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PROCESS FOR OBTAINING PRODUCTS DERIVED FROM BLOOD

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The invention relates to new methods for obtaining prothrombin from blood plasma. More particularly, the invention has to do with new, improved, and commercially valuable methods for the production of prothrombin.

It is known that blood and blood plasma contain prothrombin which is capable of being converted into thrombin, the latter being directly and quickly effective in the clotting of blood. It is also known that unpurified prothrombin is quite unstable. For this reason, large quantities of the prothrombin in the starting material have rapidly been lost in the previous purification methods, and often it has only been possible to obtain a limited purification by those means.

It is an object of the present invention to provide steps whereby blood or blood plasma or the like may be treated in a minimum number of operations and using conditions and reagents whereby little or no damaging effects are produced upon the desired prothrombin.

Another object is to provide a new anti-coagulant for blood, blood plasma and like materials which is not only highly effective in preventing coagulation but which is of particular value when preparing active blood coagulating principles such as prothrombin and thrombin.

A further object of the invention is the production of a highly concentrated prothrombin product which can be obtained in high yields.

It is customary when precipitating a prothrombin product from oxalated blood plasma by acidification to dilute the plasma with water for the purpose of lowering the electrolyte concentration. Prothrombin tends to remain in solution when electrolyte concentration is too high. On the other hand, sufficient oxalate ion must also be present to protect the prothrombin and prevent its activation by calcium. This addition of oxalate ion has heretofore been accomplished by adding a soluble oxalate alone, such as potassium oxalate, but such addition of a salt of oxalic acid raises the electrolyte concentration to such a degree that it has previously been impossible to recover more than a fraction of the original prothrombin upon dilution and acidification. One reason for this is, that prothrombin is appreciably soluble in water and, when adding a salt of oxalic acid as the only source of oxalate ion, it is necessary to thereafter dilute the oxalated plasma to such an extent before attempting to precipitate prothrombin that a large proportion of the prothrombin goes into solution and is not precipitated, even upon acidification.

We have found that oxalic acid, or similar acid, may be used in obtaining the proper concentration of oxalate or similar ion, but that oxalic acid alone is not sufficient, because of the damaging effect on prothrombin of the increased acidity brought about thereby, and the only satisfactory plasma is one in which the acid is used in combination with a soluble salt of such an acid, e. g. sodium or potassium oxalate when oxalic acid is the acid used. Thus, we have found that extraordinarily high yields of purified prothrombin can be obtained by precipitating, for example blood plasma, at certain more or less definite degrees of acidity in the presence of sufficient ions capable of depressing the calcium concentration below the point where it activates prothrombin and while avoiding a high total ion concentration.

We have further found that it is very important, when a calcium depressed plasma, such as oxalated plasma, is prepared in accordance with our invention, that about 15 to 25 volumes of blood, preferably 20 volumes, be run into 1 volume of an oxalate solution consisting of 18.5 g. of potassium oxalate monohydrate, (or corresponding amount of equivalent compound capable of removing calcium ions from solution without precipitating protein), and 5.0 g. of oxalic acid dihydrate per liter of distilled water.

In spite of the above mentioned addition of oxalic acid to the blood, the proportions of chemicals present are such that no hemolysis of the red blood cells occurs. Hence, the red blood cells or corpuscles can be completely removed by ordinary centrifugation.

After centrifuging, the resulting plasma can be treated by dilution and adjustment of the acidity within the range of pH 5.0 up to about pH 5.5 to produce a very satisfactory separation of an active prothrombin fraction from large amounts of inert proteins, while losing very little or practically none of the original prothrombin activity. This is quite remarkable, inasmuch as the previously known methods for obtaining a prothrombin product have resulted, especially in the first stages of the process, in the loss of most of, or very large proportions of, the original activity.

The invention can be illustrated by the following examples:

Example 1

A 0.5% solution of oxalic acid dihydrate is prepared and enough solid potassium oxalate monohydrate added to make its concentration 1.85% of the latter substance. 50 cc. of this new anticoagulant is mixed with a liter of blood taken from a stab wound of the cow. The mixture is centrifuged and the volume of plasma measured.

Its prothrombin content is approximately 200 units per cc. The plasma is diluted with 15 volumes of cold distilled water, or water low in electrolytes, and the pH brought to 5.1 with 1% acetic acid or 0.01 N mineral acid. As a result of having used the new anticoagulant mixture, this precipitate which forms upon acidification to pH 5.1 contains all of the prothrombin of the plasma. It can be separated from the bulk of the plasma proteins, which remain in solution by any of the ordinary means such as centrifugation.

Example 2

1 liter of beef blood is drawn into 50 to 60 cc. of a solution containing 1.704% of sodium oxalate ($Na_2C_2O_4$) and 0.5% of oxalic acid ($H_2C_2O_4.2H_2O$). The mixture is centrifuged and the plasma thereby obtained is diluted with 15 times its volume of water and acidified to a pH of 5.1 with dilute acid, such as acetic acid. The prothrombin precipitate obtained upon acidification to pH 5.1 is separated and tested for prothrombin activity. The test shows that substantially 100% of the prothrombin activity originally present in the plasma is present in the precipitate.

Whereas the first precipitation step or early stages of the best methods previously known for obtaining prothrombin from blood or plasma have resulted in very low over-all yields of prothrombin product of only moderate purity, the present invention makes possible a remarkable improvement in yields in the very first precipitation step.

The examples given above are intended to illustrate the invention without limiting it to the exact materials, proportions and conditions described therein. For example, instead of using a soluble oxalate combined with oxalic acid as the anticoagulant, one can use corresponding quantities and proportions of an equivalent compound, or compounds, capable of removing calcium ions from the plasma, either by their causing precipitation of insoluble calcium compounds or suppressing ionization sufficient to prevent activation of prothrombin, or by their production of both of these effects.

A feature of all of these anticoagulants is that free acid is always used either alone or with a salt, but always in proportions such that the amount of free acid relative to the salt is as great as possible without reaching an acidity that causes substantial damage to the prothrombin, while at the same time bringing the total ion concentration as low as possible to avoid high dilutions during the subsequent prothrombin precipitation step. One can use any suitable organic or inorganic acid capable of removing calcium ion from its part in the blood coagulation mechanism or reducing its concentration to practical ineffectiveness. Such acids may be used in combination with their salts or with salts of similarly functioning acids. By using a polybasic acid, it is possible to use an acid salt alone, or at least decrease the amount of free acid it is necessary to add, since a certain amount of free acid is essentially present when employing such an acid salt.

What we claim as our invention is:

1. In a process for obtaining prothrombin, the step which comprises adding one volume of an aqueous solution containing about 0.5% oxalic acid and about 1.8% of a soluble salt of oxalic acid to about 15 to 25 volumes of blood.

2. Process for obtaining prothrombin which comprises adding one volume of an aqueous solution containing about 0.5% oxalic acid and about 1.8% of a soluble salt of oxalic acid to about 15 to 25 volumes of blood, separating the plasma from the so treated blood, strongly diluting the plasma with water of a very low ion concentration and adjusting the acidity of the diluted plasma to an acidity between about pH 5.0 and pH 5.5 thereby obtaining a precipitate of prothrombin and separating the latter.

3. A product consisting of one volume of a solution containing about 0.5% oxalic acid and about 1.8% of a soluble salt of oxalic acid and about 15 to 25 volumes of blood.

4. A product consisting of one volume of a solution containing about 0.5% oxalic acid and about 1.8% of a soluble salt of oxalic acid and the plasma fraction of about 15 to 25 volumes of blood.

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