Migration Distances of Human Serum Protein Fractions in Agar Gel Electrophoresis Patterns (*)

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In tests of changes of serum proteins, in most cases, the quantitative relation or the relative percentage of their fractions separated by electrophoresis has hitherto been considered in praxis.

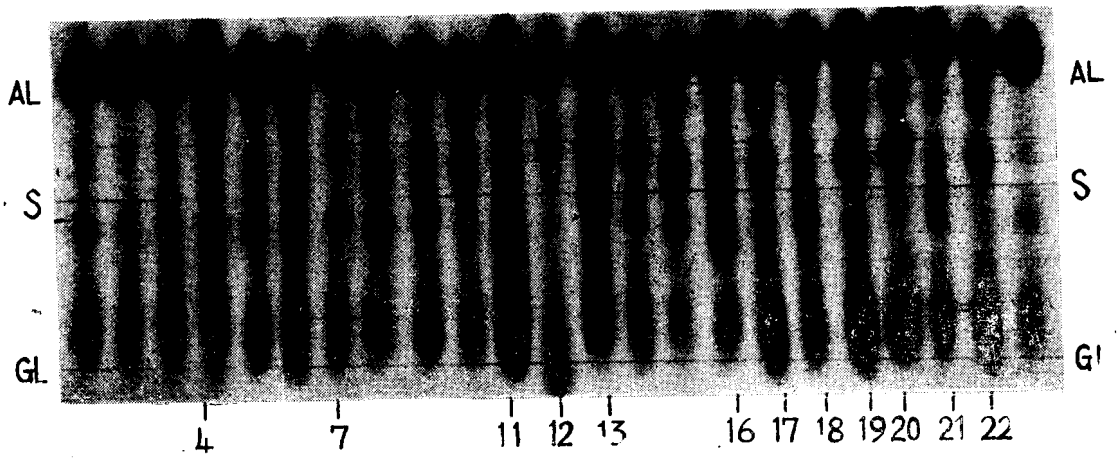
However their qualitative changes—varieties of migration distances of fractions-have very rarely been reported, and even in a contradictory way.

Ressler[7] made a comparison of 4 abnormal globulins to a normal to show distinct differences in their migration distances.

Wuhrmann and Wunderly wrote,[1]"Auffällige Veränderungen der Wanderungsgeschwindigkeit der einzelnen Fraktionen bei Hepatitis und anderen Leberleiden sahen wir nie" (ähnlich auch Malamani, Gastaldi, und Rodari[2]).

It is well known that the fraction of heterogenic gamma globulin which used to appear in cases of hyperglobulinemia shows a large shape with a round peak and a wide base at the cost of the albumin, but the knowlege about the migration distances of serum proteins, albumin and globulins is yet very primitive so far as the author knows.

Therefore this work was undertaken to elucidate the alteration of migration in abnormal cases of serum protein fractions through the method of mass observation written below.



[Fig. 1.]

Fig. 1. Photograph to show some of the various fractional migration distances of serum proteins of different cases. 23 patterns have appeared in a single agar gel plate under apparently the same conditions. S-S, start line; A_1-A_1, line for lower boundary of normal albumin fraction; G_1-G_1, for that of normal gamma globulin fraction. Pattern 1 and 23(last) represent the control. Pattern, 7, 11, 13, 16, 17, 18, 20 and 21 from left show albumin fractions located higher than others. Pattern 12, 17, 19, and 22 have gamma globulin fractions migrated farther than others. Pattern 16 shows beta globulin fraction situated farther from the start line than others.

Collection and Conservation of Test Materials

915 normal and inpatient sera(+) were obtained from The Central Laboratory of SNU Hospital, from May,

(*): The author is much obliged to Director, Prof. Tongick Kim for the courtesy shown him in granting so many of the sera.

1959 to Feb. 1960. Each of them was poured individually into a small vial containing a piece of tiny polyethylene tube(2 mm thick 10 mm long) as a carrier of serum, to be reserved in a refrigerator when not in use.

Experimental Methods

1.5% agar suspension is made with the purified[3] agar U.S.P.S.B. Penick, and the buffer containing vernonal sodium 10 gm, CH_3COONa. 3HOH 8.2 gm, N/10 HCl 60 ml, NaCl 2.92 gm, H_3BO_3 1.7 gm, N/10 CH_3COOH 18.7 ml, NaOH 0.408 gm and HOH ad 2,000 ml, resulting in PH 8.6.

70 ml of the agar suspension are heated in a water bath under vigourous stirring to produce a clear hot agar solution, which 20 minutes after being poured upon a large transparent glass plate(17 cm×25 cm) laid horizontal, is to become a smooth agar gel plate (about 2 mm thick).

The agar gel plate is set upon a sheet of mm section paper, on which 23 points were previously marked clearly at intervals of 1 cm along its longer center line to let the points appear through the agar gel Plate for materials to be individually deposited on. Point 1, 12(middle) and 23(last) are reserved for a control serum and the other points for the samples.

0.005 ml of a serum can be spotted upon a point by slightly touching an end of the polyethylene (serum filled) tube.

10 minutes after being spotted, the agar gel plate is bridged with wet wicks in buffer in an Ep. chamber to be run at 5V/cm, 2 mA/cm(width) for 3 and an half hours.

After ending electrophresis, the agar gel plate is put into a protein staining bath for 10 minutes, then into successive washing baths for 2 hours. After being washed enough, it is dried on a heating plate under 50°C.

The dried agar plate containing 23 Ep. patterns is set upon a section paper with lines for analyzing the migration distances of fractions of serum proteins.

According to the fraction positions analyzed, some groups and types can be established in the patterns observed.

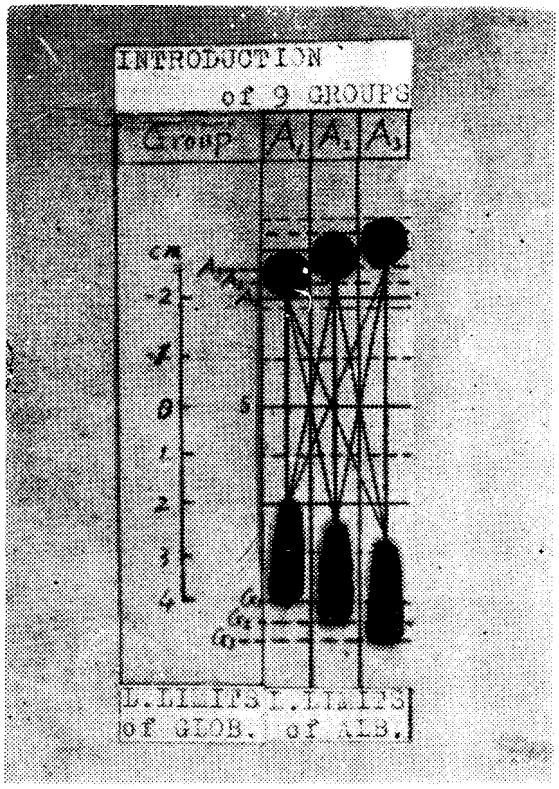
Results

A lot of observations made after this method on the Ep. patterns derived from various bedy states, normal and diseases, have resulted in the presentation of groups(Fig. 2) types(Fig, 3) and shapes (Fig. 4). Groups:

Considerable differences are found in the migration distances of the fractions of albumin and gamma globulin.

The lower boundaries of albumin are expressed in 3 grades with A_1, A_2, and A_3, while those of gamma globulin in 3 grades with G_1, G_2 and G_3.

The combinations between the varied positions of albumin and gamma globulin have produced following 9 groups, A_1G_1, A_1G_2, A_1G_3, A_2G_1, A_2G_2, A_2G_3, A_3G_1, A_3G_2, and A_3G_3,(Fig. 2)



[Fig. 2.]

Fig. 2. Diagram to introduce 9 groups, A_1G_1, A_1G_2, A_1G_3. A_2G_1. A_2G_2, A_2G_3, A_3G_1, A_3G_2, and A_3G_3 in the patterns observed.

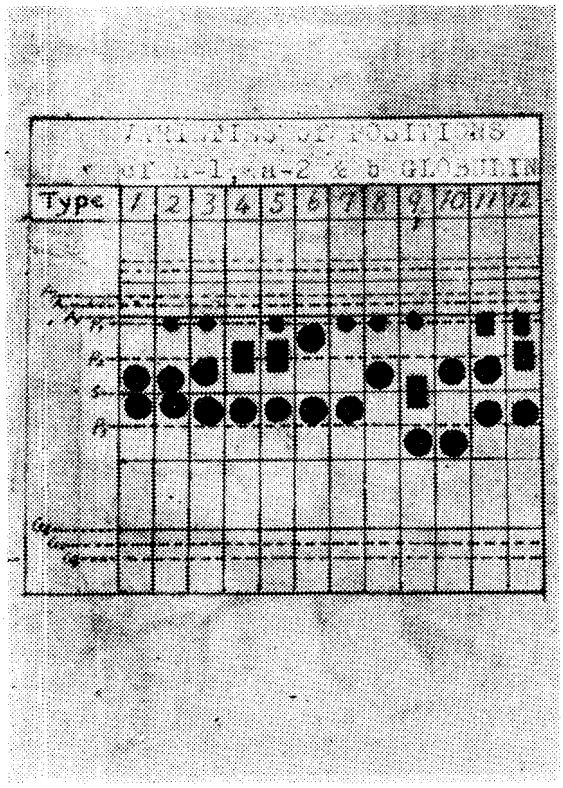
S, start point; disc, albumin; rod, gamma globuln; A_1, A_2 and A_3, lower limits of albumin; G_1, G_2 and G_3, those of gamma globulin.

$SA_1=2$ cm, $A_1A_2=A_2A_3=0.2$ cm

$SG_1=4$ cm, $G_1G_2=G_2G_3=0.3$ cm

Types: The fractions of alpha(or alpha 1 and alpha 2) and beta do not always have taken fixed

positions of their own, but shown remarkably varied ones respectively. 12 types are thus classified. (Fig. 3).



[Fig. 3.]

Flg. 3. Diagram to show all the types of patterns observed. Smaller disks at or near P-1 indicate position of alpha-1, bigger ones above S-S line, that of alpha or alpha-2 and those below S-S line that of beta. Squares across a line show the range of a fraction position. Each type has its fractions, alpha or alpha-1, alpha-2 and beta located in proper positions.

S, start point, $P_1S=2$ cm,

$P_2S=P_3S=1$ cm.

Shapes: All the fractions of gamma globulin in the Ep. patterns do not necessarily have shown the same appearance but varied ones like those in Fig. 4.

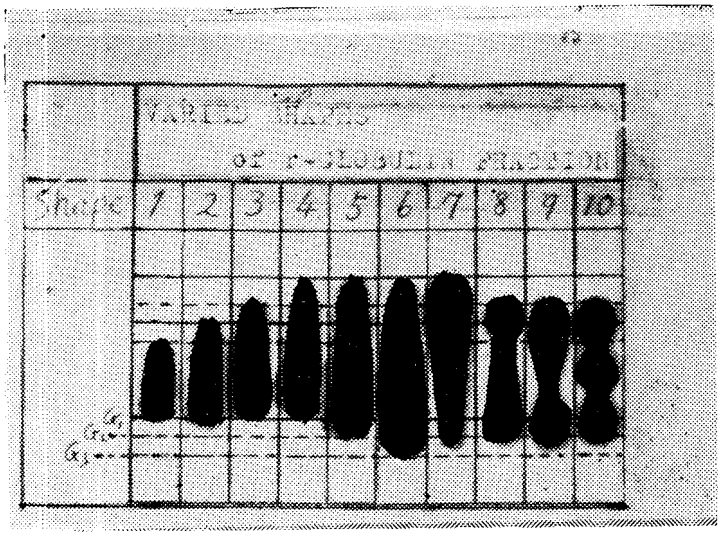
Fig. 4. Diagram to exhibit varied shapes of gamma globulin fractions observed. Shapes from 1 to 6 show the gradual lengths of gamma globulin from hypo-to hypergammaglobulinemia. Shape 7 is the reversed form of shape 6. Shape 8, 9 and 10 exhibit enforced forms of heterogenic structures.

Table 1. Distribution of the Types in the Groups of Patterns Observed

T \ G	A_1G_1	A_1G_2	A_1G_3	A_2G_1	A_2G_2	A_2G_3	A_3G_1	A_3G_2	A_3G_3
1	+				+				
2	+		+	+	+				
3	+	+			+				+
4	+	+	+	+	+	+	+		+
5	+	+	+	+	+		+	+	
6	+	+	+	+	+		+	+	
7		+		+	+	+	+		
8				+		+			
9	+	+							
10		+			+				
11	+	+							
12	+	+							

G, group; T, type; +, appearing.

Group A_1G_1 and A_1G_2 contain most of the types, while type 4, 5 and 6 are popular in most groups.



[Fig. 4.]

Discussion

As, in general, known, the migration distance(cm^2/sec. V) of serum fractions depends on a good deal of factors as electric charge, size and asymmetry of protein molecules, PH and ionic strength of buffer, characteristics of medium, temperature, electroosmosis, evaporation and side effect. And under the same or constant conditions, it is believed that a serum fraction is to migrate a proper distance[4] in accordance with its structural characteristics.

The varieties shown in Fig. 1—4 are thought to have derived from the complicated changes of normal and disease serum proteins. The buffer and apparatus used in this work are, by no means, specific for the mass observation of the migration distances of serum proteins. However this much large agar gel plate, on which more than 20 serum spots of samples can be arranged, is thought to be essential for the mass observation.

Present retults of this work are not sufficient to explain the causes or meanings of all the groups and types of patterns and shapes of gamma globulin. However, following facts obtained from the results of this work, are thought to be more or less suggestive in solving some parts of the problems.

Types in the groups:

Types 1 and 2 of any group are often met in acute severe cases(+) of 1) pyogenic infections, 2) inflammations, 3) malignant tumors with ulceration, hemorrhage, necrosis or superimposed infection, 4) bleeding into serous cavities, ruptured ectopy, 5) after surgical procedures of malignant tumors, 6) hepatic necrosis, intoxication, 7) fresh myocardial infarction etc. Thus these 2 types seem to be signals for some acute destructive processes.

Type 3 is very often seen in cases of subacute inflammations of various organs, tissues, infections, malignant tumors, over-charged professional donors etc. This type coincides very well with 7. constellation of Wuhrmann[6] which is typical for malignant tumors in metastasis.

Type 4 and 5 of group A_1G_1 are usually met at normal states or slight diseases. Therefore the two types are supposed to be standard. But when type 4 and 5 accompany with any group except A_1G_1, they mean widely chronic debilitating diseases, tuberculosis, liver cirrhosis, hypertension, heart failures, malignant tumors, all sorts of anemias, starvation, certain infections, toxemia etc.

Type 6 is often met in cases of allergic states, parasitic infestation, chronic inflammations, neoplastic diseases, cancers, Hodgkin's diseases etc.

Type 7 is very rarely observed in a few cases of infectious hepatitis, liver cirrhosis.

Type 8 is often seen in untreated or aggravated cases of nephrotic syndroms.

Type 9 and 10 are seen in cases of apoplexia, spontaneous gangrene, heart failures, hypertension, nephritis etc., probably having relation with some of circulatory disturbances.

Type 11 and 12 are met in cases of chronic inflammation, malignant tumors, eclampsia, hypertension, heart neurosis, alkalosis etc.

Shapes of gamma globulin:

Shape 1, meaning a poor production of antibodies, is found in patterns of gastric ulcer, hemorrhage, certain infections, leukemia etc.

Shape 2 and 3 are seen in cases of normal states and slight diseases.

Shape 4 has often appeared in course of chronic inflammations, and in convalescence of acute infections.

Shape 5 and 6 represent hypergammaglobulinemic states in serious diseases with circulatory disturbances.

Shape 7 is seen especially in severe damages of lymphatic system, liver cirrhosis, Hodgkin's disease etc.

Shape 8, 9 and 10 are chiefly found in end course of malignant tumors, heart failures, nephrosis etc.

In general, albumin fractions whose lower boundaries are situated at A_2 or A_3 have shown considerable decreases in quantitiy. In other words, most chronic debilitating diseases and some acute affections, which cause obstructive jaundice, have shown albumin fractions at A_2 or A_3. In many of these cases, the migration distances of albumin fractions are fouud to be proportional with the grades or severities of their proces ses

The lower boundaries of gamma globulin fractions in advanced cases of liver cirrhosis, heart failure, nephritis, infections, malignant tumor, general edema etc. are often seen at G_2 or farther at G_3, owing to the proportional hypergammaglobulinemia.

From the view points of this work, it might be of value to test an Ep pattern in 2 ways, once qualitatively as in this work and then quantitatively as hitherto, in an examination of serum proteins.

Summary

1. Mass observations were made to find some

(+) : The diagnosis of the concerned cases which are cited in this work, were originally made by the specialists in SNU Hospital through procedures needed.

significances of varieties of fraction positions of human serum proteins appeared in agar gel electrophoretic patterns.

2. According to the differences of migration distances of serum protein fractions and forms of gamma globulin, some groups, types and shapes are established in the patterns obtained.

3. Discussions are added to the fraction positions, the types and the shapes which seemed to be referable in clinical diagnosis.

國 文 抄 錄

寒天膠質電氣泳動像에 出現한 血淸蛋白成分의 泳動距離

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1) 寒天膠質 EP 型에 出現한 人血淸蛋白成分들의 位置의 多樣性의 意義를 探求하기 爲하여 集團觀察이 行하여졌다.

2) 血淸蛋白成分들의 泳動距離의, 그리고 Gamma globulin의 形像들의 差異로 因하여 若干群, 型 及 形들이 檢査된 EP 型에 設定되었다.

3) 臨床診斷에 參考될만하다고 생각되는 血淸蛋白成分들의 位置, 型 及 形들에 若干 考察이 加하여졌다.

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