CHANGES IN THE HYDROGEN ION CONCENTRATION
OF THE BLOOD WITH COAGULATION.*

BY EDWIN F. HIRSCH

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Studies inquiring into the mechanism of blood clotting have been made by many careful investigators, and while much is written regarding the factors concerned, there is offered as yet no satisfactory and uniformly accepted explanation for the fact that blood usually clots when it escapes from a wound. Some of this confusion exists, perhaps, because the more recent investigations in colloidal chemistry were not available, and because some of the factors concerned with gel formation have not been understood. These last mentioned observations, especially, are significant since with them consideration has been taken of the importance of the H ion concentration of the gel medium, a factor whose relation to blood clotting has not been apparent and which to date seems to have received little attention. The work of Loeb (1) and others regarding the importance of the H ion concentration of the medium upon the behavior (ionization) of the proteins in solution and of the electrolytes as well, brings into studies of blood clotting this factor for consideration.

Much of the work on blood clotting concerns the isolation of certain substances and the evaluation of their importance in the clotting mechanism. According to Bordet (2) coagulation of the blood is the aggregation into meshes of fibrin of particles of fibrinogen which Fredericq, 46 years ago, found in the plasma dispersed as a colloid. This substance, whether so named or otherwise, is accepted quite generally as the coagulating part of the blood clot. Another important constituent of the fibrin clot is calcium. Many years ago, Brücke (3) demonstrated that fibrin left an ash containing calcium phosphate. Pekelharing and Hammarsten (4)

*Aided by the Winfield Peck Memorial Fund.

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found that these salts are not necessary for the transformation of fibrinogen into fibrin by the thrombin, but that they are indispensable to the formation of the latter from a precursor in the blood. Bordet and Gengou (5) long ago showed that contact accomplishes in result the effect of calcium ions; i.e., that contact with a foreign solid body brings about the appearance of thrombin, but is not requisite for the coagulating influence of the latter. When blood is received into a paraffined vessel no thrombin is formed; when received into an unprotected vessel thrombin is produced at the zone of contact, a fact which explains why coagulation begins along the wall. Blood platelets are believed to furnish a lipoid substance (cytozyme) which, mixed with a substance in the serum (serozyme), gives rise in the presence of calcium ions to thrombin, the substance that gels the fibrinogen. According to Howell (6), the circulating blood contains an antithrombin which, in shed blood, is neutralized before coagulation takes place.

This brief statement regarding the theories of blood clotting makes apparent that little consideration has been given to changes in H ion concentration which follow when blood is removed from its normal position in the blood vessels and comes into new relations as regards gas equilibria (carbon dioxide especially). In the adjustment to these new relations there may be changes in base (OH) and acid (H) distribution which are fundamentally important in blood clotting.

According to present ideas (7) regarding the formation of gels,—and the clotting of plasma belongs with these reactions,—the uniform precipitation throughout the liquid of a network which takes up the solvent strongly, is necessary. The resulting network of myriads of hydrous particles constitutes the gel structure. The amount of substance dispersed in the solvent and necessary to form a firm jelly is determined by the size of these particles and the extent to which they are able to occlude the solvent. Since the presence of finely divided particles with marked hygroscopic properties is necessary for the formation of a jelly, the precipitation of hydrous substances from their colloidal solution is most favorable for the formation of a gel. This occurs, however, only when the precipitation takes place at a suitable rate in a medium without appreciable solvent action, or where the substance is
being precipitated in a less soluble or insoluble form. The effect of salts on a gel formation is determined by the precipitating or stabilizing influences of their ions, in as far as these influence the amount and velocity of the precipitation. A slow precipitation is better if there be no increase in the size of the particles as the result of the solvent action by the electrolyte. Rapid coagulation by the addition of an electrolyte to a colloidal hydrous substance usually results in a gelatinous precipitate and not a jelly because there is no uniform mixing of the colloid with the coagulant. The slow, uniform precipitation necessary for gel formation is replaced here by a rapid, uneven coagulation, and the consequent contraction which distinguishes a gelatinous precipitate from a jelly. When gel formation is prevented by the presence of a stabilizing ion, the addition of an electrolyte having a suitable precipitating ion neutralizes the stabilizing ion and a gel forms. This result obtains also when gels are formed by dialysis. Here the stabilizing ion is removed slowly and uniformly to a concentration below that necessary to hold the colloid in solution.

Weiser and Bloxsom (7), in their studies of arsenate jellies prepared with aluminum, iron, and manganese salts, observed gel formation upon the neutralization of a stabilizing H ion by either suitable acids, alkalies, or salts added in definite concentrations. If too little of the electrolyte was added no jelly or an imperfect jelly formed, while, if too much was used, a gelatinous precipitate was obtained. Their results with manganese arsenate jellies are instructive. Potassium dihydrogen arsenate ionizes as follows:

$$KH_2AsO_4 \rightleftharpoons K^+ + H_2AsO_4^-$$

On account of the solubility of Mn(H_2AsO_4), no Mn ions are permanently removed from solution by the interaction of MnSO_4 with H_2AsO_4. However, the latter ionizes secondarily to a slight degree as follows:

$$H_2AsO_4^- \rightleftharpoons H^+ + HAsO_4^2^-$$

Insoluble MnHAsO_4 is formed, with sulfuric acid as a cleavage product. Since the precipitation of MnHAsO_4 is accompanied by

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"The H-ion concentration in the solution might even be increased by the addition of an acid with a multivalent anion that is strongly adsorbed provided the solvent action of the acid is not too great."
the formation of an equivalent amount of H ions in solution, an equilibrium is set up which prevents the complete precipitation of the manganese. Using the primary (KH₂AsO₄) salt with the manganese sulfate, complete mixing was obtained regularly before precipitation started, but with the secondary and tertiary salts a gelatinous precipitate or cloudy, heterogeneous jelly was produced instantly. This emphasizes again the need of slow neutralization of the stabilizing ion for gel formation in a medium. Kraemer (8) observed, in other arsenate jelly studies, optimum ratios between the interacting manganese sulfate and the potassium dihydrogen arsenate. The sulfuric acid formed in this reaction Kraemer regards as increasing and favoring the formation of the jelly.

Other gels are commonly prepared from fruit juices. Tarr (9), in determining the acidity factor in fruit juices, finds that there is a direct relation between jelly formation (gelling of pectin) and the actual acidity or hydrogen ion concentration. The minimum H ion concentration at which jelly forms is pH 3.46 for the purest obtained pectin, and the H ion controls the formation of the jelly. When the minimum H ion concentration has been reached, the jelly forms, and as the reaction becomes more acid (up to pH 3.1), the jelly becomes stiffer. At a H ion concentration greater than pH 3.1 contraction of the gel occurs.

Similar notions regarding the transformation of the fibrin from a hydrosol to a hydrogel state have been expressed in a number of recent reports. Thus Hekma (10) subscribes to the physical theory of blood coagulation and thinks the gelling process is due to the formation of acid compounds such as nucleoproteins, globulins, and phosphatides from the platelets and leucocytes as they separate from the plasma. These withdraw alkali and water from the plasma until the fibrin is changed from a sol to a gel. With the ultramicroscope this process appears as the formation of minute particles which grow into threads. Kugelmass (11), starting with a plasma-thrombin or a fibrinogen-thrombin system of pH less than 7.0, observed a diminution in the H ion concentration which resembles an adsorption process, being very rapid at first. The fibrin clot, he says, has a lower H ion concentration than that of the original mixture. The greater the original H ion concentration the larger is the differ-
ence between the initial and final pH. On the average, about 50 per cent of the H ions disappear during the coagulation. The optimum for coagulation is between pH 5.0 and 8.0. On both the acid and the alkaline sides of the optimum range, the greater the deviation the less perfect is the coagulum and the fibrin remains in suspended shreds. In another report on changes in the H ion concentration during coagulation of the blood, Kugelmass (12) says that when the clot forms it adsorbs free ions, and that the H ion concentration of the medium in which clotting occurs is always less than the original mixture.

The results by Kugelmass on changes in the H ion concentration of the blood during coagulation, while important, were not obtained with blood in the usual process of coagulation. In systems where the normal carbon dioxide content of the blood is disregarded, significant and rapid changes in reaction may be overlooked. A method for demonstrating H ion changes in rapidly clotting blood needs as essentials, speed and the detection of minute variations. The difficulty encountered in making such determinations by the gas chain method is chiefly the time interval needed for the system to come into equilibrium. During this period coagulation takes place and the platinized electrode becomes gummed. It seemed possible, then, to obtain the speed and accuracy by means of a colorimetric system carefully standardized and suitable for detecting minute changes in reaction. Recently, Brode (13) and Holmes (14) have described a method for determining the hydrogen ion concentration of a medium by the spectrophotometer. According to their work, especially that of Brode, changes within the pH range of a 2-color indicator, as for example one with green-yellow in the acid range and red in the alkaline, are accompanied by changes in the amount of each color measured at its characteristic wave-length, rather than in a sequence of colors in transition with each other over the entire pH range. Thus with a certain indicator, a particular and characteristic wave-length is used on the spectrophotometer, and with a definite thickness of the analyzing cell in the presence of a determined concentration of the dye, the amount of color transmission is measured. Standardized by buffer solutions carefully calibrated electrometrically, the amount of color expressed as percentage or in terms of extinction coefficient may be converted into an
equivalent pH value. A Keuffel and Esser\(^2\) color analyzer was used for making the colorimetric determinations (made in measurements of color percentage), and cresol red, having a pH range of 6.4 to 9.4, was chosen because the pH values of the blood are approximately in the middle portion of the indicator range. In choosing an indicator for a certain pH range this feature ought to be considered, for, in the middle portion of the range, the color percentages and the pH values are practically linear and parallel. The amount of indicator contained is also important in order that the readings in percentage (with the spectrophotometer used) come within the range of 0 to 100. If the amount of dye used is too small the pH curve will be distributed over a range more than 0 to 100 per cent, and if too much, the percentages of color will read too low. The amount of color is dependent upon the thickness of the fluid in the analyzer cell, generally speaking, according to Beer's law.

All of the results obtained in this study are from measurements made in 1 cm. length cells, the amount of cresol red being 0.02 mg. in 1.9 cc. volume. The standardization of these measurements was accomplished with buffer solutions whose pH values were ascertained electrometrically by the gas chain method with a Leeds and Northrup, Type K potentiometer. The results of such standardization measurements in terms of color percentage measured with the color analyzer at wave-length 572 m\(\mu\) are given in Table I. These results suggest that within the pH range 7.68 to 7.25 the pH variations and the color percentage variations are practically equal and uniform and that from these values the intermediate ones may be interpolated to make a scale. So constructed the values form Table II. In making Table II, as was mentioned, the middle portion of the indicator curve is almost linear, but at each end this relation does not hold, and where percentage values are obtained in these portions corrections are necessary. However, in the work reported here most of the values obtained are in the middle portion of the indicator curve.

The blood of rabbits was taken from the heart under oil through a paraffined needle into a paraffined syringe, transferred quickly under oil to a paraffined centrifuge tube, and centrifugalized. The

\(^2\) This instrument was purchased with a sum of money given by the Winfield Peck Memorial Fund.
uncotted plasma was removed and without delay mixed with the indicator in a closed color analyzer cell. The escape of carbon dioxide was prevented as much as possible by transferring the serum under oil in a paraffined pipette. The cell was then closed quickly, and readings were made at once. After clotting under

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Cresol red, 1 cm. length tube.

0.02 mg. in 1.9 cc. volume.
These results, obtained according to the methods given, demonstrate that blood clotting under the conditions mentioned is accompanied by a slight diminution in alkalinity. The contention may be made that plasma changes in reaction in a like manner simply upon standing. This, however, has not been observed in these experiments, for with some plasmas where clotting did not take place for 30 minutes the pH values obtained were the same at the beginning and at the end of that period, while readings of unclotted and clotted plasma made during like intervals of time had the pH differences mentioned. Serum obtained from clotted plasma and measured at intervals for 7 hours remained unchanged.

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<td>7.39</td>
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<td>7.41</td>
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<tr>
<td>7.56</td>
<td>7.46</td>
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Average...................................... 0.09

| 7.58*              | 7.34             | 0.24          |

*Dog serum.
†These plasmas clotted poorly.

**DISCUSSION.**

The results detailed here may have significance in correlating the clotting of blood with other gelling processes. In the formation of these other gels, acids in proper concentrations are able to cause the gelling process to take place. With some gels, of course, the cleavage of an acid radical from the hydrosol is part of the process. This possibility has been considered in the clotting of blood. Ruppel (15) has suggested that the euglobulins, to which class the fibrinogen belongs, are split from an acid constituent of the serum at their isoelectric point, and Kugelmaß (11) has de-
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termined the isoelectric point of fibrin to be at pH 7.2. The fact
remains, however, that acids in proper concentration do bring about
the gelling process and, in terms of gel formation, are regarded as
neutralizing the stabilizing ion of the hydrosol, thus permitting the
hydrogel to form. The experiments by Kugelmass show that H
ions are used up or bound in the gelling process. As regards the
source of this acidity, taking into account that it forms with great
rapidity, carbonic acid as a possible source deserves considera-
tion. Bordet, in his review, has emphasized the importance of
contact with rough surfaces or particles in blood clotting, and that
the clotting of plasma begins in those portions touching these
surfaces. Similar reactions are commonly observed in liquids
supersaturated with a crystalloid, when simply scratching the
side of the containing vessel or introducing a crystal or particle
sets into action the whole crystallization process. The separation
of fibrin, which is formed from its precursor, fibrinogen, probably
should be considered as the precipitation of a relatively insoluble
compound. When the blood is brought into conditions of atmos-
pheric tension, as regards carbon dioxide there is a supersat-
uration. Although bound in the blood largely as BHCO₃⁻ a
rearrangement takes place under the conditions of atmospheric
tension and CO₂ escapes. This reaction may be represented in
the following way.

\[ 2\text{BHCO}_3 \rightleftharpoons \text{B}_2\text{CO}_4 + \text{H}_2\text{CO}_3 \]

\[ \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}_2\text{O} + \text{CO}_2 \]

The instability of the carbonic acid, however, permits only a
transient effect, but since the reaction of serum exposed so as to
allow carbon dioxide to escape becomes considerably more alka-
line (0.5 pH or more), appreciable H₂CO₃ is liberated under the
altered conditions. This liberation occurs without base to hold
it neutralized during the process. In other words unbound, transi-
tently present acid of considerable strength (H ion) is formed.
Conditions of this sort, of course, are favorable for gel formation
in the presence of a dispersed hydrosol, as has been observed in
the experiments mentioned with other gelling processes.

³ The letter B indicates in the formulas a single valence of a base.
Changes in H Ion Concentration

The intravascular clotting of blood post mortem may follow along similar lines and may resemble the rigor appearing in muscles after death. It is known generally, and has been for some time, that in striated muscle tissue a postmortem rigor is accompanied by the appearance of an acid, by some regarded as lactic acid. Studies of rigor mortis in smooth muscle tissue (fibromyomas) by Hirsch (16) revealed that in those tissues not manifesting such a change in reaction no rigor appeared. Since acid substances appear in tissues after death, it is possible that these become important in causing the intravascular blood clotting post mortem.

Perhaps of some significance are those observations on delayed blood clotting in animals during peptone and anaphylactic shock, as well as after intravenous injections of foreign proteins or bacteria. With all of these a variation of the hydrogen ion concentration of the blood is reported. With peptone shock Menten (17) observed a marked increase in the acidity of the blood and blood plasma. Under conditions such as these the alkaline reserve is markedly diminished, and the acidosis, so called, is compensated, the concentration of free H ions being no greater. When such blood is exposed to air the amount of BHCO₃ is relatively small, as is also the amount of H₂CO₃ liberated under the diminished carbon dioxide tension. This difference in the amount of available H ions may be important in the slow clotting or the incoagulability of such bloods.

SUMMARY.

The clotting of blood plasma under conditions where the escape of carbon dioxide is largely prevented is accompanied by a sudden slight diminution of its alkalinity.

With rabbit plasma an average pH difference of 0.09 was determined between clotted and unclotted plasma.

Those plasmas with only slight changes in pH clotted poorly.

The possibility of sudden changes in carbon dioxide tension initiating the normal clotting of blood is considered.

Dr. E. R. LeCount suggested to me the possibility of there being pH changes with the clotting of blood. We have discussed the results obtained as the work progressed. His helpful suggestions are hereby acknowledged.
BIBLIOGRAPHY.

4. Pekelharing and Hammarsten, see Bordet (2).
5. Bordet, J., and Gengou, see Bordet (2).
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