Studies on the Changes of Crossing Diagrams of Human Sera in Diseases against Concanavalin A

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Nakamura and his colaborators[1] have found a specific protein in the extract of jack bean meal, which reacts with serum proteins to form precipitate. This protein was first named provisionally Protein J by them, but later[2] it was identified with concanavalin A, by crystallizing them.

The precipitation reactions of concanavalin A with serum globulins can be demonstrated by the gel diffusion method. Then by the technique of two-dimensional crossing electrophoresis, "crossing diagrams"[3] of serum against concanavalin A could be obtained, and the diagrams were shown to be characteristic for each animal species[1,4]. In the present report, the same technique has been applied to sera in diseases, to investigate the change of "crossing diagrams".

EXPERIMENTAL

Material: Sera from 40 normal adults were used as control. Sera from 161 patients without or before x-ray irradiation or operation were made available from the Hospital of Yamaguchi Medical School. All sera were carefully prepared not to be contaminated with hemoglobin, as it combines with haptoglobin to reduce α_2-globulin fraction and to increase β-fraction, thus to change the crossing diagram[5].

As a solution of concanavalin A, jack bean extract was used. It was prepared as follows: To 1 g. of jack bean meal 5ml. of water were added, stirred occasionally for 30 min. at room temperature, and centrifuged. Somewhat yellowish extract contained about 8 mg. of protein N/ml., i.e., 5% protein. Protein concentration of the extract remained almost constant in all batches. It could be reserved in a refrigerator for about a week.

Buffer solution of pH 8.5 and ionic strength 0.05 was used according to Miller and Golder[6].

Filter paper, Whatman No. 1 or Toyo No. 52 of 30×30 cm. area was used.

Apparatus: Electrophoresis apparatuses of horizontal type or hanging-paper type were used as already reported by Nakamura and colaborators[3].

Procedure of two-dimensional crossing paper electrophoresis: After the lines for application of serum and jack bean extract were drawn on a filter paper, it was

soaked with buffer solution and set in the apparatus. Then, the serum to be tested was applied on the prearranged line and 0.1% solution of bromophenol blue or bromocresol green was applied on spots behind the line of serum to visualize the migration of serum albumin. The first electrophoresis was carried out in direction 1, at 160–200 V., 14–20 mA. for 7–9 hours (or at 50–80 V., 5.5–8.5 mA. for 15–17 hours) at room temperature. After the first electrophoresis had been finished, the filter paper was turned 90° to the direction of electrical field. Jack bean extract was then applied on the prearranged line, and the second electrophoresis was carried out in direction 2, which is perpendicular to the first, at 60–100 V., 8–11 mA. for 17–20 hours (or at 50–80 V., 5.5–8.5 mA. for 20–22 hours) at room temperature.

Quantities of solutions applied were usually either 0.005 ml./cm. of serum and 0.01 ml./cm. of jack bean extract or 0.00375 ml./cm. of serum and 0.0075 ml./cm. of jack bean extract. The ratio of serum to jack bean extract per cm. line length was 1:2. In the cases of sera with highly increased α and/or β globulin fractions, the ratio of jack bean extract was increased.

After electrophoresis, filter paper was dried at 110°C and stained with bromophenol blue or amidoblack 10B. Concentration of serum protein was determined by a refractometer (Hitachi) and the relative concentrations of protein fractions were determined by an automatic recording densitometer after one-dimensional paper electrophoresis.

RESULTS AND DISCUSSIONS

I. Crossing Diagrams of Normal Human Serum Against Concanavalin A.

In Fig. 1, an example of the "crossing diagram" of human serum against concanavalin A is shown. The diagram was made as follows: At first normal human serum was applied on the line AB and the first electrophoresis was carried out in direction 1. Then jack bean extract was applied on the line XY and the second electrophoresis was carried out in direction 2. As can be seen, the line of concanavalin A showed no change at the crossing point with serum albumin, but showed groovings at those with α_1-, α_2-, and β-globulins. In some cases γ-globulin also showed a groove. But it could not yet be settled, if the groove was caused by the physicochemical nature of γ-globulin *per se* or by some other reasons, for instance, changes in reserving the blood samples. Therefore the groove formed at crossing point of γ-globulin was set aside and not considered further in this report.

The grooves (peaks) in the line of concanavalin A at the crossing points with α_1-, α_2-, and β-globulins must be made by the formation of addition complexes of concanavalin A with the globulins, as they encountered each other.

Nakamura and Wakeyama[7] tried to calculate the relative concentrations of trypsin

inhibitors in serum from the "crossing diagram" against trypsin. But their theoretical treatment can hardly be applied here, because the complexes formed precipitate on the filter paper, and thus the mass action of the two reactants, i.e., concanavalin A and the corresponding serum protein, and the equilibrium of the reaction can not validly be assumed here. But if the pattern of the crossing diagram is very constant for healthy serum and characteristic for diseased cases, the "crossing diagram" may be utilized as an aid in blood analyses.

Crossing diagrams of sera obtained from 40 healthy normal adults showed three peaks of about the same height, corresponding to α_1-, α_2-, and β-globulins. In Table I, their relative hights are shown, taking the length of crossing of albumin with concanavalin A line as standard (here numerically as 10), their relative fractional heights to the sum of them, and the relative areas enclosed by the peaks and the line of concanavalin A.

Table I. Relative Heights and Areas of the Three Peaks of Crossing Diagrams.

	α_1-Peak	α_2-Peak	β-Peak
Relative height*) (mean of 28)	6.95±0.77	7.39±0.76	6.77±0.67
Relative fractional height**) (mean of 28)	32.9±1.10	35.0±1.21	32.1±1.18
Relative area***) (mean of 20)	26.5±2.16	37.0±1.87	36.5±2.25

*) Taking the length of crossing of albumin with concanavalin A as 10.
**) Ratio of the height of each peak to the sum of them.
***) Ratio of each area to the sum of three areas.

In Table II is shown the results obtained from diagrams of one serum, when the duration of elctrophoresis was varied. As can be seen from the table, with the increase of the electrophoresis time (i.e., the higher the peak), α_1-peak seems to become relatively lower and β-peak higher. But the change is little.

Table II. Change of Relative Areas of the Three Peaks with Length of Crossing Time.

Duration of 2nd electrophoresis	α_1-Peak	α_2-Peak	β-Peak
17 hours	29.1	36.8	34.1
6	32.1	34.6	33.3
3.5	35.1	35.6	29.3

As mentioned above, the height and the area of a peak can not be related theoretically to the concentration of the corresponding protein fraction. However, from the results shown in Table I and II, the pattern of the "crossing diagram" is obviously constant, provided the experimental conditions were fixed.

The relative concentrations of smaller fractions of serum proteins (α_1-, α_2-, and

β-globulins) measured by the usual paper electrophoresis, show considerable fluctuation, as already demonstrated by Sugimoto and colaborators[8], and can be seen also from our results presented in Table III. On the other hand, the "crossing diagram" of serum against concanavalin A shows very characteristic and constant peaks corresponding to them. Therefore this method may serve as a sensitive procedure to investigate the change in α_1-, α_2-, and β-globulins, although their quantitative change can not be calculated from the diagram.

Table III. Relative Concentration of Protein Fractions of Normal Serum (Mean of 20).

Albumin	α_1-Globulin	α_2-Globulin	β-Globulin	γ-Globulin
54.3±2.45	4.2±0.73	7.7±0.96	12.7±1.29	21.0±1.47

II. Changes of the "Crossing Diagrams" of Sera in Diseases.

a) Classification of varied crossing diagrams.

Of 161 sera of patients investigated, 59 showed crossing diagrams similar to the normal one, whereas the other 102 sera showed diagrams evidently changed from the normal one.

Provided that each peak corresponding to α_1-, α_2-, and β-globulin might change independently, the types of diagrams may be classified in 27, as shown in Table IV.

Table IV. A Theoretically Possible Classification of the Types of Crossing Diagrams.

No.	α_1	α_2	β	No.	α_1	α_2	β	No.	α_1	α_2	β
1	O	O	O	10	+	+	+	19	−	−	−
2	−	O	O	11	−	+	+	20	O	+	+
3	O	−	O	12	+	−	+	21	+	O	+
4	O	O	−	13	+	+	−	22	+	+	O
5	+	−	−	14	+	−	−	23	O	−	−
6	O	+	O	15	−	+	−	24	−	O	−
7	O	O	+	16	−	−	+	25	−	−	O
8	O	−	+	17	+	−	O	26	−	+	O
9	O	+	−	18	+	O	−	27	−	O	+

O: no change, +: rise, −: fall of the peak.

First seven types in the first column correspond to and can not be distinguished from those of the other two columns. Further, No. 8 and 17 might not be distinguished from No. 3, 7, or 5; No. 9 and 18 from No. 4, 6, or 5; and No. 26 and 27 from No. 2, 6, or 7, depending on the degree of changes. Thus the crossing diagrams of serum against concanavalin A may be practically classified into eight

types:

(1) Normal (Fig. 1)
(2) Low α_1-peak (Fig. 2)
(3) Low α_2-peak (Fig. 3)
(4) Low β-peak (Fig. 4)
(5) High α_1-peak (Fig. 5)
(6) High α_2-peak (Fig. 6)
(7) High β-peak (Fig. 7)
(8) Appearance of unusual peak (Fig. 8).

b) Characteristics of crossing diagrams in various diseases.

Typical examples of each type of crossing diagram are shown in Fig. 1 to 8. In Table V is shown the results of classification of all 161 cases.

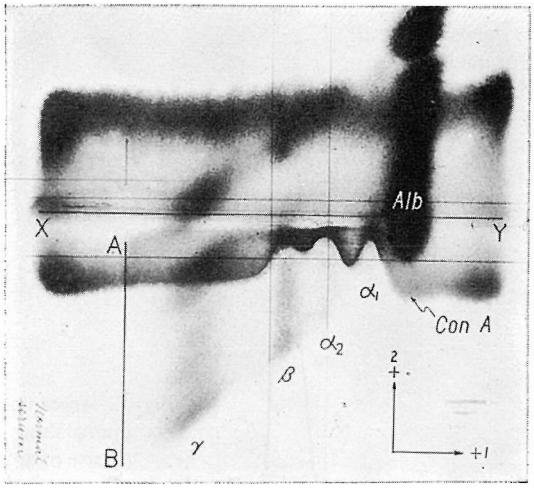

Fig. 1. An example of crossing diagrams of normal adult serum against concanavalin A.

First electrophoresis: 0.03 ml./8 cm. of the serum was applied on the line AB, and the 1st electrophoresis was carried out in direction 1, at 200 V., 15 mA./30 cm. for 7.5 hours, at room temperature. Second electrophoresis: 0.12 ml./16 cm. of jack bean extract was applied on the line XY, and the 2nd electrophoresis was carried out in direction 2, at 90 V., 9 mA./30 cm. for 20 hours.
Buffer, pH 8.5, ionic strength 0.05, barbiturate, according to Miller and Golder[6]. Filter paper, Toyo No. 52. Stained with bromophenol blue.

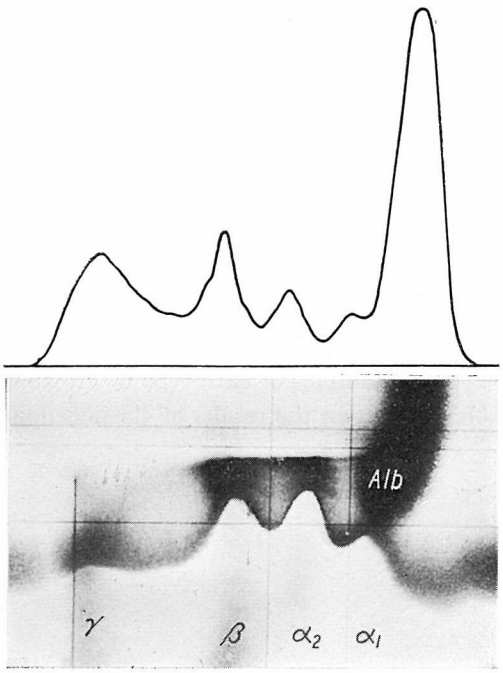

Fig. 2. An example of the crossing diagrams showing the fall of α_1-peak (Basedow's disease).

First electrophoresis: 0.004 ml./cm. of the investigated serum was applied and the 1st electrophoresis was carried out at 55 V, 6 mA. for 16 hours. Second electrophoresis: 0.01 ml./cm. of jack bean extract was applied on the line perpendicular to that of serum, and the 2nd electrophoresis was carried out in the direction perpendicular to the first, at 60 V., 7.8 mA. for 21 hours. Other conditions were the same as in Fig. 1. (The figure shows only the main part of the concanavalin line.)
The above line diagram shows the usual electrophoretic pattern of the investigated serum. Relative concentrations the of protein fractions were: Albumin 47.0, α_1-globulin 5.5, α_2-globulin 8.3, β-globulin 15.0, γ-globulin 24.2.

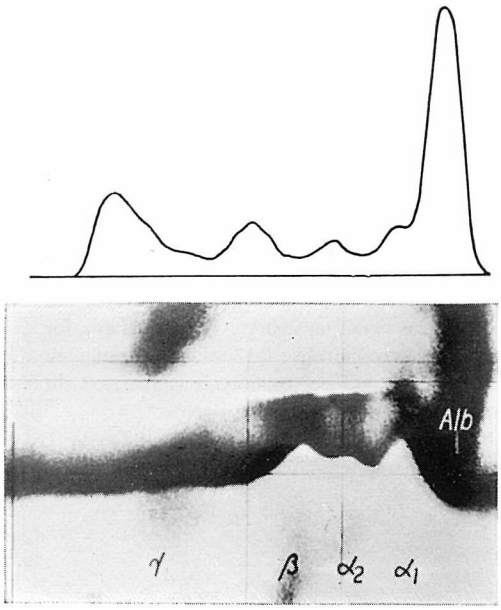

Fig. 3. An example of the crossing diagrams showing the fall of α_2-peak (a case of aplastic anemia).

First electrophoresis: 0.028 ml./8 cm. of the serum was applied and the 1st electrophoresis was carried out at 170 V., 15 mA. for 8 hours. Second electrophoresis: 0.018 ml./16 cm. of jack bean extract was applied and the 2nd electrophoresis was carried out in the direction perpendicular to the first, at 60 V., 9 mA. for 18 hours. Other conditions were the same as in Fig. 1.
Line diagram: Usual electrophoretic pattern of the investigated serum. Albumin 49.7, α_1-globulin 6.8, α_2-globulin 7.2, β-globulin 8.9, γ-globulin 27.4.

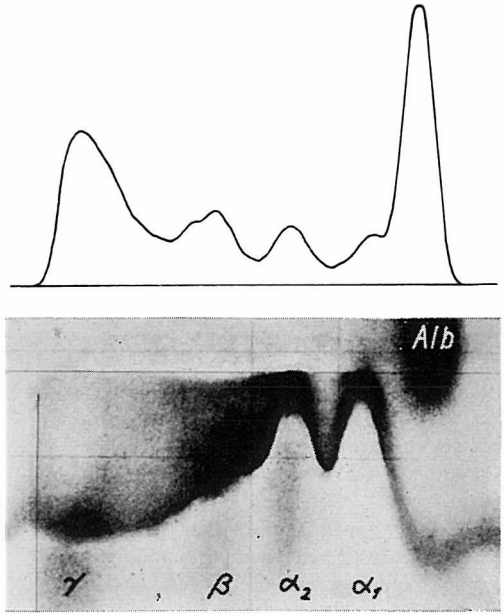

Fig. 4. An example of the crossing diagrams showing the fall of β-peak (a case of uterine cancer).

First electrophoresis: 0.005 ml./cm. of serum was applied and the 1st electrophoresis was carried out at 60 V., 7 mA. for 16 hours. Second electrophoresis: 0.01 ml./cm. of jack bean extract was applied and the 2nd electrophoresis was carried out in direction perpendicular to the first, at 60 V., 7.5 mA. for 22 hours. Other conditions were the same as in Fig. 1.

Line diagram: Usual electrophoretic pattern of the investigated serum. Albumin 33.9, α_1-globulin 5.2, α_2-globulin 8.5, β-globulin 15.6, γ-globulin 36.8.

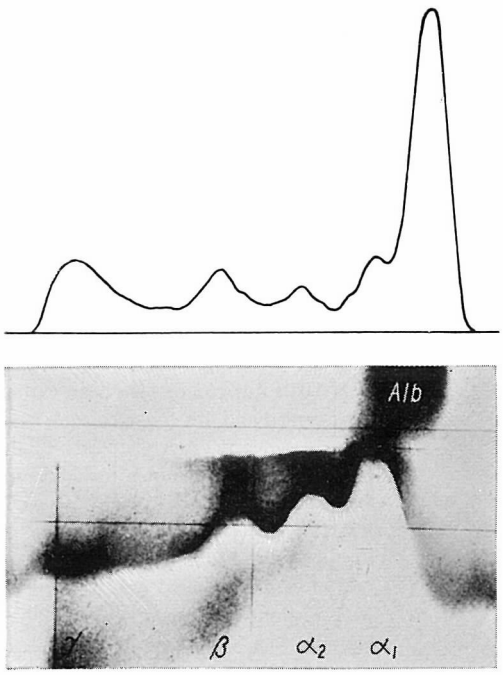

Fig. 5. An example of the crossing diagrams showing the rise of α_1-peak (a case of ovarian cystoma).

First electrophoresis: 0.005 ml./cm. of the serum was applied and the 1st electrophoresis was carried out at 60 V., 8 mA. for 16 hours. Second electrophoresis: 0.01 ml./cm. of jack bean extract was applied and the 2nd electrophoresis was carried out perpendicularly to the first run, at 60 V., 8 mA. for 22 hours. Other conditions were the same as in Fig. 1.

Line diagram: Usual electrophoretic pattern of the investigated serum. Albumin 50.2, α_1-globulin 9.1, α_2-globulin 8.0, β-globulin 14.2, γ-globulin 18.5.

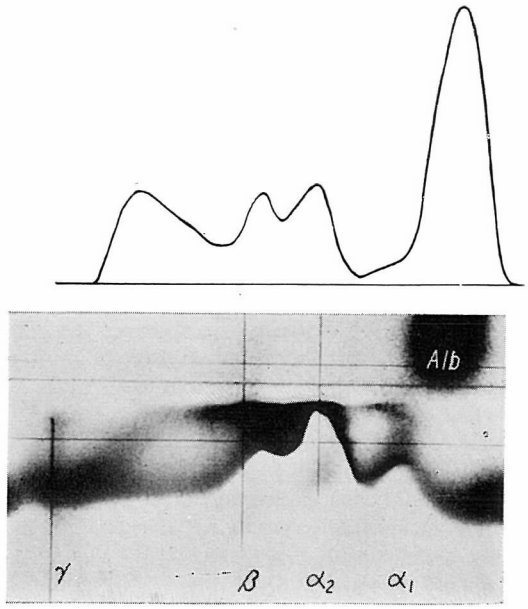

Fig. 6. An example of the crossing diagrams showing the rise of α_2-peak (a case of uterine cancer)

First electrophoresis: 0.003 ml./cm. of the serum was applied and the 1st electrophoresis was carried out at 70 V., 8 mA. for 15.5 hours. Second electrophoresis: 0.0075 ml./cm. of jack bean extract was applied and the 2nd electrophoresis was carried out perpendicularly to the first run, at 60 V., 7 mA. for 22 hours.
Line diagram: Usual electrophoretic pattern of the investigated serum. Albumin 47.6, α_1-globulin 3.2, α_2-globulin 14.6, β-globulin 10.0, γ-globulin 24.7.

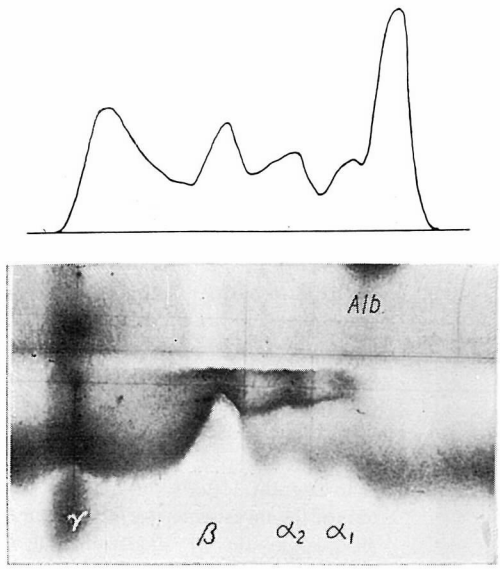

Fig. 7. An example of the crossing diagrams showing the rise of β-peak (a case of hypercholesterolemic xanthomatosis).

First electrophoresis: 0.005 ml./cm. of the serum was applied and the 1st electrophoresis was carried out at 50 V., 5.5 mA. for 17 hours. Second electrophoresis: 0.01 ml./cm. of jack bean extract was applied and the 2nd electrophoresis was carried out perpendicularly to the first run, at 50 V., 5.5 mA. for 24 hours. Other conditions were the same as in Fig. 1.
Line diagram: Usual electrophoretic pattern of the investigated serum. Albumin 29.3, α_1-globulin 8.1, α_2-globulin 14.0, β-globulin 15.4, γ-globulin 33.2.

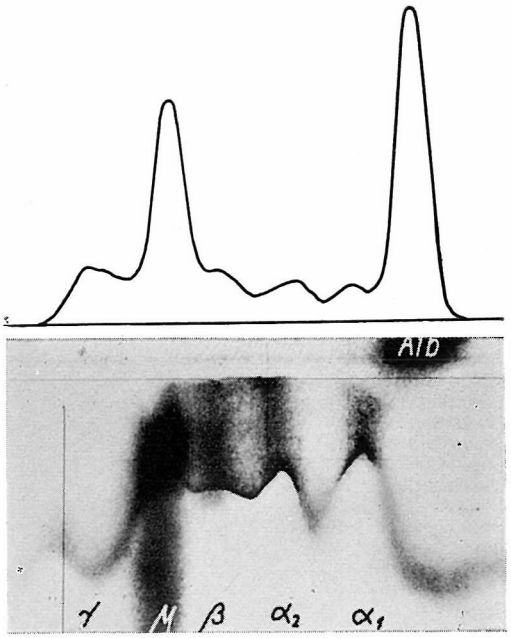

Fig. 8. An example of the crossing diagrams showing an unusual 4th peak (a case of multiple myeloma).

First electrophoresis: 0.005 ml./cm. of the serum was applied and the 1st electrophoresis was carried out at 60 V., 6.5 mA. for 16.5 hours. Second electrophoresis: 0.01 ml./cm. of jack bean extract was applied and the 2nd electrophoresis was carried out perpendicularly to the first run, at 60 V., 7 mA. for 23 hours. Other conditions were the same as in Fig. 1.

Line diagram: Usual electrophoretic pattern of the investigated serum. Albumin 44.4, α_1-globulin 2.9, α_2-globulin, 6.4, β-globulin 6.1, M-component 32.6, γ-globulin 7.6. Total protein concentration 8.6 g./100 ml.

As can be seen from Table V, eight types of diagram do not always show specificity in regard to diseases. Some comments will be given in the following.

(1) Nephrosis.

The electrophoretic pattern of the serum in nephrosis is very often characteristic[9] in showing evident decrease of albumin fraction and enormous increase of α_2-globulin fraction. In typical cases this is also reflected in the crossing diagram; the crossing diagram of a nephrotic serum presented in Fig. 9 shows a enormously large α_2-peak with weak α_1- and β-peaks as its shoulders. It might be noteworthy, that the relative concentration of β-globulin remains rather in the normal range, although the β-peak of the crossing diagram appears low, as shown in the figure.

(2) Gastritis, gastric and duodenal ulcers.

Among 20 investigated cases of gastric and duodenal diseases except cancer, about 1/4 of them showed slight decrease in albumin and 1/3 showed slight increase in γ-globulin. A few of them showed also slight changes of other fractions, and one case showed an abnormal fractions between β- and γ-fractions. These changes except the appearance of the abnormal fraction, of which mention will be made later, were almost insignificant. Thus they do not contradict with the results obtained by Minamizono[10], Goto[11], Hatashita[12], Puls[13] and Jencks[14], who observed no remarkable change.

As to the crossing diagrams, 13 cases of investigated 20 patients showed normal diagrams, and the rest showed changed ones. One of the latter showed a very little

Table V. Types of Crossing Disgrams of Sera in Diaeases Against Concanavalin A and Number of Observed Cases.

Diseases	N	$\alpha_1(-)$	$\alpha_2(-)$	$\beta(-)$	$\alpha_1(+)$	$\alpha_2(+)$	$\beta(+)$	4th peak	Total
Gastric cancer	1	—	—	7	—	10	—	—	18
Cancer of liver	—	—	—	3	—	1	—	—	4
Cancer of gallbladder	—	—	—	1	—	—	—	—	1
Cancer of large intestine	—	—	—	—	—	1	—	—	1
Cancer of rectum	1	—	—	—	—	1	—	—	2
Cancer of thyroid	—	—	—	—	—	1	—	—	1
Uterine cancer	7	—	—	3	—	6	—	—	16
Vaginal cancer	—	—	—	—	—	1	—	—	1
Cancer of breast	1	—	—	—	—	—	—	—	1
Malignant chorioepithelioma	—	—	—	—	—	1	—	—	1
Osteosarcoma	—	—	—	—	—	1	—	—	1
Melanosarcoma	1	—	—	—	—	—	—	—	1
Leucosarcoma	—	—	—	—	—	—	—	1	1
Leukemia	—	—	—	1	2	1	—	—	4
Multiple myeloma	—	—	—	—	—	—	—	2	2
Gastric ulcer	6	—	—	2	—	2	—	1	11
Duodenal ulcer	3	—	—	1	—	1	—	—	5
Chronic gastritis	3	—	—	—	—	—	—	—	3
Benign pyloric stenosis	1	—	—	—	—	—	—	—	1
Acute hepatitis	3	—	1	1	1	—	—	—	6
Chronic hepatitis	1	—	—	—	—	2	—	—	3
Liver cirrhosis	3	—	—	2	3	—	—	1	9
Ileus	1	—	—	—	—	—	—	—	1
Intestinal fistula	—	—	—	—	—	1	—	—	1
Dystrophia (after stomach resection)	—	—	—	—	—	1	—	—	1
Uterine myoma	10	3	1	1	—	2	1	—	19
Ovarian cystoma	2	—	—	—	1	—	—	—	3
Hemangioma of liver	—	1	—	—	—	—	—	—	1
Vesicular mole	—	—	—	—	1	—	—	—	1
Acute nephritis	—	—	—	—	—	1	—	—	1
Chronic nephritis	3	—	—	—	—	1	—	—	4
Nephrotic syndrome	—	1	—	—	—	4	—	—	5
Malignant cirrhosis of kidney	—	—	1	1	—	1	—	—	3
Renal hypertension	—	—	—	—	—	1	—	—	1
Essential hypertension	4	—	—	—	—	—	—	—	4
Aplastic anemia	—	—	1	—	—	—	—	—	1
Hemolytic jaundice	—	—	—	—	1	—	—	—	1
Thrombopenic purpura	1	—	—	—	—	—	—	—	1
Banchi's disease	1	—	—	—	—	—	—	—	1
Xanthomatosis	—	—	—	—	—	—	2	—	2
Rheumatic fever	—	—	—	1	—	1	—	—	2
Rheumatic endocarditis	1	—	—	—	—	—	—	—	1
Chronic rheumatic arthritis	—	—	—	—	—	1	—	—	1
Pulmonary fibroma	—	—	—	—	—	1	—	—	1
Silicosis	2	—	—	—	—	—	—	—	2
Acute pleuritis	—	—	—	—	—	1	—	—	1
Tuberculous cervical lymphadenitis	—	—	—	—	—	1	—	—	1
Wilson's disease	—	—	—	—	1	—	—	—	1
Basedow's disease	—	1	—	1	—	—	—	—	2
Softening of brain	—	—	—	1	—	—	—	—	1
Adrenal insufficiency	1	—	—	—	—	—	—	—	1
Diabetes mellitus	—	—	—	1	—	—	—	—	1
Porphyrinuria	—	—	—	1	—	—	—	—	1
Hemochromatosis	1	—	—	—	—	—	—	—	1
Total	59	6	4	29	10	45	3	5	161

N: Normal type, (+): rise, and (−): fall of peak.

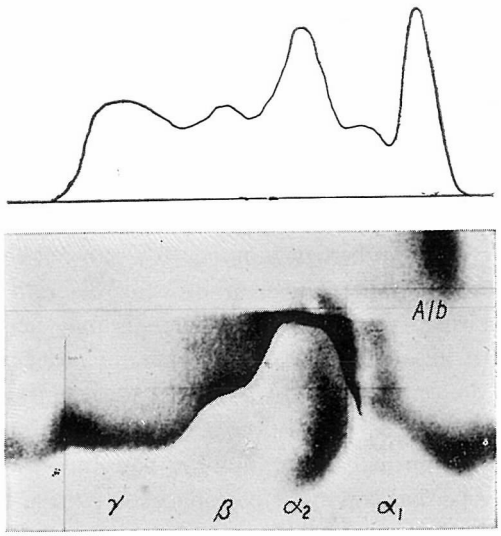

Fig. 9. An example of the crossing diagrams of sera from patients showing nephrotic syndrome.

First electrophoresis: 0.005 ml./cm. of the serum was applied and the 1st electrophoresis was carried out at 60 V., 7 mA. for 16 hours. Second electrophoresis: 0.01 ml./cm. of jack bean extract was applied and the 2nd electrophoresis was carried out perpendicularly to the first run, at 60 V., 7 mA. for 22 hours. Other conditions were the same as in Fig. 1.
Line diagram: Usual electrophoretic pattern of the investigated serum. Albumin 20.3, α_1-globulin 9.4, α_2-globulin 31.3, β-globulin 14.5, γ-globulin 24.4.

4th peak, corresponding to the unusual fraction. One of the 13 cases with normal diagram showed increase in β-globulin concentration, and the rest showed normal pattern. The change of crossing diagram could also be correlated to that of reacting globulin fractions in 3 of 7 cases with changed diagrams.

(3) Benign tumors.

Twenty-one cases of benign tumors, including 17 cases of myoma of uterus, 3 of ovarian cystoma, and one of hepatic hemangioma, were investigated. In one third of them slight increase in γ-globulin concentration was observed. This result does not contradict with those obtained by Hatashita[12], Jencks[13], and Yoshida[15].

Crossing diagrams of the sera were normal in 12 cases and in 9 other cases slight changes of peaks were observed, which could hardly be correlated with the changes of protein concentration.

(4) Malignant tumors.

In regard to the change of serum protein fraction in diseases with various malignant tumors, numerous investigations[16-21] have been made; usually decrease in albumin and increase in α-globulin were observed. Several authors[10,12,21,22] observed increase in α_2-globulin and others[10,13,23,24] increase in γ-globulin. The 60 sera investigated in the present experiment showed, as a whole, decrease in albumin,

increase in α_2- and γ-globulins. But it is not self-evident that in all types of malignant tumors the same change of serum proteins would occur.

In the crossing diagrams of the 60 investigated sera, rise of α_1-peak was found in 2 cases, rise of α_2-peak in 27 cases, fall of β-peak in 15 cases, appearance of unusual 4th peak in 3 cases, and normal diagram in 11 cases. Thus the rise of α_2-peak amounts to 45%, which might differentiate the cases with malignant tumors from those with benign tumors. As a whole about 81% showed changes in crossing diagram, whereas in cases with benign tumors, 43% showed changes.

As to the correlations of the change in crossing diagram to that in the concentration of protein fractions, following comments may be made: Of 25 cases with high α_2-peak, 19 showed increase in α_2-globulin concentraction; of 15 cases with low β-peak 12 showed decrease in β-globulin concentration; rise of α_1-peak corresponded to increase in α_1-globulin concentration; and 11 cases with normal crossing diagram showed normal electrophoretic pattern. Thus in most cases correlation between crossing diagram and electrophoretic pattern was confirmed.

Seibert and colaborators[23] and Mehl and colaborators[25] correlated the increase in α-globulin fraction in cancer to the increase in mucoproteins. Sumner and colaborators[26] investigated the reaction of concanavalin A with glycoproteins. In regard to our experiments of the crossing diagram of serum against concanavalin A, carbohydrate-free serum albumin did not react with concanavalin A. Further, by the technique of crossing electrophoresis, the reaction of haptoglobin, a glycoprotein, with concanavalin A could be demonstrated.[5] Thus the peaks formed in the crossing diagram of serum against concanavalin A may probably be due to the reaction of glycoproteins in serum.

(5) Appearance of unusual fourth peak.

As shown in Table 5, in the crossing diagrams of 5 cases an unusual 4th peak was observed. The crossing diagrams of them are shown in Fig. 8 and 10 to 13. The 4th peak in three of them corresponded to an unusual protein fraction, which could be separated by the usual paper electrophoresis, whereas in the other two cases no or only hardly detectable unusual fraction could be observed. But as a matter of course, the 4th peak in the crossing diagram must in these cases also be ascribed to an unusual protein fraction.

It is not easy to determine, whether an unusual protein fraction which appeared in a electrophoretic pattern of a pathological serum is abnormal or an ircreased normal fraction, as the electrophoretic components of a serum consist of numerous subfractions. Final conclusion can only be arrived utilizing various techniques including immunological ones.

M-Component in multiple myeloma: As to the abnormal components which appear in multiple myeloma, they could not be detected immunologically in normal serum[27], and referred to "M-component". According to the results of Wuhrmann

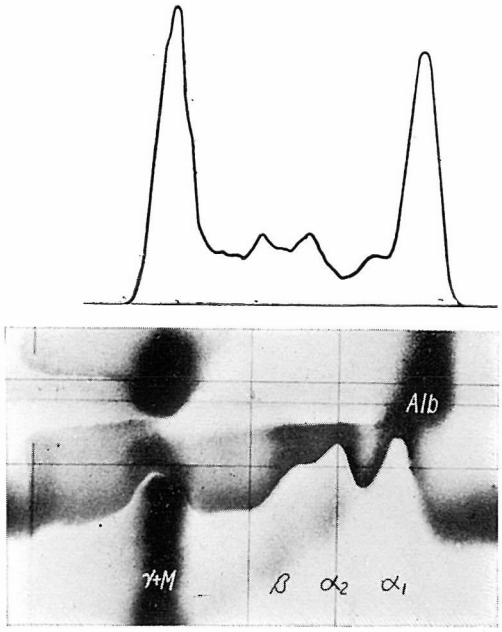

Fig. 10. The crossing diagram of the serum of a case of multiple myeloma, showing an unusual 4th peak.

First electrophoresis: 0.12 ml./8 cm. of the serum was applied and the 1st electrophoresis was carried out at 160 V., 15 mA. for 8.5 hours. Second electrophoresis: 0.12 ml./16 cm. of jack bean extract was applied and the 2nd electrophoresis was carried out perpendicularly to the first run, at 65 V., 10.5 mA. for 21 hours. Other conditions were the same as in Fig. 1.
Line diagram: Usual electrophoretic pattern of the investigated serum. Albumin 27.7, α_1-globulin 9.9, α_2-globulin 13.6, β-globulin 10.3, γ-globulin 38.5. Total protein concentration 8.1 g./100 ml.

and colaborators,[28] M-component of each patient seems to show "individual specificity." However, the abnormality of a myeloma protein can not be established even by the immunological tests, as they fail to detect minute amount of an antigen. The unusual protein fractions observed in the two cases of myeloma presented here (Fig. 8 and 10) are probably the abnormal M-component. The M-component of the case shown in Fig. 8 migrated with a mobility between β- and γ-globulin whereas that of the case shown in Fig. 10 migrated with a mobility equal to γ-globulin.

In regard to the other three cases (Fig. 11, 12 and 13), it is not altogether clear whether the unusual fractions observed were abnormal or not.

A case of gastric ulcer and fibroma: As to the characteristics of myeloma protein, Putnam[29] referred to the electrophoretic homogeneity in accord with the standpoint of European auther[16] referring to the sharpness of the zone of "paraprotein" in general. The unusual component of the case of gastric ulcer and fibroma shown in Fig. 11 was separated as a fairely sharp zone. From its shape this fraction resembles to M-component of myeloma. But in this case no myeloma cell was de-

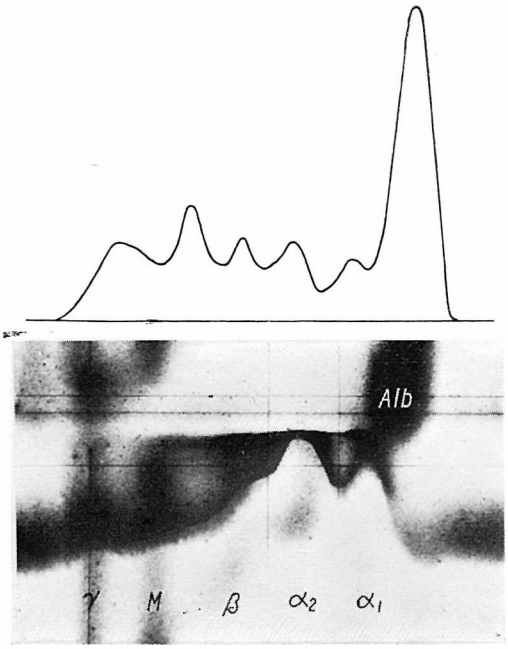

Fig. 11. The crossing diagram of the serum of a case of gastric ulcer, showing an unusual 4th peak.

First electrophoresis: 0.004 ml./cm. of the serum was applied and the 1st electrophoresis was carried out at 70 V., 6.5 mA. for 16 hours. Second electrophoresis: 0.01 ml./cm. of jack bean extract was applied and the 2nd electrophoresis was carried out perpendicularly to the first run, at 70 V., 7 mA. for 22 hours. Other conditions were the same as in Fig. 1.

Line diagram: Usual electrophoretic pattern of the investigated serum. Albumin 47.6, α_1-globulin 6.1, α_2-globulin 10.8, β-globulin 6.9, unusual fraction 14.4, γ-globulin 14.8. Total protein concentration 6.5 g./100 ml.

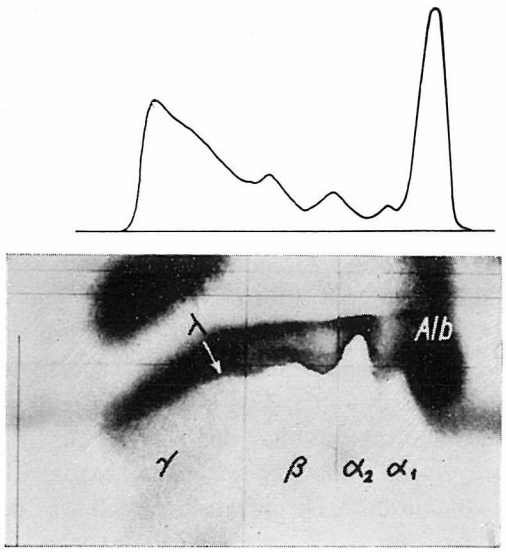

Fig. 12. The crossing diagram of the serum of a case of leucosarcoma, showing an unusual 4th peak.

First electrophoresis: 0.12 ml./8 cm. of the serum was applied and the 1st electrophoresis was carried out at 180 V., 20 mA. for 8 hours. Second electrophoresis: 0.12 ml./16 cm. of jack bean extract was applied and the 2nd electrophoresis was carried out perpendicularly to the first run, at 80 V., 9.5 mA. for 24 hours. Other conditions were the same as in Fig. 1.

Line diagram: Usual electrophoretic pattern of the investigated serum. Albumin 31.3, α_1-globulin 2.4, α_2-globulin 7.6, β-globulin 9.6, γ-globulin 49.1. Total protein concentration 7.7 g./100 ml.

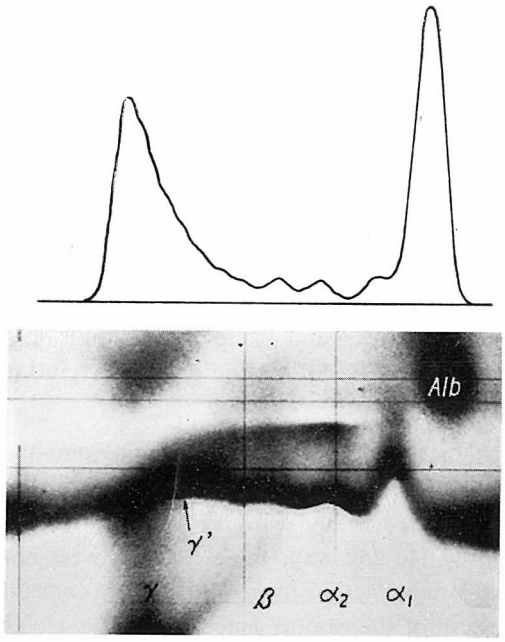

Fig. 13. The crossing diagram of the serum of a case of liver cirrhosis, showing an ususual 4th peak.

First electrophoresis: 0.03 ml./8 cm. of the serum was applied and the 1st electrophoresis was carried out at 160 V., 16 mA. for 7.5 hours. Second electrophoresis: 0.13 ml./16 cm. of jack bean extract was applied and the 2nd electrophoresis was carried out perpendicularly to the first run, at 70 V., 9 mA. for 21 hours. Other conditions were the same as in Fig. 1.
Line diagram: Usual electrophoretic pattern of the investigated serum. Albumin 33.8, α_1-globulin 3.6, α_2-globulin 4.9, β-globulin 6.4, γ-globulin 51.3. Total protein concentration 4.4 g./100 ml.

tected. Case history is as follows:

R. T. 64y. (1961), male. Since August 1961 dull appetite, pain in the epigastric region, blood vomiting. On the 3rd, October, resection of the stomach. On the serous membrane of front wall of the stomach resected, a tumor, 3 cm in diameter with stem; a benign fibroma. In mucous membrane of lesser curvature of pyloric part, ulcer. No malignant change. In blood and sternal puncture no myeloma cell. Erythrocyte 3.15×10^6, leucocyte $4,900/mm^3$. Lymph-nodes, spleen and liver were not touchable. After 15 months from the operation, the patient was apparently healthy.

Various cases with unusual serum fractions similar to M-component were already reported[30-32]. Among them were also suspected cases of idiopatic paraproteinemia [32]. However, it is not certain whether the present case can be included in them, although the crossing diagram differs from that of myeloma. As can be seen from Fig. 11, the unusual component in this case formed scarecely a peak in the line of concanavalin A, i.e., they reacted very weakly, if any. On the other hand M-component of myeloma of the cases of Fig. 8 and 10, formed evident peak in the crossing diagram. In this respect, the unusual fraction of this case might be different from that of myeloma, and the formation of a peak in the crossing diagram against concanavalin A might serve to differentiate them. However, Baker and Martin[33] reported a case of hypergammaglobulinemia, which developed myeloma 4 years

later. Thus again it is also not altogether certain, that the case shown in Fig. 11 will not develop into myeloma in the future.

A case of leucosarcoma (the case shown in Fig. 12): H. F. 79y. (1961), male. In March swelling of cervical, axillary, inguinal and abdominal lymph-nodes of the size of pea to that of hen's egg. Hematocrit 28.9, hemoglobin 10.0 g/dl. Erythrocyte 3.34×10^6, leucocyte 14,000/mm^3. Sarcoma cells occupied about half of the latter. Sternal puncture, nemerous lymphoid sarcoma cells. Deceased in July 1961.

A case of liver cirrhosis (the case shown in Fig. 13): Y. N., 55y. (1962), female. Edema of leg and ascites. Admission on the 11th April. Ascites puncture on the same day, 4,000 ml and on 17th, 3,500 ml. Biopsy, portal cirrhosis. Deceased on 29th April.

In these two cases, the crossing diagrams show the characteristic that β-peak has a broad shoulder culminating to a peak toward starting point. This peak was named here provisionally λ in the case of leucosarcoma, and γ' in the case of liver cirrhosis. In any way, these findings were not encountered in other cases, and show that some fraction reacted here with concanavalin A. In the usual electrophoretic pattern of the case of leucosarcoma, γ-globulin showed a slight shoulder, indicating a second fraction of it. It is not improbable that this second fraction of γ-globulin had reacted, although it is not established whether it was an abnormal protein or not. On the other hand in the case of liver cirrhosis no subfraction of γ-globulin was observed in the usual electrophoretic pattern, though the unusual peak in the crossing diagram shows some subfraction to have reacted.

By the usual electrophoretic method, unusual protein fractions were already observed[16, 32], not only in multiple myeloma, but also in various diseases including macroglobulinemia Waldenström[34], lymphatic leukemia[30], malignant lymphoma[30, 31], amyloidosis[35] and idiopathic cryoglobulinemia[29, 36]. Therefore it would not be extraordinary to find unusual protein fractions in the three cases discussed here, except multiple myeloma. But it would be interesting, that in one case the unusual fraction observed by the usual electrophoresis could scarecely be detected by the technique of crossing diagram against concanavalin A, whereas in the other two cases unusual fractions scarecely observed by the usual technique were distinguished by our method.

III. Relationship Between the Change of Crossing Diagram and That of the Concentration of Serum Proteins.

As already mentioned, in most cases the change of crossing diagram corresponds to that of the concentration of serum proteins. But in some cases correlation could not be observed. As shown in Table V, of 161 sera investigated here, 59 showed normal crossing diagrams. In about one third of them decrease in albumin and in

about half of them increase in γ-globulin were observed. In some cases concentration of other protein fractions was changed considerably. In Table VI three cases of evidently high β-globulinemia, but with normal crossing diagram were shown.

Table VI. Relative Concentration of Serum Proteins in Cases Showing Normal Crossing Diagram.

Case	Disease	Albumin	α_1	α_2	β	γ
K. I.	Epidemic hepatitis	47.2	3.2	7.3	21.6	17.3
T. M.	Liver cirrhosis	38.3	4.4	10.4	20.4	26.5
H. I.	Thrombopenic purpura	41.2	4.4	13.8	21.4	19.2

Therefore in these cases, the increase in the concentration of β-globulin fraction as a whole did not caused the β-peak rise in the crossing diagram. There were also some 24 cases, where the change of crossing diagram and that of protein fraction did not correlate with each other, some of them are shown in Table VII.

Thus it may be inferred that α_1-, α_2-, and β-globulins do not as a whole, but only some specific components in them react with concanavalin A. And these re-

Table VII. Cases in Which Relative Concentrations of Serum Proteins and Crossing Diagrams did not Correlate.

Case	Disease	Type of cross. diagr.	Albumin	α_1	α_2	β	γ
M. U.	Malignant sclerosis of kidney	α_2-	41.8	5.8	11.6	18.5	22.3
M. S.	Liver cancer	$\beta-$	38.9	5.5	9.4	12.4	33.8
S. O.	〃 〃	〃	51.3	4.6	7.4	10.6	26.1
Y. N.	Uterine cancer	〃	46.2	4.8	9.2	11.6	28.2
F. K.	Gastric cancer	〃	53.3	4.5	9.9	13.5	18.8
H. H.	Duodenal ulcer	〃	47.4	4.8	9.6	14.0	24.2
A. M.	Uterine myoma	〃	45.3	4.1	10.8	15.1	24.7
C. M.	Liver cirrhosis	〃	39.2	4.0	7.1	11.3	38.4
T. S.	Basedow's disease	〃	49.5	4.0	9.5	13.8	23.2
M. S.	Hemolytic jaundice	α_1+	53.2	4.7	6.4	11.1	24.6
S. S.	Gastric cancer	α_2+	49.7	5.8	7.6	10.8	26.1
N. H.	〃 〃	〃	51.7	3.1	8.3	11.7	25.2
K. U.	〃 〃	〃	43.9	4.4	8.9	11.9	31.0
K. T.	Uterine cancer	〃	46.6	3.7	9.0	10.6	30.2
M. A.	Gastric ulcer	〃	55.2	3.9	8.5	12.7	19.7
E. M.	Duodenal ulcer	〃	49.9	2.9	9.1	13.8	24.3
K. N.	Uterine myoma	〃	58.0	4.1	9.0	11.0	17.1
T. S.	Renal hypertension	〃	48.8	5.3	8.6	13.4	23.9
Y. A.	Chronic hepatitis	〃	49.7	3.2	7.7	11.3	28.1
A. N.	Uterine myoma	$\beta+$	55.4	3.6	5.7	12.4	22.8

acting components seem to be glycoproteins, as already mentioned. But it must also be reminded that the change in the concentrations of minor protein fractions are susceptible to a larger fluctuation than that of the peaks in crossing diagrams, hence it might also be possible that in some cases the change of crossing diagram would reveal the real estate of the serum protein fraction concerned.

On the other hand, Table V shows that in the cases with changed crossing diagram, the fall of β-peak and the rise of α_2-peak were rather often encountered, making 29% and 45%, respectively, of the total changed cases. Each other case did not reach 5%, except the case with the rise of α_1-peak. The reason of these facts remains to be investigated, although the rise or fall of a peak can not always be correlated to the concentration change of the corresponding globulin fraction.

SUMMARY

Two-dimensional crossing paper electrophoresis of human blood serum against concanavalin A of jack bean was carried out, to obtain "crossing diagram" of serum. Sera of 161 patients and of 40 healthy normal adults were studied.

Human serum, except in some diseases, showed "crossing diagram" consisting of three peaks, which correspond to three globulins, α_1-, α_2- and β-globulins. The peaks were formed due to the precipitation reaction between concanavalin A and serum proteins.

Sera of 59 patients showed crossing diagrams which can not be distinguished from those of normal serum. On the other hand, sera of 102 patients showed evidently different crossing diagrams from that of normal. The changes in the crossing diagrams were classified into 8 types, including normal. Sera of 5 patients showed crossing diagrams with an unusual peak besides the three.

Specific correlation between types of crossing diagrams and the diseases was hardly observed, except the appearance of the 4th peak in multiple myeloma and the rise of α_2-peak in nephrosis.

In most cases the relative concentrations of α_1-, α_2- and β-globulins determined by the usual paper electrophoresis were related to the changes in crossing diagrams, but in some cases not.

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