Specific Reaction of a Protein of Jack Bean with Sera of Various Animals

Tomoharu ZAIZEN

Institute for Medical Chemistry, (Director: Prof. Dr. S. Nakamura) Yamaguchi Medical School, Ube, Japan.

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NAKAMURA and TANAKA[1] found a specific protein of jack bean which reacted specifically with various animal proteins to form a precipitate. This protein was named by them Protein J and similar ones were found also in some other beans.

As discussed by them, it is already known that some plants contain proteins which react with erythrocytes and can be regarded as agglutinins. The isohemagglutinins in man are thought to be produced by the direct participation of blood group genes, although there are as yet some discussions which ascribe them to immunization. The proteins, however, which are contained in some plants and agglutinate erythrocytes can not be a result of immunization. They are sometimes called, in this sense, "natural" antibodies. But an antibody is defined as a substance produced in response to an antigen which intruded into an organism. Thus a "natural" antibody produced in response to no antigen is self-contradictory. To avoid such semantic confusion, BOYD[2] proposed the term "lectin" for such substance as is produced not in response to an antigen but reacts more or less specifically with certain proteins or antigens.

It is already well established that the agglutination and the precipitation can be caused by one and the same antibody.[3,4] Therefore the agglutinating "lectin" can probably precipitate appropriate substances, and the distinction between agglutinating and precipitating "lectins", if they exist, may be unnecessary. In this sense the observation of the precipitating "lectin" by NAKAMURA and TANAKA may have little theoretical bearing on the reaction. However, it is interesting that observations were made hitherto only on the agglutinating ones of plants. One of the reasons lies probably in the fact that the agglutination of erythrocytes is a very sensitive reaction, which can be detected by a minute amount of an agglutinating substance. Whether the precipitation reaction of two proteins, "lectins", is as sensitive as that between antigen and antibody, can not be predicted. On the other hand, the precipitation of a protein can also be caused by other reasons than the direct reaction of it with a specific protein; thus when a solution of a protein is mixed with the other protein solution, the precipitation of the protein may occur by the change in pH and/or ionic strength, which may be caused by the mixing. The second reason that observations were made hitherto only on the agglutinating "lectin", might

be that the expectation was to use such substances for the test of blood groups. The precipitating "lectin" seems in this respect also to be disadvantageous, as no direct practical application could be expected. Protein J of jack bean which was found by NAKAMURA and TANAKA reacted with various animal proteins. Especially its reaction with serum proteins seems to be different from fraction to fraction. The crossing diagram of the serum of an animal against Protein J is specific for each species. The sera of patients of some diseases showed characteristic patterns in the diagrams. Thus it is also hoped to utilize Protein J to the differential diagnosis of some diseases.

EXPERIMENTAL

I. Apparatuses for the two-dimensional crossing paper electrophoresis: Hitherto two sorts of apparatuses were used: a horizontal type apparatus modified from that of GRASSMANN[5] and a hanging paper type one modified from that of DURRUM.[6]

A. Horizontal type of electrophoresis apparatus for the two-dimensional crossing electrophoresis was shown diagrammatically in Fig. 1. It consisted of three parts, namely base section (H), rack (W), and cover (F). The cover is not flat but slightly saddle-backed to prevent the falling of water drops on the filter paper. The cover can be set by its four edges in the gutter (T) made around the base section. The space

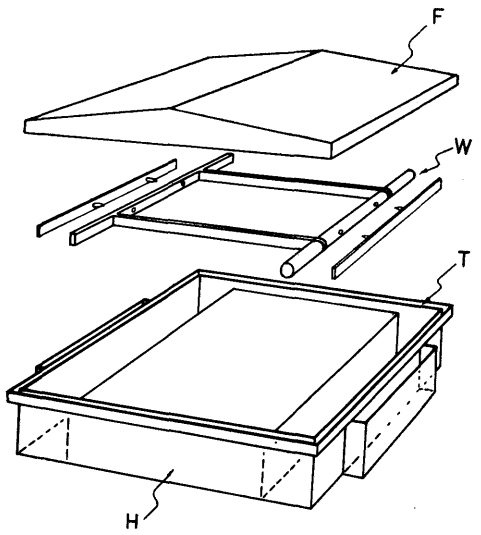

Fig. 1. Two-dimensional electrophoresis apparatus of horizontal type.

for setting filter paper can be isolated from the external air by filling the gutter with buffer solution or water.

The rack consists of four plastic bars, one of which can be rotated. For two-dimensional procedure a filter paper sheet of 30×30 cm is used. The paper is placed on the rack after it is dipped into the buffer solution and drained a little. It can be appropriately stretched by rotating the lateral bar, and pinned with pin-bars. The first electrophoresis is carried out in the direction perpendicular to the lateral bars of the rack. Then the filter paper is cut short on both sides, each 2–3 cm under the bar, and the rack with the paper as a whole is rotated 90°. The upper and the lower ends of the paper are cut in and hang down to be dipped into corresponding electrode compartments.

B. The hanging paper type apparatus was shown diagrammatically in Fig. 2. It also consisted of three parts: Base section (H), rack (W), and cover (F). The cover can be set by its edges into the gutter (T) around the base section. In each electrode compartment, a intermediate feed wick made of plastic plate was placed. The wick was wrapped with filter paper. The both ends of the filter paper hanged

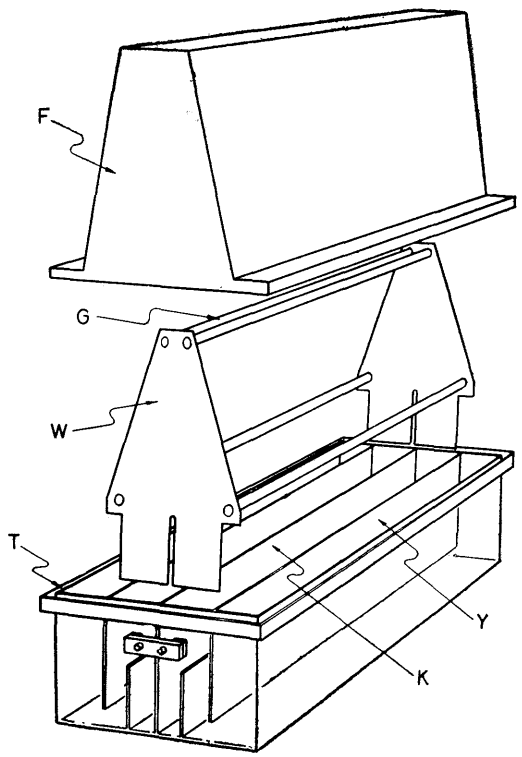

Fig. 2. Hanging paper type of two-dimensional electrophoresis apparatus.

on the rack (W) were pressed on to the feed wicks.

C. Use of a capillary tube in applying solution on the filter paper. As reported by NAKAMURA, *et al.*[7] the damage of the filter paper with the capillary tube in applying solutions may cause a disturbance in the line of the other reacting substance. Thus capillary tubes provided with some cotton threads at the tip were used to avoid the damage of the filter paper.

II. Procedure of the two-dimensional crossing paper electrophoresis.

The two-dimensional technique of crossing paper electrophoresis was the same as described by NAKAMURA *et al.*[7] The filter paper used was Toyo No. 52 of 30 × 30 cm area. The effective area for electrophoresis was 22 × 22 cm for the horizontal type apparatus and somewhat larger for the hanging paper type. The paper was of a rather heavy brand, in order to prevent breakage, especially in staining.

At first a design for two successive runs of electrophoresis was drawn, as shown in Fig. 3. For use in the hanging paper type apparatus, two perpendicullarly crossing lines were drawn in addition, to secure the correct position of the paper on the rack (W). Then one of the reactants, for instance, serum, was applied on the line AB, and the first electrophoresis was carried out in direction 1. The duration of

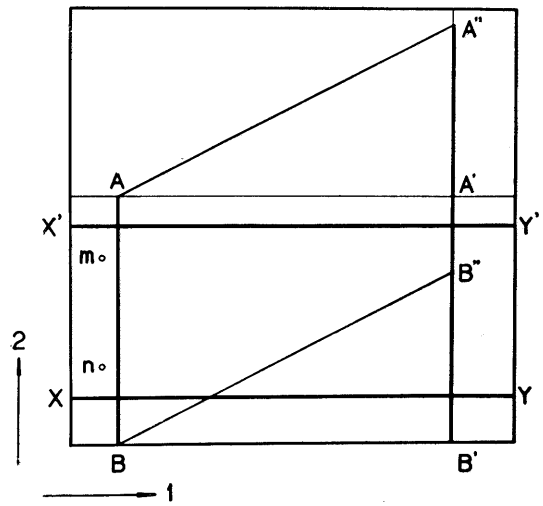

Fig. 3. An example of the design for two-dimensional electrophoresis.

the electrophoresis was tested in anticipation by other runs, and was fixed as to assure the appropriate separation of the applied material into its components. As for serum, bromophenol blue was mixed to follow the serum albumin. After the first electrophoresis was finished, the filter paper was turned 90° with the rack in the case of horizontal type apparatus, and in the case of hanging paper type one, the

filter paper was removed from the rack and turned. Then the other reactant, extract of jack bean meal, was applied on the line XY, and the second electrophoresis was carried out in direction 2. After the two runs of electrophoresis the filter paper was dried at 110°C for 10 min. and stained with amido black 10 B or with bromophenol blue.

III. MATERIAL.

Extract of jack bean meal was prepared by adding an amount of water 5 times the weight of the meal. After one hour of mild stirring, the suspension was centrifuged. A clear yellowish brown solution was obtained. After being stored in a refrigerator, some precipitate gradually occurred, which could not be dissolved by the addition of sodium chloride. The supernatant could be used for the reaction, as well as the original extract.

Sera of various animals were obtained by clotting the bloods in test tubes.

RESULTS AND DISCUSSIONS

1. Protein J of jack bean.

The electrophoretic pattern of jack bean extract was shown in Fig. 4. It contained two main fractions; one, charged negatively at pH 8,6 and the other charged positively. The former moved toward anode and the latter toward cathode. Provisionally the former was named Protein A and the latter Protein J. The content in Protein J of Jack bean appeared to be somewhat larger in a kind cultivated in Japan than in the jack bean meal imported from the U. S. A. As the protein reacting specifically with serum proteins was Protein J, the Japanese jack bean was preferred.

2. Crossing diagram of jack bean extract against serum.

In Fig. 5. an example of two-dimensional crossing electrophoreses of jack bean extract with bovine serum was shown. In the figure. the jack bean extract was first applied on the line AB and the first electrophoresis was carried out in direction 1. Then the bovien serum was applied on the line XY and the second electrophoresis was carried out in direction 2. A clear cut peak of precipitate can be seen in the region of Protein J of jack bean extract. It rises from the zone of α-globulin of the serum, indicating the reaction of Protein J with α-globulin. This result can be interpreted as follows: Protein J migrated toward cathode and α-globulin toward anode and encountered each other on the way. The complex formed by the interaction of the two proteins did not migrate or migrated only slightly when dissolved in the excess of reactant. Thus the line of α-globulin became hollow at the crossing with the line of Protein J, to form a "crossing diagram". As defined

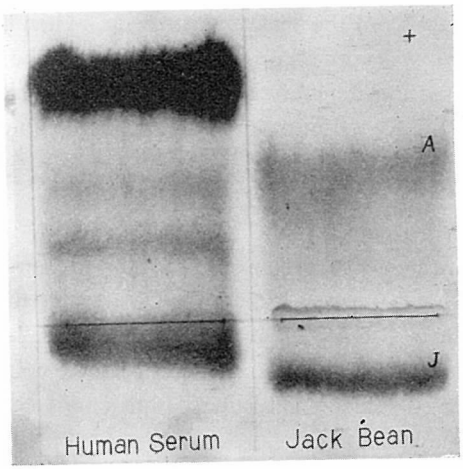

Fig. 4. Electrophoretic pattern of the extract of jack bean.

0.02 ml/4.5 cm of human serum, and of the jack bean extract, respectively, were applied on the original lines. Electrophoresis at 60 v. for 12 hrs. Barbiturate buffer of pH 8.6 and ionic strength 0.05. Filter paper, Toyo No. 51. Stained with bromophenol blue.

by Nakamura a "crossing diagram" is a specific pattern of the line of a reactant formed in accordance with the concentration of the other reactant in the two-dimensional crossing electrophoresis. Usually peaks are formed in the line of the second reactant, corresponding to the fractions of the first reactant. Thus two

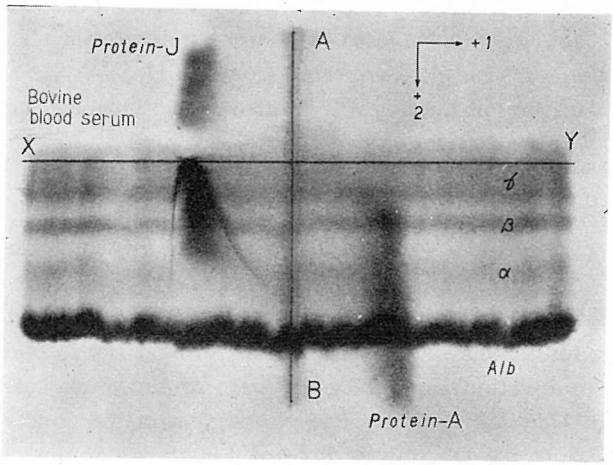

Fig. 5. Crossing diagram of jack bean extract against bovine serum.

First electrophoresis was carried out in direction 1, at 60 v. for 18 hours, after 0.06 ml/14 cm of jack bean extract were applied on the line AB. Second electrophoresis was carried out in direction 2 at 160 v. for 7 hours, after 0.06 ml/21 cm of the bovine serum were applied on the line XY. Stained with amide black 10B. Other conditions were the same as in Fig. 4.

sorts of "crossing diagrams" must be distinguished according to the nature of the peaks on the diagrams: "crossing diagram of the first reactant" against the second one, or conversely "crossing diagram of the second reactant" against the first one. This can be most clearly shown in the case of antigen-antobody reactions. For examele, a "crossing diagram of an antigen" against antiserum can be obtaind by first applying an antigen, then the peaks on the resulting diagram correspond to the distribution of antigens. On the contrary, a "crossing diagram of an antiboy" against an antigen can be obtained by reversing the order of the application of the two reactants: first the antibody and then the antigen. Then the peaks correspond to the distribution of antibodies. In the case shown in Fig. 5, the crossing diagram obtained is "that of the extract of jack bean" against bovine serum.

The fact that the crossing diagram of Protein J was obtained on the line of α-globulin indicates that the reacting partner of Protein J was α-globulin. But this can be most clearly shown by making a "crossing diagram of bovine serum" against the extract of jack bean, as can be seen in the following section.

3. Crossing diagram of serum against Protein J.

In order to determine the distribution of the serum proteins which react with Protein J, the order of the two successive runs of electrophoresis was reversed from that shown in Fig 5. In the crossing diagram shown in Fig. 6, serum proteins were

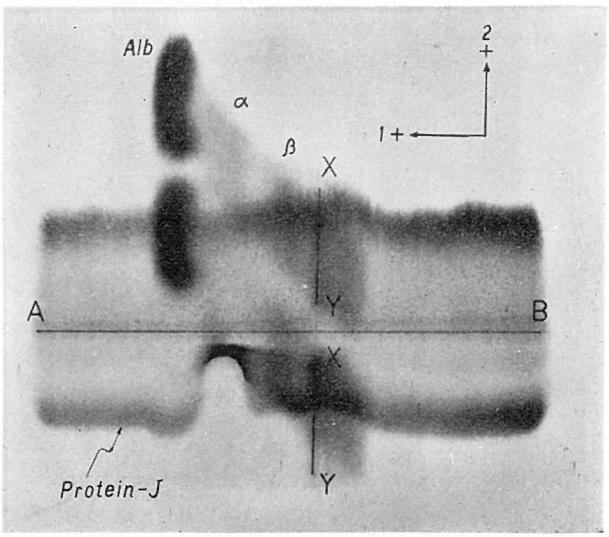

Fig. 6. Crossing diagram of bovine serum against Protein J.

First electrophoresis: 0.0125 ml/4 cm of bovine serum were applied on each XY line. Toward direction 1, at 80 v. for 12 hours 15 min. Second electrophoresis: 0.045 ml/18 cm of jack bean extract were applied on the line AB. Toward direction 2, at 100 v. for 10 hrs. 30 min.

first separated in the direction 1, then the jack bean extract was applied perpendicularly to the former and the second electrophoresis was carried out. The curves which appear on the line of Protein J belong to the "crossing diagram of serum proteins" against the extract of jack bean, or more specifically against Protein J.

From the diagram at least three peaks can be seen. The first peak lies on the zone of α_2-globulin, the second on that of β-globulin, and the third on that of γ-globulin. It is worthy of note that α_2-globulin seems to have reacted wholly to leave almost no trace of protein to be stained, while β- and γ-globulins penetrated the corresponding peaks to appear above them. This fact shows clearly that β- and γ-globulins reacted only partly with Protein J.

As discussed at the beginning it is very interesting that the precipitation of blood serum with jack bean extract has hitherto been overlooked. It can be more clearly demonstrated by the technique of Ouchterlony[8] as shown in Fig. 7. In the agar plate appeared lines of precipitate between jack bean extrct (J) and bovine serum (B) as well as sheep serum (S). Continuity of the line between bovine and sheep

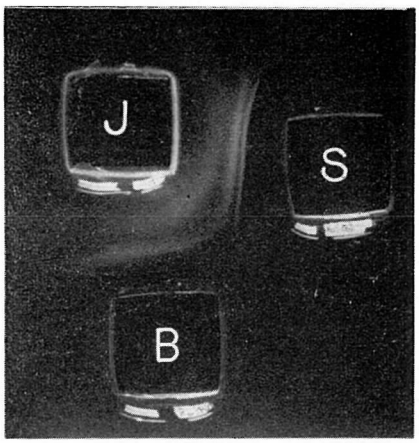

Fig. 7 Reaction of Protein J with sheep and bovine sera by double diuffsion in agar gel.
J: Jack bean extract. S: Sheep serum. B: Bovine serum.

sera indicates that the reacting proteins are of the same nature.

The height of the peak in the crossing diagram is determined chiefly by the ratio of the two reactants, as discussed by NAKAMURA.[7] In the case of usual antigen-antibody reactions, both antigen and antibody migrate under usual experimental conditions towards the same pole, the anode. Thus the peak will be highest when the two reactants react in the epuivalence zone. But if the directions of the migration of the two reactants are different from each other, the relationship will be changed,

and a peak formed in the epuivalence zone is not always highest. In Fig. 8, the highest peak of the crossing diagram of bovine serum against Protein J is Peak 1′. It rises from the base-line of Protein J above the original line of application, XY. If the peaks were made of precipitate, it would remain at the point of formation, as the precipitate can not migrate. In the crossing diagram shown in Fig. 8, a peak made of precipitate must be formed under the line, XY, where Protein J was originally applied, since the latter migrated downward. Hence it is clear that peak 1′ is not made of precipitate, but of dissolved complex of a part of α_2-globulin and Protein J. As the chief mass of Peak 1 corresponding to α_2-globulin remained undissolved, only a part of α_2-globulin must be included in the formation of peak 1′.

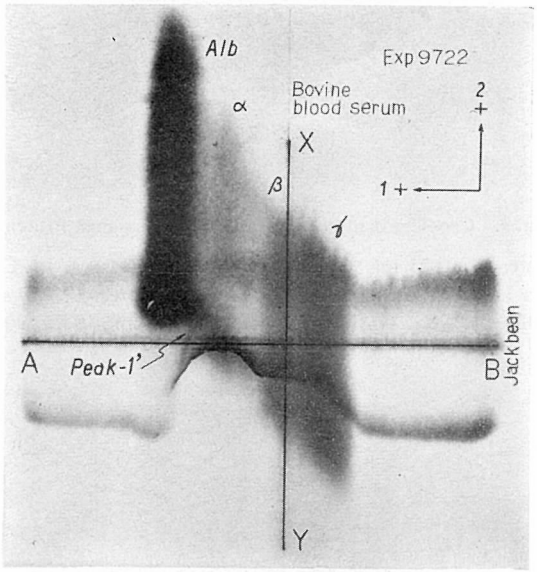

Fig. 8. Crossing diagram of bovin serum against Protein J showing Peak 1′.
First electrophoresis: 0.016 ml/16 cm of bovine serum were applied on the line XY. Toward direction 1, at 60 v. for 14.5 hrs. Second electropeoresis: 0.06 ml/18 cm of jack bean extract on the line AB. Toward direction 2, at 160 v. for 8 hrs.

4. Species specificity of crossing diagram of serum against Protein J.

The homogeneity of Protein J is not yet established. But from the crossing diagram shown in Fig. 6, 9, and so on, it can be seen that Protein J reacted as a whole with each serum protein. On the other hand serum globulins, which can be distinguished electrophoretically are not homogenous and each comprises many components. From the fact that Protein J reacted as a whole to form peaks, corresponing to α_1-, α_2-, β-, and γ-globulins, the specificity of the reaction of Protein J with

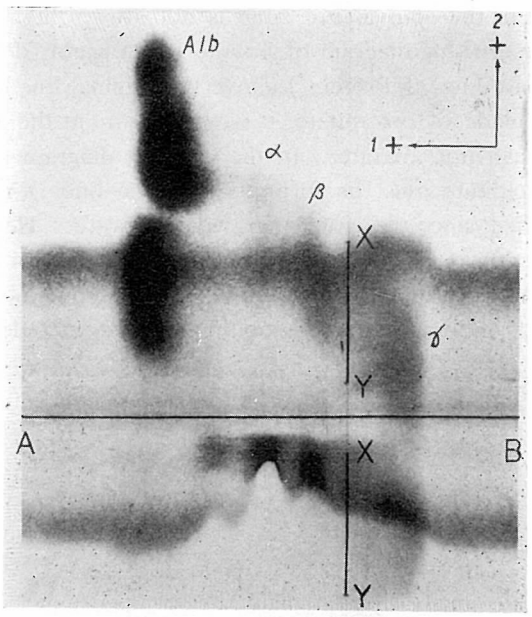

Fig. 9. Crossing diagram of human serum against Protein J.

First electrohoresis: 0.025 ml/8 cm of human serum were applied on the line XY. Toward direction 1, at 80 v. for 14 hrs. Second electrophoresis: 0.10 ml/20 cm of jack bean extract on the line AB. Toward direction 2, at 120 v. for 8 hrs. 20 min.

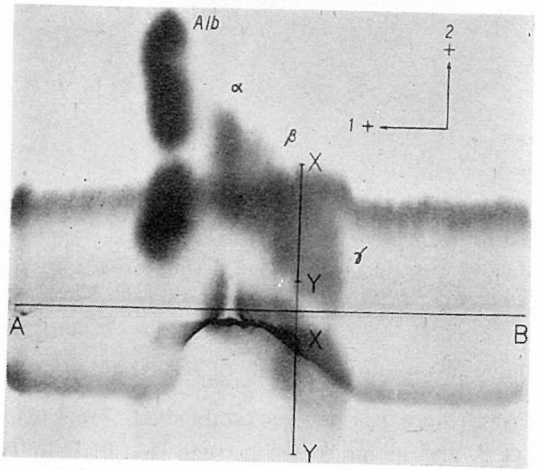

Fig. 10. Crossing diagram of hog serum against Protein J.

First electeophoresis: 0.0125 ml/4 cm of hog serum were applied on each XY-line. Toward direction 1, at 80 v. for 12 hrs. Second electrophoresis: 0.06 ml/18 cm of jack bean extract on the line AB. Toward direction 2, at 120 v. for 6 hrs.

serum proteins seems to be rather less strict. Moreover, the stoichiometry of the reaction can not be determined merely from the "crossing diagram". However, the "crossing diagram of the serum" appears to be characteristic of the animal species: Fig. 9 shows an example of the crossing diagrams of human serum against protein J, Fig. 10 that of hog, Fig 11 that of sheep, and Fig. 12 that of rat serum.

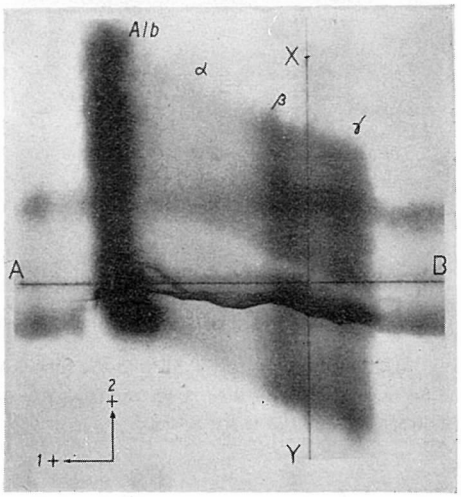

Fig. 11. Crossing diagram of sheep serum against Protein J.

First electrophoresis: 0.08 ml/16 cm of sheep serum on the line XY. Toward direction 1, at 80 v. for 16 hrs. Second electrophoresis: 0.08 ml/20 cm of jack bean extract on the line AB. Toward direction 2, at 120 v. for 8 hrs. 10 min.

As can be seen from the figures, the crossing diagram of human serum (Fig. 9) showed rather sharply separated peaks compared with sera of other animals. It shows at least four peaks, of which the first one corresponds to α_1-globulin and could not be observed in the crossing diagram of bovine serum. The fourth peak in the region of γ-globulin was missed occationally. Fig. 10 shows the crossing diagram of hog serum. There appeared only one flat peak, which seems to be a fused peak, consisting of several peaks corresponding α_1-, α_2-, β-, and γ-globulins. Fig. 11 shows the crossing diagramns of sheep serum. As in the case of human serum, at least four peaks can be seen, which correspond to α_1-, α_2-, β-, and γ-globulins. But the first peak which corresponds to α_1-globulin is flat in contrast to the crossing diagram of human serum. However, the height of a peak in the crossing diagram varied with the ratio of quantities of both reactants. Hence one can expect to obtain crossing diagrms of clearly separated peaks under other conditions. In the crossing diagram of rat serum shown in Fig 12, the first peak appeared in the broad region of albumin and α_1-globulin. Moreover, the line of precipitate is

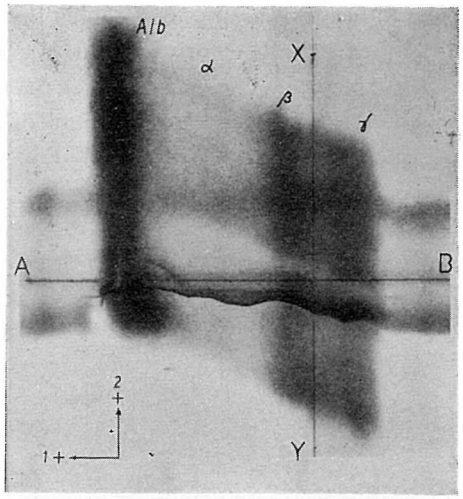

Fig. 12. Crossing diagram of rat serum against Protein J.

First electrophoresis: 0.08 ml/16 cm of rat serum on the lime XY. Toward direction 1, at 80 v. for 16 hrs. Second electrophoresis: 0.08 ml/20 cm of jack bean extract on the line AB. Toward direction 2, at 120 v. for 8 hrs.

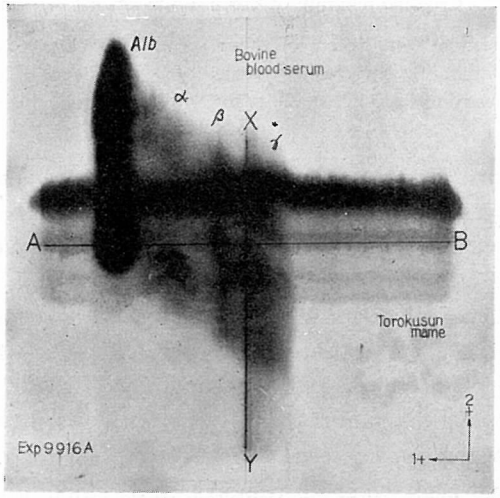

Fig. 13. Crossing diagram of bovine serum against the extract of Torokusun-beans.

First electrophoresis: 0.025 ml/15 cm of bovine serum were applied on the line AB. Toward direction 1, at 60 v. for 13 hrs. Second electrophoresis: 0.06 ml/20 cm of the extract of Torokusun-beans were applied on line XY. Toward directlon 2, at 120 v. for 6 hrs.

here very strong. In regard to these results, the albumin of rat serum seems to contain a fraction which reacts with protein J, whereas humn, bovine, hog, sheep sera did not contain such a fraction.

5. Specific proteins of beans other than jack bean.

The discovery of a specific protein in jack bean has led to the investigation of other beans. Of the beans tested, kidney bean (uzura-bean, phaseolus vulgaris Linn.), torokusun-bean (a variation of the former), azuki-bean (adzukia angularis Ohwi), cow-pea (vigna sinensis Savi), soy bean, and peanut, it was found that torokusun-bean and kidney bean each contained a specific protein. Fig. 13 and 14 show the crossing diagrams of bovine serum against the extract of torokusun-bean,

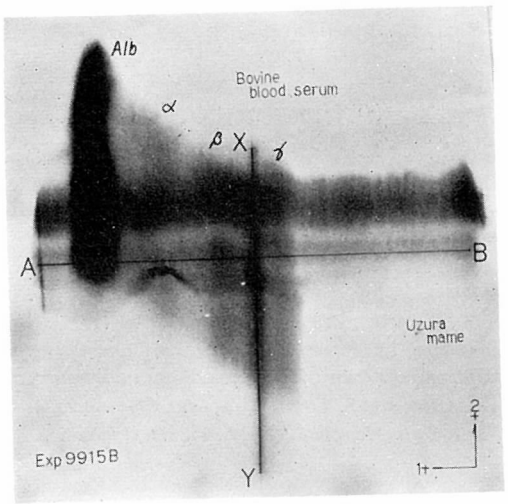

Fig. 14. Crossing diagram of bovine serum against the extract of Uzura-beans.

First electrophoresis: 0.025 ml/15 cm of bovine serum on the line XY. Toward direction 1, at 60 v. for 13 hrs. Second electrophoresis: 0.06 ml/20 cm of the extract of Uzura-beans on the line AB. Toward direction 2, at 120 v. for 6 hrs.

and that of uzura-bean, respectively. From the crossing diagrams, it can be seen, that these specific proteins also migrated toward the cathode under usual experimental conditions, and reacted chiefly with α-globulin of serum, in the same manner as Protein J. It is suspected they are of the same nature, and that such proteins are distributed fairly widely in legumes.

SUMMARY

Jack bean contained a protein, which reacted specifically with serum proteins. This specific protein was named provisionally Protein J.

Specific precipitation of serum proteins with Protein J was confirmed by simply mixing them in test tube or by the technipue of agar gel diffusion.

By the two-dimensional crossing paper electrophoresis, crossing diagrams of the extract of jack bean against sera on the one hand, and those of sera against the extract of jack bean on the other hand, were obtained. The former diagrams showed a peak in the region of Protein J, indicating that the reactant was Protein J; the the latter diagrams showed usually four peaks correspondig to α_1-, α_2-, β-, and γ-globulin of serum.

The crossing diagrams of sera of various animals showed characteristic figures.

Some other beans than jack bean contained also a specific protein, which reacted in the same manner.

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