Agar Gel Electrophoresis of the Hybrid of Canine and Human Hemoglobins: A simple convenient Means for the Determination of Chain Anomaly of Abnormal Hemoglobins.

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During the recent several years a remarkable progress has been made in the field of the chemistry of hemoglobin. The gate to this new domain was opened by Schroeder and his associates (1958),[1] who discovered two sorts of polypeptide, α and β chains, in the globin of normal adult hemoglobin. A chemical formula $\alpha_2\beta_2$ was thus given to its molecule. At about the same time a great steps forward were taken also with regard to the abnormal hemoglobins by the epoch-making work of Ingram (1958, 1959)[2,3]. He invented fingerprinting, an ingenious procedure for the study of polypeptide anomaly of hemoglobins, and demonstrated that hemoglobin S of sickle cell anemia had normal α chain and abnormal β chain. Similar studies with other hemoglobins have soon revealed that abnormal hemoglobins are abnormal because of the anomaly of either α chain or β chain. It is accordingly thought at the present time that detection of abnormal chain becomes a task which is mandatory for the identification as soon as a new abnormal hemoglobin is discovered.

About fifteen years before the discovery of α and β chains, dissociation of hemoglobin molecule was noticed by Reiner and his associates,[4] who demonstrated by Tiselius electrophoresis that bovine hemoglobin split into two halves in acid medium (pH 2.6–3.7). Similar observation was also made with human hemoglobin by Field and O'Brien.[5] They showed by ultracentrifugation that the hemoglobin was dissociated reversibly in acid medium (pH 3.5–6.0), forming two submolecules which could be restored to the original molecule in alkaline medium.

Recently Itano and Robinson[6] successfully produced the hybrids of abnormal human hemoglobins by dissociation and recombination in acid and neutral media. One of their experiments was concerned with the formation of normal human hemoglobin (Hb A: $\alpha_2^A\beta_2^A$) from a hemoglobin possessing α chain anomaly (Hb I: $\alpha_2^I\beta_2^A$) and hemoglobin of β chain anomaly (Hb S: $\alpha_2^A\beta_2^S$). These hemoglobins were purified as carbon monoxy-type pigment, mixed, dissociated at pH 4.7, and finally recombined at pH 6.5. Tiselius electrophoresis of the mixture treated in this way disclosed four peaks which corresponded to original two hemoglobins (Hb I: $\alpha_2^I\beta_2^A$

and Hb S: $\alpha_2^A\beta_2^S$) and two hybrids. The two hybrids were a hemoglobin of double chain anomaly, $\alpha_2^I\beta_2^S$ and a normal hemoglobin, $\alpha_2^A\beta_2^A$. The process was designated hybridization, and its principle was immediately applied to the detection of chain anomaly of various hemoglobins so far described.[7] Demonstration of Hb A in the hybrids was a reliable criteria for the determination of chain anomaly. When hybrid Hb A was formed by the hybridization between a known hemoglobin of β chain anomaly was presumed to have α chain anomaly, and vice versa. Unfortunately hybridization is not useful in our laboratory, because there is a great difficulty in obtaining control sample of abnormal hemoglobin in Japan where hemoglobinopathy is rarely encountered.[8]

Accordingly we were earnestly hoping for another method to which we were accessible when we wanted to examine the chain anomaly of Hb M_{Iwate},[9] the abnormal hemoglobin of hereditary nigremia. Shortly before the discovery of this hemoglobin in 1960 Satake and Take separated α and β chain of bovine hemoglobin by paper electrophoresis with a buffer solution containing large amount of urea.[10] We improved their technique so that it might be useful for the study of human hemoglobin, and were successful in demonstrating the α chain anomaly of Hb M_{Iwate}.[11] The procedure was called urea-dissociation paper electrophoresis. It was employed for the study of several abnormal hemoglobins discovered in Ube with satisfactory results.[8] However, it proved insensitive when it was applied to the detection of abnormal chain in Hb M_{Kurume}.[12] The need of more sensitive method which does not require control sample of abnormal human hemoglobin was felt urgently at that time.

In our laboratory attempts have been made for these two years to obtain a new method for the detection of chain anomaly. Special attention was paid to the study of Itano and Robinson[13] who reported the hybrids of human hemoglobin with canine hemoglobin, since canine hemoglobin was thought to serve as a convenient control hemoglobin in the hybridization of human abnormal hemoglobins. Formation of hybrids was studied with canine hemoglobin employing human abnormal hemoglobins of known chain anomaly (β chain: Hb S and Hb C; α chain: Hb M_{Iwate} and Hb Ube 2) as hybrid-mate.

Unlike the original method of Itano and Robinson[13] in which Tiselius electrophoresis was used agar gel electrophoresis[14] was adopted for the demonstration of individual hybrids because this technique was simple and rapid, and required very small amount (a few drops) of purified abnormal hemoglobin and canine hemoglobin.

Last year (1962) Huehns and his associates[15] published a procedure of hybridization which was essentially the same in principle as ours. They employed starch gel electrophoresis for the analysis of hybrids. In our experience agar gel electrophoresis was more convenient and easier.

The present paper aims to describe our new method of hybridization test together

with some examples of its application.

MATERIALS AND METHODS

Samples of human and canine blood were collected and prevented from coagulation with citrate or oxalate. Following centrifugation packed red cells were washed three times with physiological saline. Aliquots of the packed cells were hemolysed according to the method of Drabkin[16] to get hemoglobin solutions of approximately 10 g/dl.. In order to obtain purified abnormal human hemoglobins the hemolysate was subjected to starch block electrophoresis[17] (Tris-EDTA borate buffer of pH 8.6 or 7.0). The portion of starch block which contained purely the abnormal hemoglobin was cut out and introduced into a funnel. To this was added a small amount of water. The funnel was aspirated to collect the eluate of the abnormal hemoglobin.

The abnormal hemoglobins used in this experiment were hemoglobin Ube 1,[18] hemoglobin Ube 2,[8] hemoglobin Tokuchi,[19] hemoglobin Hikari[20] and hemoglobin M_{Iwate}.[11]

Two buffer solutions necessary for the hybridization were prepared as follows:

1) 0.1 M acetate buffer solution (pH 4.7): 13.6 gm of sodium acetate (CH_3-COONa. $3H_2O$) were dissolved in distilled water and made to 1000 ml., and 1300 ml. of 0.1 N acetic acid were added to it. The pH of the mixture was adjusted to 4.7 accurately by addition of either acid or alkali.

2) Tris-EDTA borate buffer solution (pH 8.6): 55.0 gm of Tris (hydroxymethyl) aminomethane 7.0 gm of EDTA and 9.0 gm of boric acid were dissolved in the order mentioned into a small amount of distilled water and made finally to 1000 ml. with distilled water. This stock buffer solution was diluted 10-fold with distilled water before use for the hybridization and three-fold for agar gel electrophoresis.

A 1:1 mixture of canine hemoglobin (Hb Can) and normal human hemoglobin (Hb A) approximately equaling in concentration was introduced in a sealed cellophane (Visking) tube. In another cellophane tube canine hemoglobin (Hb Can) and abnormal human hemoglobin of unknown chain anomaly (Hb X) were mixed in proportion of 1:1. Both tubes were placed in a beaker containing one liter of acetate buffer solution of pH 4.7 for 24 hours in a refrigerator. The canine and the human hemoglobins dissociated into their submolecules (α and β chains). They were taken out and transferred into another beaker which had 1 liter of Tris-EDTA borate buffer of pH 8.6 and kept 24 hours again in a refrigerator (4C) to recombine the dissociated hemoglobin submolecules.

The mixture of the two cellophane tubes were subjected to agar gel electrophoresis on the same slide glass. The Hb Can-Hb A mixture served as a standard control for the demonstration of abnormal human-canine hybrid in the Hb Can-Hb X mixture.

RESULTS

In the hybrid mixture of normal human adult hemoglobin (Hb A) and canine hemoglobin (Hb Can), two new pigments in addition to two original hemoglobins (Hb A and Hb Can) were clearly demonstrated by agar gel electrophoresis at pH 8.6 as shown in Figures 1 and 2. Canine hemoglobin migrated more slowly than hemoglobin A to the anode; one component (II) of the two newly produced pigments moved more slowly than canine hemoglobin, and the other new pigment (I) migrated more rapidly than hemoglobin A (to the anode side). The order of migration from the anode to the cathode was, therefore, hybrid (I), Hb A, Hb Can and hybrid (II). These hybrids were thought to be produced by the process expressed by the following equation.

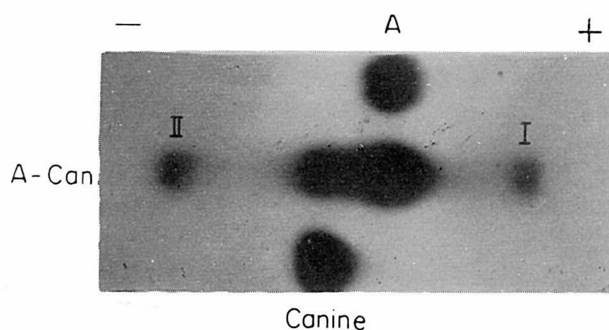


Fig. 1. Hybridization of normal human hemoglobin (Hb A) with canine hemoglobin (Hb Can) as demonstrated by agar gel electrophoresis.

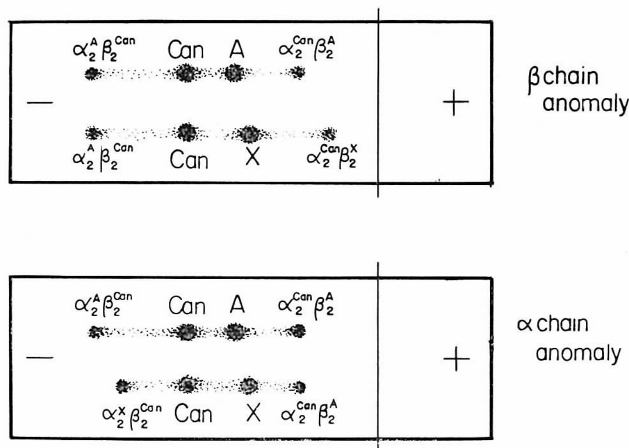
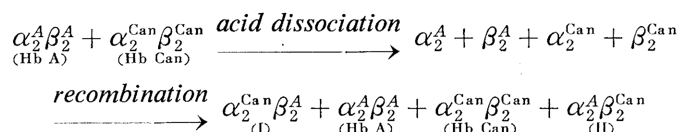


Fig. 2. Criterion for the determination of chain anomaly of an abnormal hemoglobin (Hb X: $\alpha_2{}^X\beta_2{}^A$ or $\alpha_2{}^A\beta_2{}^X$) by hybridization with canine hemoglobin (Hb Can: $\alpha_2{}^{Can}\beta_2{}^{Can}$).



$$\underset{\text{(Hb A)}}{\alpha_2^A\beta_2^A} + \underset{\text{(Hb Can)}}{\alpha_2^{\mathrm{Can}}\beta_2^{\mathrm{Can}}} \xrightarrow{\textit{acid dissociation}} \alpha_2^A + \beta_2^A + \alpha_2^{\mathrm{Can}} + \beta_2^{\mathrm{Can}}$$

$$\xrightarrow{\textit{recombination}} \underset{\text{(I)}}{\alpha_2^{\mathrm{Can}}\beta_2^A} + \underset{\text{(Hb A)}}{\alpha_2^A\beta_2^A} + \underset{\text{(Hb Can)}}{\alpha_2^{\mathrm{Can}}\beta_2^{\mathrm{Can}}} + \underset{\text{(II)}}{\alpha_2^A\beta_2^{\mathrm{Can}}}$$

Which one of the two hybrids is in reality $\alpha_2^{\mathrm{Can}}\beta_2^A$, and which is $\alpha_2^A\beta_2^{\mathrm{Can}}$? This question will be solved by a concomitant electrophoresis of the Hb A-Hb Can hybrid solution and Hb S-Hb Can hybrid solution on the same agar slide glass. (Plate, No, 1) Hb S ($\alpha_2^A\beta_2^S$) is a hemoglobin of established β chain anomaly, and its human-canine hybrid $\alpha_2^{\mathrm{Can}}\beta_2^S$ is expected to have an electrophoretic mobility different from that of Hb A's hybrid $\alpha_2^{\mathrm{Can}}\beta_2^A$. Hb S has the same α chain as Hb A, and therefore hybrid $\alpha_2^A\beta_2^{\mathrm{Can}}$ is common to both hemoglobins. Hence it is presumed that any hybrids showing a different migration in this system of Hb A-Hb Can and Hb S-Hb Can will refer to the hybrid hemoglobins made of canine α chain and human β chain ($\alpha_2^{\mathrm{Can}}\beta_2^A$ and $\alpha_2^{\mathrm{Can}}\beta_2^S$), and those exhibiting the same migration are concerned with the hybrid molecule of human α chain and canine β chain ($\alpha_2^A\beta_2^{\mathrm{Can}}$).

The result of such an experiment is presented in Plate, No. 1, which reveals discrepancy and agreement of the hybrid spots on the anode and the cathode side, respectively. It is therefore thought that a hybrid hemoglobin composed of human β chain and canine α chain presents itself near the anode, and that consisting of human α chain and canine β chain comes out close to the cathode.

The validity of this presumption was substantiated further by similar electrophoretic experiments of the hybrids of Hb Can and various abnormal hemoglobins of known chain anomaly, such as Hb C ($\alpha_2^A\beta_2^C$), Hb $\mathrm{M_{Iwate}}$ ($\alpha_2^{MI}\beta_2^A$) and Hb Ube 2 ($\alpha_2^{U2}\beta_2^A$), using Hb Can-Hb A hybrid as control. Disagreement of the migration of spots on the anode side analogous to that obtained by Hb Can-Hb S hybrid was observed in Hb Can-Hb C hybrid. In contrast, difference in migration between the two hybrid spots was demonstrable on the cathode side by Hb Can-Hb $\mathrm{M_{Iwate}}$ and Hb Can-Hb Ube 2 hybrid.

Thus it seems justified to conclude that comparative electrophoresis of Hb Can-Hb A hybrid with the hybrid of Hb Can and unknown abnormal hemoglobin (Hb X) on the same agar gel slide glass is useful for the detection of chain anomaly of the abnormal hemoglobin. (Fig. 2) In this test disagreement of migration of hybrid spots on the anode side indicates β chain anomaly (β^X) and, on the cathode side, α chain anomaly (α^X).

The above method was successfully applied to the determination of chain anomaly of abnormal hemoglobins which were recently discovered in Ube and in other cities. The study disclosed α chain anomaly in Hb Ube 2, and Hb $\mathrm{M_{Iwate}}$, and β chain anomaly in Hb Ube 1, Hb Tokuchi and Hb Hikari as illustrated.

A) Hb Ube 1 (Plate, No. 2)

Hb Ube 1 was discovered from a 11-year-old girl with hemolytic anemia. There

were many erythrocytes holding Heinz bodies in her peripheral blood. The abnormal hemoglobin had approximately the same electrophoretic mobility as hemoglobin S. It migrated slower than hemoglobin A producing a tongue-like projection extending from it cathode-ward in starch block electrophoresis at pH 8.6. Plate (No. 2) illustrates the hybridization of this hemoglobin with the canine hemoglobin in which difference in mobility between the hybrid spots to the anode side is clearly realized. This indicates β chain anomaly in hemoglobin Ube 1. Therefore, the hemoglobin is expressed by $\alpha_2^A\beta_2^{\mathrm{Ube1}}$.

B) Hb Ube 2 (Plate, No. 3)

This abnormal hemoglobin was discovered from a 44-year-old house-wife with uterine cancer and from her daughter. This hemoglobin was electrophoretically faster than hemoglobin A at pH 8.6. It had been already known to us from the result of fingerprinting that the hemoglobin had α chain anomaly.

The electrophoregram of the Hb Ube 2-Hb Can hybrid mixture was quite different from that of Hb A-Hb Can hybridization. Only two spots were seen in the former in contrast to four spots in the latter. Hybrid $\alpha_2^{\mathrm{Ube2}}\beta_2^{\mathrm{Can}}$ and canine hemoglobin $\alpha_2^{\mathrm{Can}}\beta_2^{\mathrm{Can}}$ were almost equal in electric charge. So they produced a single spot on the electrophoregram by mutual overlapping. Similar overlapping took place between hybrid $\alpha_2^{\mathrm{Can}}\beta_2^A$ and hybrid $\alpha_2^{\mathrm{Ube2}}\beta_2^A$. Accordingly another single spot was formed to the anode side. There was discrepancy between the hybrid spots to the cathode side when the two electrophoregram were compared with each other.

C) Hb Tokuchi (Plate, No. 4)

Hemoglobin Tokuchi was a fast-moving hemoglobin which was demonstrated in a 65-year-old man with cardiac failure and in his son. Hybridization test revealed discrepancy in mobility between anodic hybrid $\alpha_2^{\mathrm{Can}}\beta_2^{\mathrm{Tok}}$ and the control anodic hybrid $\alpha_2^{\mathrm{Can}}\beta_2^A$. It is therefore thought that Hb Tokuchi has β chain anomaly. This was reconfirmed also by fingerprinting.

D) Hb Hikari (Plate, No. 5)

Hb Hikari was detected from a 22-year-old man with hepatitis and neurotic symptoms. The hemoglobin was fast-moving. Plate (No. 5) represents the result of hybridization test. It is apparent that Hb Hikari has β chain anomaly because there is disagreement of the mobility of hybrid spots $\alpha_2^{\mathrm{Can}}\beta_2^{\mathrm{Hik}}$ and $\alpha_2^{\mathrm{Can}}\beta_2^A$ which are seen to the anodic extreme of the electrophoregrams.

E) Hb M_{Iwate} (Plate, No. 6)

Hb M_{Iwate} is an abnormal hemoglobin with dark brown color which was demonstrated in the blood of hereditary nigremia. This disease is endemic in a district of Iwate Prefecture. Oxyhemoglobin form of Hb M_{Iwate} was separable from Hb A by means of agar gel electrophoresis. Hb M_{Iwate} solution (oxyhemoglobin type) puri-

fied by agar gel electrophoresis and Hb A solution were mixed with Hb canine solution separately for the purpose of hybridization. The result of the experiment is shown in Plate (No. 6). The migratory discrepancy between hybrid $\alpha_2^{MI}\beta_2^{Can}$ and control hybrid $\alpha_2^{A}\beta_2^{Can}$ on the cathode side is clearly visible, while such disagreement was not seen with respect to the two hybrid spots coming out on the anode side. This is interpreted as indicating α chain anomaly in Hb M_{Iwate}. The α chain anomaly was reconfirmed also by fingerprinting.

DISCUSSION

Itano, Singer and Robinson[6] are credited with the invention of hybridization that is a technique of producing new hemoglobins from a mixture of two different hemoglobins by the mutual exchange of each α and β chain. In their original experiment Hb S and Hb I were mixed, dissociated in acid medium, and subsequently recombined in neutral medium. The recombined products were examined by moving boundary electrophoresis. Four peaks corresponding to Hb S, Hb A, Hb I and a new hemoglobin were demonstrated in the electrophoregram. Hb A was the product of the recombination of the normal α chain of Hb S and the normal β chain of Hb I, namely a hybrid of Hb S and Hb I, while the new hemoglobin was another hybrid which was formed by the abnormal α (Hb I) and β (Hb S) chain of these hemoglobins.

This technique was soon introduced to the practical study of abnormal hemoglobins for the determination of their chain anomaly by Lehmann,[7] who employed paper electrophoresis for the demonstration of hybrids and examined the appearance of Hb A in the hybrid mixture as a criterion. When Hb A was demonstrable, it was presumed that there would be opposite chain anomalies between the known abnormal hemoglobin (used as the control mate of hybridization) and the hemoglobin possessing unknown abnormal chain. In contrast, when Hb A was not detectable, unknown hemoglobin was thought to have the same kind of chain abnormality as that of the known, although in the latter case, failure in the performance of hybridization should also be taken into consideration as a possibility. It is accordingly convenient to select abnormal hemoglobins which are expected to have opposite chain anomalies for this kind of hybridization test.

Soon after the introduction of this technique, another more convenient technique in which Hb Can was used as a hybrid-mate of abnormal hemoglobin was invented for the study of abnormal hemoglobin.[13] This ingeneous technique has enabled every laboratory to detect the chain anomaly of abnormal hemoglobins, because Hb Can is easily available everywhere in contrast to the difficulty of keeping incessantly fresh abnormal hemoglobins of abnormal α and β chain in a stocker. Several hybridization techniques with Hb Can using Tiselius moving boundary (original method), [6,13] paper[7] and starch gel[15] electrophoreses have been published.

Usually, the migratory difference in electrophoresis of the questioned hybrid formed from the abnormal hemoglobin and Hb Can and the corresponding hybrid seen in the control arising from Hb A and Hb Can is small. Therefore, Tiselius and paper electrophoresis which have unsatisfactory separating effect are not ideal for such hybrid analysis. Starch gel electrophoresis[21] gave satisfactory separation of the recombined hemoglobins into each component. However, its manipulation was troublesome, and purification of starch and preparation of starch gel were not easy.

In contrast, agar gel electrophoresis (on a slide glass[14]) which was developed in our laboratory is simple and rapid and gives a sharp separation effect equaling to that of starch gel electrophoresis. Its electrophoregram can be preserved indefinitely after the agar slide glass has been stained and dried. This is the reason why agar gel electrophoresis was used in our hybrid test.

In the early stage of our work Itano's hybridization[13] with canine hemoglobin as tested by agar gel electrophoresis did not always give satisfactory results. Therefore, basic conditions necessary for the successful formation of hybrid were studied scrupulously.

In order to know the optimal pH for acid dissociation of hemoglobin molecule, hemoglobin mixtures of Hb A and Hb Can in Visking tubes were separately dialysed against a series of buffer solutions varying in pH from 3.2 to 5.3 with 0.3 pH intervals for 24 hours in a refrigerator and then put into Tris-EDTA borate buffer solution of pH 8.6 for 24 hours to recombine the dissociated molecules. Ideal hybrid formation between the two hemoglobins was observed when buffer solutions of rather narrow range of pH, namely from 4.4 to 4.7 were employed for dissociation. No hybrid was formed when the pH of the buffer solutions used for dissociation was above 5.0. Below pH 4.4, hemoglobins were partially precipitated in the Visking tubes. The precipitate did not dissolve completely by the process of recombination in alkaline buffer solution. Consequently the supernatant solution of the hemoglobin mixture (after centrifugation) which was to be examined by agar gel electrophoresis for the hybrids became very poor in hemoglobin content. This resulted in the difficulty of demonstration of hybrids in agar slide glass.

It is worth mentioning that Hb Can is more easily deteriorated in acid medium than Hb A. For instance, Hb Can precipitates so completely at a pH below 4.4 that it yields no spot on agar electrophoregram. Canine hemoglobin is also susceptible to denaturation during storage in a refrigerator for a certain period of time. It is therefore necessary to filter or centrifuge the canine hemolysate every time when hybridization test is made so that an excellent solution of canine hemoglobin may be used for it.

Itano[13] recommended that twenty-four hours are sufficient for the acid dissociation of hemoglobins. However, a study by light scattering method[22] disclosed that dissociation and recombination of hemoglobin molecule took place within a few

minutes when it was exposed to sufficient amount of acid or alkali. In our experience the time necessary for acid dissociation yielding a maximum proportion of hybrids in hemoglobin mixture was 48 to 72 hours when acetate buffer solution of pH 4.7 was employed. Acid dissociation for 12 hours was around the limit of hybrid formation, where faint vestige of hybrid spots were noticeable. When the acid incubation time was less 9 hours, no hybrids were obtained. These findings are consistent with observation made by Antonini.[23]

It is plausible that hemoglobin molecule dissociates or recombines immediately when it is exposed to acid or alkali. However, in practice, it takes a certain length of time to get effective dissociation and recombination which secure the successful hybridization, because hemoglobin solution in the Visking tube undergoes gradual change in pH, being indirectly influenced by the acid or alkaline solution which is outside the membrane. Cellophane membrane of the Visking tube does not permit instant change of pH.

The production of hybrid hemoglobin attains maximum level when human hemoglobin (either normal or abnormal) is mixed with canine hemoglobin in proportion of one to one. When the mixing proportion of the two hemoglobins is far from 1 : 1, the hybrids produced in the mixture becomes so small in amount that can not be demonstrated by electrophoresis on account of the adverse effect of the predominant original hemoglobins.

Huehns[15] interpreted the electrophoretic migration of hybrids and their original hemoglobins on starch gel from the view point of the difference of electric net charges of subunits (α and β chain) of hemoglobin molecule. However, straightforward application of this idea to the agar electrophoregram may be thoughtless, because agar gel and starch gel electrophoreses are somewhat different in mechanism as seen in the case of plasma haptoglobin analysis. Another instance which illustrates the difference between starch and agar gel electrophoreses is that the two hybrids produced between Hb C and Hb Can overlap on the two original hemoglobins, thus yielding only two spots on starch gel, whereas three spots indicating β chain anomaly of Hb C were clearly seen on agar gel. Accordingly, when agar gel electrophoresis is used for the demonstration of hybrids, it is recommended to watch the anodic and cathodic ends of the electrophoregrams, where hybrid hemoglobins make their appearance. Usually abnormal hemoglobin is a combination of either abnomal α or abnormal β chain (except for Hb G/C) and normal β^A or α^A chain. Therefore, one of the two hybrids referring to the combination of Hb Can chain and normal chain of the abnormal hemoglobin should have the same electrophoretic mobility as one of the hybrids of the control (Hb A-Hb Can mixture), and another hybrid should show a migration different from that of the control. If the anodic hybrid of the unknown sample (Hb X-Hb Can mixture) is different in migration from that of the control, it is interpreted that the unknown hemoglobin has β chain anomaly, and if discrepancy is apparent on the cathode side, α chain anomaly

is conceived. This criteria invariably gave satisfactory results when it was applied to the abnormal hemoglobins discovered in Ube, in good agreement with the findings obtained by urea dissociation paper electrophoresis and fingerprinting.

An interesting abnormal hemoglobin examined by our method was Hb Ube 2. Its two hybrids ($\alpha_2^{\text{Ube2}}\beta_2^{\text{Can}}$ and $\alpha_2^{\text{Can}}\beta_2^{A}$) overlapped the two original hemoglobins. However, the anodic hybrid had the same electrophoretic migration as its control ($\alpha_2^{\text{Can}}\beta_2^{A}$), while the cathodic hybrid was not equal to its control hybrid ($\alpha_2^{A}\beta_2^{\text{Can}}$). Hb Ube 2 was therefore, presumed to have α chain anomaly.

Although our procedure has not yet been applied to such an abnormal hemoglobin as Hb I which is extremely fast-moving, this criterion will not fail to detect its chain anomaly.

A great difficulty in determining the abnormal chain of Hb M_{Iwate}. Since Hb M_{Iwate} (oxy-Hb form) was clearly separated from Hb A by agar gel electrophoresis, the cathodic hybrid ($\alpha_2^{MI}\beta_2^{A}$) was expected to be demonstrated at its proper position, being distinctly differentiated from the original Hb M_{Iwate}. Actually the separation was unsatisfactory for unknown reason.

Anyhow, our hybridization technique is easy in performance, and recommendable as a screening test for the detection of chain anomaly of newly discovered abnormal hemoglobins.

CONCLUSION

A new method for the detection of chain anomaly of abnormal hemoglobins has been divised by combined use of hybridization and agar gel electrophoresis. An equal amount of hemoglobin and another control mixture of canine hemoglobin and human normal hemoglobin were placed separately in cellophane tubes, they were dialysed against 0.1 M acetate buffer solution of pH 4.7 for 24 hours in a refrigerator, and then the cellophane tubes were transferred into Tris-EDTA borate buffer solution of pH 8.6 to be dialysed for 24 hours for the recombination of acid-dissociated hemoglobin subunits. At the end of dialysis the recombined hemoglobin mixtures (Hb X-Hb Can and Hb A-Hb Can) were subjected to agar gel electrophoresis at pH 8.6.

Disagreement in electrophoretic mobility of hybrid spots of the mixture on the anode side indicates β chain anomaly, on the cathode side refers to α chain anomaly of the abnormal hemoglobin.

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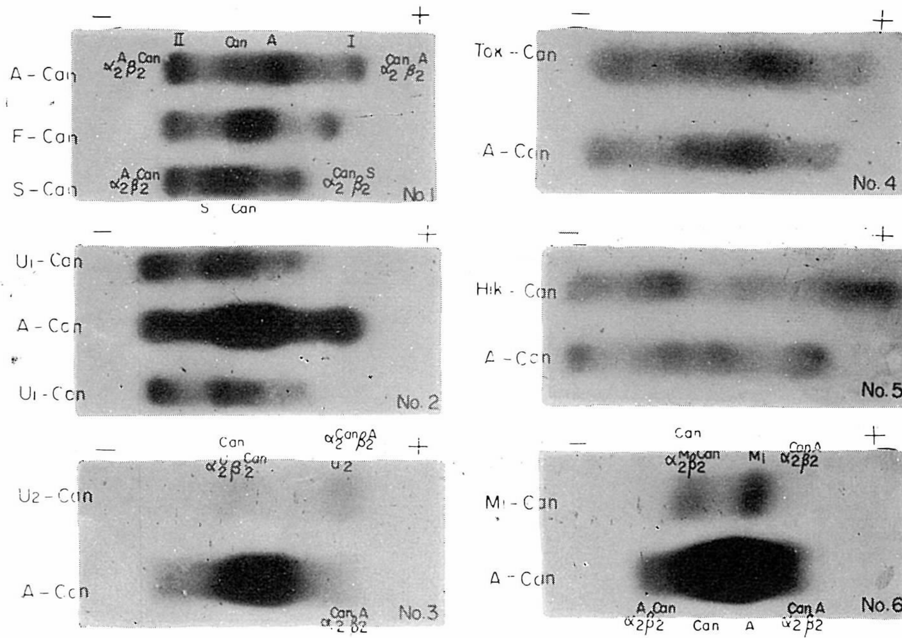
PLATE



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