a monoclonal gammopathy. For this purpose is the serum electrophoresis alone quite enough and immunoelectrophoresis merely for confirmation and typing of an M-component.

One of the interesting cases encountered was a case of ataxia teleangiectasia which appeared to lack not only IgA but also IgG. This type of case has never been reported.

Finally, as to light chain type of M-components, the ratio of kappa to lambda was 1.21 in the present study. This ratio is in accord with 1.13 observed by Miyoshi[10] in Japan but is far less than the ratio observed by Mannick and Kunkel[11], Fahey and Solomon[12], or Williams *et al.*[13] that ranges from 1.5 to 2.1. This discrepancy may be explained by a racial difference. It is well known that the incidence of myelomatosis among Japanese is much lower than that among Caucasian.

ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to Prof. Susumu Hibino. Many thanks are also expressed to Dr. Saburo Ono, Dr. Akira Kunii, and the author's colleagues in the Hematology Section for their guidance and encouragement.

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