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# UNITED STATES PATENT OFFICE

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## PRODUCTION OF PLASMA

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5 Claims. (Cl. 167—78)

This invention relates to improvements in the methods of bleeding immune animals and is more particularly directed to the production of increased yields of plasma.

In the production of antitoxins and the like, the customary procedure entails the bleeding of immunized animals such as horses, pigs, rabbits, etc. For bleeding as serum, the blood is allowed to flow into a suitable vessel where it is permitted to coagulate and clot, subsequently separating a liquid fraction which is removed from the clotted material as serum. The yield of serum is usually 40% to 50% by volume of the original blood.

To obtain plasma, the blood is led into a receptacle containing a suitable anticoagulant which maintains the fibrin in solution. The blood does not coagulate but the blood cells settle to the bottom of the vessel and transparent plasma collects in the layer over the blood cells. The plasma layer may be siphoned off and treated as desired. As anticoagulants, one customarily uses potassium oxalate, sodium citrate and the like. In an efficient processing the yield of plasma represents about 60% to 70% of the volume of the original blood, and in some cases, in the bleeding of hogs, plasma bean extract has been used to pack the blood cells more firmly and the yield of plasma may increase to as much as 80%.

It is one of the objects of my invention to facilitate the treatment of the blood in order to increase the yields of plasma.

It is a still further object to facilitate the production of plasma without destroying any of the immune property thereof.

These and other objects are primarily obtained by the use of sodium pyrophosphate as an anticoagulant for the production of plasma. Bleeding into a vessel containing sodium pyrophosphate as an anticoagulant and washing the blood cells increases the yield of plasma from 20% to 30% over the yield obtained with the usual bleeding as oxalated or citrated plasma, or an increase of 40% to 50% in yield over the usual methods for bleeding as serum. The product contains the same degree of immune property (number of protecting units per gram of nitrogen) as the prior methods.

In bleeding to obtain plasma, I generally prepare an aqueous solution containing about 1% of sodium pyrophosphate and about 1.5% of sodium chloride. This solution is adjusted to a pH of 7.0 to 7.2 with phosphoric acid, mono- or disodium phosphate. To a suitable quantity of this solution, an equal volume of blood is added. This mixture is kept in the cold room for twenty-four hours after which the supernatant plasma is siphoned off from the settled blood cells. To obtain additional plasma, the residue of blood cells is centrifuged or passed through a cream separator. Centrifuging gives a good product without any hemolysis. The use of a cream separator is somewhat faster than the centrifuge method but with the former, the liquid obtained is sometimes dark colored, indicating hemolysis, breaking down of some of the blood cells. The blood cells may also be washed by a suitable washing method such as will be described.

In order to avoid dilution, I may utilize much less of the anticoagulant solution so that only $\frac{1}{10}$ as much solution is used as compared with the added blood. More specifically, I may add 8 liters of blood to 800 cc. of a 5% sodium pyrophosphate solution containing 1.5% sodium chloride, the pH of the solution having been adjusted to 8.2 prior to the addition of the blood. After twenty-four hours in the cold room, plasma is siphoned off and to the remaining blood cells, there may be added an equal volume of washing solution containing 0.5% sodium pyrophosphate and 1.5% sodium chloride, the solution having

been adjusted to a pH 7 prior to use. The washing liquid may be separated from the blood cells by centrifuging or by use of a cream separator as described above.

The washing solution is greatly improved if there is added to the solution a small amount of a gum. A typical washing solution contains 0.5% sodium pyrophosphate, 0.85% sodium chloride and 0.5% gum tragacanth. The solution is adjusted to pH 7–7.5 prior to use. The presence of the gum facilitates sedimentation of the blood cells and assists in separation of the liquid plasma from the cells.

In place of the gum tragacanth, I may use 0.5% to 1.0% gum acacia, gum arabic and the like I have found that 0.2% or even less of tragacanth is effective. The use of gum tragacanth broadly in the treatment of blood for obtaining plasma is claimed in my copending application Serial No. 300,594, filed October 21, 1939.

COMPARISON OF BLEEDING HORSES IN OXALATE AND PYROPHOSPHATE

*Diphtheria horses*

| Horse number | Diluent | Volume of blood taken | Units of antitoxin | Increase in yield by bleeding in pyrophosphate |
|---|---|---|---|---|
| | | *Liters* | | *Percent* |
| 7,855 | Pot. oxalate | 8 | 3,922,500 | 35 |
| 7,855 | Na pyrophosphate | 8 | 5,325,000 | |
| 7,865 | Pot. oxalate | 8 | 3,445,000 | 33 |
| 7,865 | Na pyrophosphate | 8 | 4,593,600 | |
| 7,901 | Pot. oxalate | 16 | 6,661,000 | 29 |
| 7,901 | Na pyrophosphate | 16 | 8,612,000 | |
| 7,903 | Pot. oxalate | 4 | 1,029,000 | 22 |
| 7,903 | Na pyrophosphate | 4 | 1,257,325 | |
| 7,863 | Pot. oxalate | 16 | 6,373,000 | 17 |
| 7,863 | Na pyrophosphate | 16 | 7,488,000 | |
| Summary: | | | | |
| | Potassium oxalate | | 21,430,500 | 27 |
| | Sodium pyrophosphate | | 27,275,325 | |

*Staphylococcus horses*

| Horse number | Diluent | Volume of blood taken | Units of antitoxin | Increase in yield by bleeding |
|---|---|---|---|---|
| | | *Liters* | | *Percent* |
| 7234, 7601, 7608, 7748 | Potassium oxalate | 16 | 1,009,500 | 25 |
| 7234, 7601, 7608, 7748 | Sodium pyrophosphate | 16 | 1,265,970 | |
| 5715, 6737, 7025, 7233, 7604, 7607, 7724, 7759. | Potassium oxalate | 32 | 2,208,000 | 29 |
| 5715, 6737, 7025, 7233, 7604, 7607, 7724, 7759. | Sodium pyrophosphate | 32 | 2,864,250 | |
| Summary: | | | | |
| | Potassium oxalate | | 3,217,500 | 28 |
| | Sodium pyrophosphate | | 4,130,220 | |

The use of sodium pyrophosphate as the anticoagulant for the blood has the further advantage that it is possible to wash the blood cells after the supernatant plasma has been separated from the settled cells. When oxalate or citrate is used as an anticoagulant, the blood cells are very fragile and any washing treatment causes a breaking up of the cells with bad hemolysis. When sodium pyrophosphate is used, the blood cells, after the siphoning of the plasma, can be washed with isotonic or hypertonic salt solutions without noticeable hemolysis. The use of the pyrophosphate also serves to prevent oxidation and especially oxidation due to the presence of iron in the blood.

The following is typical of the results obtained with sodium pyrophosphate and indicates clearly the increase in yield obtained as compared with potassium oxalate as the anticoagulant.

The increase of yield of antitoxin by bleeding in sodium pyrophosphate was achieved by bleeding in equal volume of diluent (sodium pyrophosphate), or in cases in which solution of sodium pyrophosphate was used in the amount of 11% against the volume of drained blood, the blood cells after siphoning of plasma were washed with salt solution (0.85%) containing 0.5% sodium pyrophosphate. The washing liquid was used in an amount equal to the volume of settled blood cells. Cells suspension was passed through cream separator, or cells settled themselves by the addition to washing liquid of 0.5% gum of tragacanth

The following indicates the effectiveness of my method utilizing a dry anticoagulant wherein the dry sodium pyrophosphate or the like is placed in a suitable vessel and the blood is added. In calculating the drop of plasma potency as compared with serum potency, allowance for dilution of plasma by anticoagulant was made.

BLEEDING IN DRY AND LIQUID ANTICOAGULANT

| | Material | Anticoagulant | Volume of blood | Potency | Percent drop of potency compared to serum | Note |
|---|---|---|---|---|---|---|
| | | | *Cc.* | *Units* | *Percent* | |
| Tetanus, horse No. 7622. | Serum | | 50 | 875 | 100 | Mixture t |
| | Plasma | Dry 120 mg. Na pyrophosphate, 25 mg. prim. Na phosphate. | 50 | 900 | 0 | Do. |
| | ---do--- | 2.7% sol. 120 mg. Na pyrophosphate, 25 mg. prim. Na phosphate, 5 cc. 1% NaCl. | 45 | 725 | 8 | Do. |
| | ---do--- | Dry 50 mg. 1% potassium oxalate | 50 | 900 | 0 | Do. |
| | ---do--- | 1% sol. 50 mg. potassium oxalate, 5 cc. 1% NaCl. | 45 | 725 | 8 | Do. |
| | ---do--- | Dry 100 mg. Na citrate | 50 | 850 | 3 | Do. |
| | ---do--- | Sol. 100 mg. Na citrate, 5 cc. 1% NaCl | 45 | 725 | 8 | Do. |
| | Serum | | 50 | 1,100 | | Flocculation test. |
| Diphtheria, horse No. 8284. | Plasma | Dry 120 mg. Na pyrophosphate, 10 mg. prim. Na phosphate. | 50 | 1,045 | 6 | Do. |
| | ---do--- | Sol. 120 mg. Na pyrophosphate, 10 mg. prim. Na phosphate, 5 cc. 1% NaCl. | 45 | 875 | 12 | Do. |

BLEEDING IN DRY AND LIQUID ANTICOAGULANT—Con.

| | Material | Anticoagulant | Volume of blood | Potency | Percent drop of potency compared to serum | Note |
|---|---|---|---|---|---|---|
| | | | *Cc.* | *Units* | *Percent* | |
| Diphtheria, horse No. 8284. | Plasma | Dry 200 mg. prim. Na phosphate, 700 mg. second Na phosphate. | 50 | 970 | 12 | Flocculation test. |
| | do | Sol. 200 mg. prim. Na phosphate, 700 mg. second Na phosphate, 5 cc. 1% NaCl. | 45 | 870 | 13 | Do. |
| | do | Dry 50 mg. potassium oxalate | 50 | 1,050 | 5 | Do. |
| | do | Sol. 50 mg. potassium oxalate, 5 cc. 1% NaCl. | 45 | 885 | 11 | Do. |
| | do | Sol. 50 mg. germanin, 5 cc. 1%NaCl | 45 | 880 | 11 | Do. |

**The above-described samples were tested from horse #8284 for potency on guinea pigs, with the following results:**

| Material | Anticoagulant | Potency | | | Estimated units |
|---|---|---|---|---|---|
| Serum | | 1,000+ | 1,200 | 3.5 | 1,100 |
| Plasma | Dry Na pyrophosphate. | 1,000+ | 1,100 | 3.5 | 1,050 |
| Do | Sol. Na pyrophosphate. | 900+ | | 5.5 | 950 |
| Do | Dry mixture prim. and second Na phosphate. | 900+ | 1,000 | 3.5 | 950 |
| Do | Dry potassium oxalate. | 1,100+ | | 5.5 | 1,100 |
| Do | Sol. potassium oxalate. | 900+ | 1,000 | 3.5 | 950 |

| | Material | Anticoagulant | Volume blood | Potency test, flocculation pig | | Drop in potency in Lf. pig test | |
|---|---|---|---|---|---|---|---|
| | | | *Cc.* | | | *Percent* | *Percent* |
| | Serum | | 50 | 830 | 900 | | |
| Diphtheria, horse No. 8284. | Plasma | Dry mixture 120 Na pyrophosphate, 100 mg. prim. Na phos. | 50 | 775 | 850 | 7 | 6 |
| | do | Sol. 5 cc. Na pyrophosphate, 1% NaCl pH 7.2 | 45 | 625 | 700 | 17 | 14 |
| | do | Sol. 5 cc. 1% potassium oxalate and 1% NaCl | 45 | 655 | 725 | 13 | 11 |

| | Material | Anticoagulant | Volume of blood | Potency | Drop in potency of plasma |
|---|---|---|---|---|---|
| | | | *Cc.* | | *Percent* |
| | Serum | | 50 | 595 | |
| Diphtheria, horse No. 8284. | Plasma | Dry mixture 120 mg. Na pyrophosphate, 100 mg. prim. Na phos. | 50 | 545 | 9 |
| | do | Sol. 5 cc. 5% Na pyrophosphate, 1% NaCl prim., pH 7.2 | 45 | 440 | 18 |
| | do | Dry mixture 200 mg. prim. Na phos. and 700 mg. second Na phos. | 50 | 495 | 17 |
| | Serum | | 50 | 60 | |
| Diphtheria, horse No. 8358. | Plasma | Dry mixture 120 mg. Na pyrophosphate, 25 mg. prim. Na phos. | 50 | 55 | 9 |
| | do | Sol. 120 mg. Na pyrophosphate, 25 mg. prim. Na phos., 5 cc. 1% NaCl. | 45 | 35 | 35 |
| | do | Dry potassium oxalate 50 mg | 50 | 55 | 9 |
| | do | Sol. 50 mg. potassium oxalate, 5 cc. 1% NaCl | 45 | 35 | 35 |
| | do | Dry 100 mg. Na citrate | 50 | 55 | 9 |
| | do | Sol. 100 mg. Na citrate, 5 cc. 1% NaCl | 45 | 35 | 35 |
| | Serum | | 50 | 900 | |
| Diphtheria, horse No. 8200. | Plasma | Dry mixture 720 mg. Na pyrophosphate, 100 mg. prim. Na phosphate. | 50 | 830 | 8 |
| | do | Sol. 120 mg. Na pyrophosphate, 100 mg. prim. Na phos., 5 cc. 1% NaCl. | 45 | 755 | 7 |
| | Serum | | 50 | 445 | 0 |
| Diphtheria, horse No. 8084. | Plasma | Dry mixture 120 mg. Na pyrophosphate, 25 mg. prim. Na phosphate. | 50 | 445 | 0 |
| | do | Sol. 120 mg. Na pyrophosphate, 25 mg. prim. Na phos., 5 cc. 1% NaCl. | 45 | 380 | 6 |

| | Material | Anticoagulant | Volume of blood | Potency type II | Percent increase or decrease of potency in plasma toward serum | Note C.S. test |
|---|---|---|---|---|---|---|
| | | | *Cc.* | *Units* | *Percent* | |
| | Serum | | 50 | 250 | | |
| Pneumo, horse No. 6776. | Plasma | Dry mixture 120 mg. Na pyrophosphate, 25 mg. prim. Na phos. | 50 | 300 | 20 | Increase. |
| | do | Sol. 120 mg. Na pyrophosphate, 25 mg. prim. Na phos., 5 cc. 1% NaCl. | 45 | 275 | 22 | Do. |
| | do | Dry second Na phos. 1050 mg | 50 | 275 | 10 | Do. |
| | do | Dry potassium oxalate 50 mg | 50 | 325 | 30 | Do. |
| | do | Sol. 50 mg. potassium oxalate, 5 cc. 1% NaCl | 45 | 300 | 33 | Do. |
| | do | Dry Na citrate 100 mg | 50 | 300 | 20 | Do. |
| | do | Sol. 100 mg. Na citrate 5 cc. 1% NaCl | 45 | 275 | 22 | Do. |

TYPE II PNEUMO HORSE

| Horse number | Units | | | | (Corrected for dilution) liquid pyro | |
|---|---|---|---|---|---|---|
| | Serum | | Dry pyro | | | |
| | Photo-elec. | C. S. | Photo-elec. | C. S. | Photo-elec. | C. S. |
| 8,004 | 95 | 100 | 95 | 100 | 98 | 87 |
| 8,005 | 70 | 70 | 65 | *45 | 68 | 71 |
| 8,006 | 50 | 55 | 58 | 45 | 44 | 49 |
| Average | 70.7 | 75 | 72.7 | 63.3 | 70 | 69 |

*One C. S. test showed 60.

BLEEDING STAPHYLOCOCCUS HORSES IN DRY AND WET SODIUM PYROPHOSPHATE

| Horse number | Anticoagulant | Volume of blood | Volume of plasma | Potency | Drop in potency in cases bled in solution of Na pyrophosphate |
|---|---|---|---|---|---|
| | | | | *Units* | *Percent* |
| 5,715 | 400 cc. sol. 1% NaCl 8% Na pyrophosphate prim. 8.8 | 4,000 | 2,600 | 160 | |
| 5,715 | Dry mixture 10 gm. Na pyrophosphate, 2 gm. prim. Na phosphate | 4,000 | 2,400 | 180 | |
| 7,122 | 400 cc. sol. Na pyrophosphate (see above) | 4,000 | 2,900 | 70 | 8 |
| 7,122 | Dry mixture Na pyrophosphate (see above) | 4,000 | 2,700 | 90 | |
| 7,224 | 400 cc. sol. Na pyrophosphate | 4,000 | 2,600 | 110 | |
| 7,224 | Dry mixture Na pyrophosphate | 4,000 | 2,500 | 120 | |
| 7,604 | 400 cc. sol. Na pyrophosphate | 4,000 | 2,600 | 130 | |
| 7,604 | Dry mixture Na pyrophosphate | 4,000 | 2,500 | 150 | |
| 7,608 | 400 cc. sol. Na pyrophosphate | 4,000 | 2,700 | 130 | |
| 7,608 | Dry mixture Na pyrophosphate | 4,000 | 2,800 | 150 | |

Horse #50K was bled out in dry sodium pyrophosphate.
Potency of serum, 90 units; potency of plasma, 100 units.

The preceding tables contain comparisons of potencies for sera and plasmas prepared with different anticoagulants. For close comparison, sera and plasmas in each experiment were taken from small portions of the same bleeding. As can be seen from the tables, the potency of the material is, in many cases, a little higher in plasma prepared from dry anticoagulant. Using anticoagulant solution, plasmas were obtained that, after allowing for the dilution, were of the same potency as serum or, in some cases, slightly lower. Even in such cases, when using the solution of sodium pyrophosphate, the total yield of potency represents a considerable increase, due to a more complete recovery of plasma achieved by the washing process. The use of sodium pyrophosphate as an anticoagulant increases the yield of plasma content from 20% to 30% over the customary bleeding using oxalates or citrates, and an increase of 40% to 50% over the bleeding as serum.

The pyrophosphate has the further advantage that the blood cells are in such condition as to permit washing thereof without substantial hemolysis. The pyrophosphate also serves to prevent oxidation of not only the plasma but also whole blood, serum and refined antitoxin. The pyrophosphate is sufficiently effective as an anticoagulant so that only 10% of the pyrophosphate solution may be used as compared with the volume of drained blood. Following the washing of blood cells with an equal volume of washing liquid, the total volume of pooled liquid (plasma + washings) is equal to or only slightly larger than the original volume of drained bood.

It wil be further noted that the use of dry sodium pyrophosphate produces higher potencies of the order of 5% to 10% greater than when the blood is drawn into solutions of anticoagulants.

The sodium pyrophosphate is preferably adjusted to pH 8.3 to 8.8 prior to the addition of the blood since the buffer action of the blood readily compensates for the alkalinity of the pyrophosphate solution since the pyrophosphate is alkaline. The pH adjustment may be made by the addition of concentrated phosphoric acid, monobasic (primary) sodium phosphate or dibasic (secondary) sodium phosphate, the monosodium phosphate usually being preferred when the pyrophosphate is used in the dry form. In this case the pyrophosphate is best used as powder and the monobasic sodium phosphate in very small crystals. Positive anticoagulating effect on blood is obtained by final concentration of phosphates as low as 0.3%. The solutions which are used are previously sterilized as in an autoclave at 20 pounds pressure for thirty minutes. The dry mixtures may be sterilized by dry heat at 185°–200° C.

It will be apparent that my invention is subject to some change or modification in the details set forth above without departing from the spirit and scope thereof.

I claim:

1. The process of producing plasma which comprises mixing blood with sodium pyrophosphate as an anticoagulant, allowing the mixture to stand until the blood cells settle out, separating the supernatant plasma from the blood cells and subjecting the blood cells to centrifugal action to remove additional plasma associated with the blood cells.

2. The process of producing plasma which comprises mixing blood with dry sodium pyrophosphate as an anticoagulant and separating plasma from the blood cells.

3. The process of producing plasma which comprises mixing blood, dry sodium pyrophosphate and dry sodium phosphate and separating plasma from the blood cells.

4. The process of producing plasma which comprises mixing blood with an aqueous solution at about pH 8 and containing sodium pyrophosphate and sodium chloride, allowing the mixture to stand until the blood cells settle and separating the supernatant plasma from the blood cells.

5. The process of producing plasma which comprises mixing blood with sodium pyrophosphate as an anticoagulant, allowing the mixture to stand until the blood cells settle out, separating the supernatant plasma from the blood cells and washing the blood cells with a solution containing sodium pyrophosphate and gum tragacanth.

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