

## GROUP-SPECIFIC APPEARANCE OF PLASMA CLOTS

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**T**HIS ACCOUNT deals with the detection of a new factor associated with the clotting mechanism of plasma. Our investigations were prompted by certain observations made during routine laboratory work, when large quantities of blood grouping test sera were prepared by recalcifying plasma according to the method of Clegg and Dible,<sup>4</sup> in which plasma of the same group from several bottles is pooled, recalcified by the addition of calcium salt and filtered after the fibrin clot has formed. It was observed that there were differences in the appearance of these fibrin clots, that these differences were related to the blood group of the original plasma and were constant for a particular blood group. On the basis of this observation, investigations were carried out in an endeavor to elucidate the correlation.

### TECHNIC OF PREPARATION OF PLASMA CLOTS

The following method has been found to give optimal results: Plasma is siphoned off as soon as the blood cells have settled, this usually being on the second day after the withdrawal (300 ml. blood into 25 ml. of 5 per cent sodium citrate solution). Ten ml. of plasma are pipetted into a penicillin bottle, 2 ml. of a 2 per cent solution of calcium lactate are added, and the bottle is gently shaken. The mixture is incubated at 37 C. for five hours and kept in the refrigerator overnight at 6 C. The fibrin clot formed is inspected for consistency, and its size determined by subtracting from 12 ml. the quantity of free fluid remaining.

The number of plasma samples tested and the number of tests performed are seen in table 1. Plasma samples were taken from healthy adults and showed normal values for platelets, total protein, albumin and globulin.

### OBSERVATION OF CHARACTERISTICS OF THE PLASMA CLOT

We found size and consistency of the clots to be different in plasma samples belonging to different blood groups. This group-specific appearance of the clot was more distinct when plasma was reconstituted on the second to fifth day after the blood was taken, disappearing in plasma allowed to stand for about two weeks. At this time the clot was no longer specific in appearance, being larger and translucent, and filling the whole bottle so that no fluid could be expressed.

Group O plasma produced a clot which was smaller and firmer than the clots produced by plasma of the other groups. The clot of group B plasma was the largest and most translucent. Plasma of the groups A and AB gave clots intermediate in size (fg. 1).

Similar findings were obtained on repeating the experiments with the A subgroups, as shown on figure 2.

### EXPERIMENTAL FINDINGS

Some of the factors known to influence clotting were studied in turn, in an endeavor to elucidate the mechanism concerned in the production of the group-specific clot.

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*Thromboplastin*, prepared from rabbit brain<sup>15</sup> or placenta<sup>22</sup> or as "Difco"-thromboplastin, was added to plasma<sup>16</sup> which was subsequently recalcified (5 ml. thromboplastin extract to 5 ml. plasma; 1 ml. calcium lactate 2 per cent). No differences could be observed between the clots so obtained and control clots formed without the addition of thromboplastin.<sup>11</sup>

*Prothrombin-Thrombin*. To test this factor, we made use of the observation that citrated plasma clots on the addition of serum.<sup>6, 10</sup> Five ml. of citrated plasma which had lost the property of producing a group-specific clot were mixed with 5 ml. of fresh serum from the same blood group, previously confirmed in its clotting activity by the method of Smith et al.<sup>17, 21</sup> One ml. of 2 per cent calcium lactate was added and the test performed as usual. The addition of the fresh serum did not, however, restore the specific clot-forming property.

TABLE 1.—Testing of Plasma Samples

	Samples	Tests
Group O	106	324
A	108	310
AB	79	145
B	83	176
Groups, total	376	955
Subgroup A <sub>1</sub>	52	One test performed on each sample on the 3-7 day after bleeding
A <sub>2</sub>	38	
A <sub>1</sub> B	26	
A <sub>2</sub> B	24	
Subgroups, total	140	

*Fibrinogen*. Fraction I prepared from plasma of one group according to the fractionation method given by Cohn<sup>5</sup> was added to fresh and old plasma samples respectively (5 ml. of Fraction I and 5 ml. of plasma of the respective group), and subsequently recalcified. No group-specific influence could be found on clot formation.

*Isoagglutinins*. After exclusion of these substances as possible factors responsible for the group specificity of the clots, interest was directed to the isoagglutinins. The following observations were made:

(a) Agglutinin-free plasma samples were prepared by absorption according to standard technic,<sup>19</sup> using thrice-washed red cells, and incubating at 37 C. for thirty minutes. Group A plasma was absorbed with group B cells, group B plasma with group A cells, and group O plasma with AB or groups A and B cells. On recalcification the fibrin clots were not group-specific.

(b) Plasma treated exactly as above, but with group O cells and thus retaining its isoagglutinins, again produced a nonspecific clot on recalcification.

(c) Plasma of group AB, and therefore free of group specific agglutinins, was

treated as above with cells of any blood group. In each case the plasma was shown to have lost its specific clot-forming property.

From these experiments it seems that the group-specific factor is not connected with the isoagglutinins, but is removed or rendered inactive by admixture with

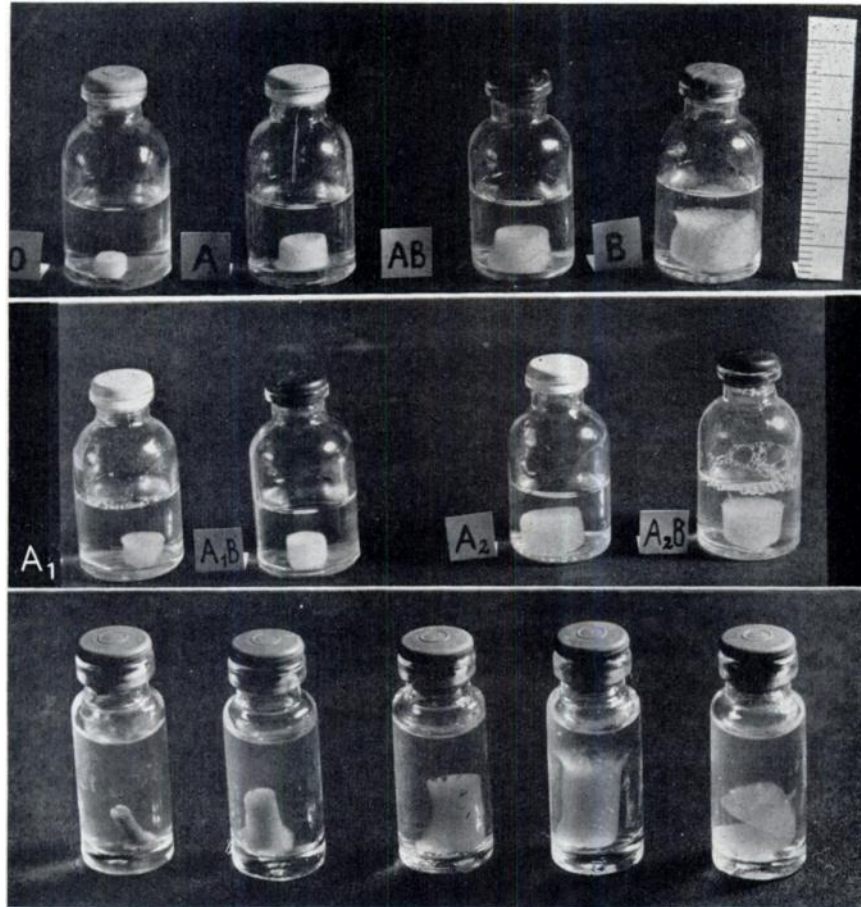


Fig. 1.—(top) Fibrin clots O, A, AB, B, left to right.

Fig. 2.—(center) Fibrin clots A<sub>1</sub>, A<sub>1</sub>B, A<sub>2</sub>, A<sub>2</sub>B, left to right.

Fig. 3.—(bottom) Dilution of plasma with saline. Left to right: undiluted, 1:3, 1:5, 1:7, 1:9. (Micro-method.)

red cells. Thromboplastin, prothrombin-thrombin and fibrinogen alike, seem to play no part in the phenomenon.

Some physical properties of the group-specific factor were investigated next.

Freezing, cooling without freezing to zero degrees C., and heating above body temperature destroyed the factor. (The factor could not be examined in samples heated over 56 C. for ten minutes, as no clot was formed after inactivation.) Plasma samples subjected to shaking in the Kahn shaking apparatus for twenty minutes,

and later examined in the usual way, no longer produced clots showing a group-specific appearance. Centrifugation (even at lower speeds) gave the same result; no difference could be observed in tests performed with plasma of the various layers. Plasma samples which were hemolytic initially showed a group-specific clot only up to three days after blood taking.

Diluting the plasma with saline leads to dilution of all the factors of the blood clotting mechanism, and so smaller clots of the same consistency were expected, but we can see in figure 3 that the loss of clot-forming substances is overcompensated by the increasing loss of contraction and in spite of the loss of clot-forming material the size of the clot rises on dilution. Figure 3 also shows that the group-specific appearance of the clot decreases on progressive dilution.

The degree of dilution required to render the clot nonspecific in appearance varies with the age of the plasma, it being greatest with fresh plasma; the degree of dilution is approximately the same with plasma samples of all groups of the same age. This finding, applicable both to dilution with saline and with serum, indicates that a special factor for each group must be postulated rather than an identical factor present in different amounts in the various groups. In each case the minimal effective concentration of this unknown factor was found to be about one third of its concentration in plasma three to seven days old.

#### *Summary of Experimental Observations*

1. The clot formed on recalcification of citrated plasma shows differences in size and consistency which are group-specific.
2. These properties are exhibited best by plasma samples tested on the third to seventh day after bleeding.
3. The specificity of the appearance is not only confined to the main groups A, B, AB and O, but is shown also by the subgroups A<sub>1</sub>, A<sub>2</sub>, and A<sub>1</sub>B, A<sub>2</sub>B.
4. Experiments failed to correlate this factor with thromboplastin, prothrombin-thrombin, fibrinogen, or the isoagglutinins.
5. The group-specific factor has the physical property of thermostability in the range of zero to 42 degrees C., is absorbed by washed red blood corpuscles, is labile to physical reagents and is inactivated by hemolysis and by dilution.

#### DISCUSSION

Differences in the appearance of fibrin clots have been noted by a number of investigators in the past. Ferry and Morrison<sup>8</sup> distinguished between a transparent gelatinous clot which does not readily synerize, and an opaque doughy clot which does synerize; intermediate types were also recognized. However, neither these investigators nor others<sup>1, 2, 13</sup> appear to have noted any relationship between clot appearance and blood groups.

The failure to relate the specific agent to such factors concerned with coagulation as thromboplastin, prothrombin-thrombin, fibrinogen and the isoagglutinins suggests by exclusion, that it may be connected in some way with platelets. Here we may note that Wintrobe<sup>20</sup> points out that clots in spleen and marrow suspensions occur only in the regions where platelets and megakaryocytes collect, re-

spectively. Tocantins<sup>18</sup> states that the structure of fibrin clots is modified by platelets which enhance the tendency to syneresis. Hayem<sup>9</sup> in 1891 related poor clot retraction and absence of exudation of serum with decreased platelet count. Pagniez,<sup>14</sup> Le Sourd and Pagniez<sup>12</sup> and Bordet and Delange<sup>3</sup> observed that intact platelets were necessary for clot retraction. Furthermore it is well known that cases of thrombocytopenic purpura exhibit poor clot retraction. Our own observation that the specific appearance of the clot decreases with increasing age of the plasma is in accordance with this line of thought, as platelets are known to disintegrate on storage.<sup>7</sup> Calcium lactate, selected after trial of various salts of calcium, could hardly be responsible for group-specificity, though oxalated plasma did not give constant results.

The plasma used in the above investigations was derived from healthy adults. It is hoped to extend the field of study to children,\* to states of disease, and also to further physical and chemical properties of the clots. These will be the subject of further communications.

#### SUMMARY

Clots formed on recalcification of citrated plasma show differences in appearance which are specific for the original blood groups.

The differences in appearance are considered to be due to a group-specific factor, some of the properties of which are described.

The group-specific factor is tentatively related to the platelets.

Further lines of investigation are suggested.

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\* A "micromethod" requiring only 1 ml. of plasma and using colloidal stains has been worked out.

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