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THE POLYVINYL ALCOHOLS AS BLOOD SUBSTITUTES*

By N. W. Roome, M.D., M.Sc., Capt. Lawrence Ruttle, R.C.A.M.C., Leverne Williams, Ph.D. and Ward Smith

A SATISFACTORY blood or plasma substitute, for use in the treatment of hæmorrhage and shock, must have certain physical properties resembling those of plasma, and must be innocuous to the recipient.

The essential physical property is a suitable colloid osmotic (oncotic) pressure. This must be provided by a colloid of such molecule size and structure that (1) the oncotic pressure is similar to that due to the plasma proteins, and (2) its rate of loss from the blood stream approximates the rate of regeneration of the recipient's plasma proteins. It would also appear desirable that the viscosity and hydrion concentration of the substitute be similar to those of plasma or blood, and that the contained colloid be metabolized or excreted rather than stored in the body. The harmful effects to be avoided include the production of fever or anaphylactic reactions on injection, vasodilator effects, and tissue or metabolic injury.

In addition to the above biological requirements, the component materials should be reasonably priced, and easily available; the mixture should be easily prepared with standard effectiveness; and there should be no deterioration on storage or on sterilization.

The following experiments were carried out regarding the suitability of a polyvinyl alcohol (PVA) as the required colloid. Rather small numbers of cases were studied in some series, as the experiments were intended to be exploratory rather than exhaustively conclusive. Further experiments have been carried out by other workers, following a report of our findings to the committee on shock, the National Research Council (Surgeon-Captain C. H. Best, Chairman) in 1942. Of these, Locke[1] has recently reported briefly on his experiments on tourniquet shock in rats, stating that the effectiveness of polyvinyl alcohol (type RH623) was "striking", and Haist[2] and associates have also found it effective in tourniquet shock in dogs.

Physical Properties and Oncotic Pressure

The polyvinyl alcohols are a series of water-soluble colloids produced commercially in a number of grades.[3] Each grade is a mixture of various sized macromolecules, the product of polymerization to various degrees, and the properties vary with the average molecule size. For example, the viscosity (in 4% aqueous solution at 20° C.), ranges from about 5 to 55 times that of water. They are whitish powders, with slight odour or taste, stable to heating indefinitely in air at 140° C. Solutions from 1 to 5% do not gel on standing at ordinary temperatures nor change viscosity after heating. The materials are quite inert chemically, but may be precipitated from solution by concentrated electrolytes, as are other lyophile colloids. They are best dissolved by mixing the powder thoroughly in a small quantity of chilled water, warming, and finally adding water to full volume; any salts to be added should be dissolved in the second portion of water. Solutions prepared in this way may contain small numbers of microscopic particles; hence for intravenous use, Seitz filtration is desirable. Sterilization may be by standard autoclaving.

The colloid osmotic (oncotic) pressure of PVA grade RH623 was studied by placing in collodion sacs attached to vertical glass tubes, suitable amounts of 5, 8, and 10% solutions in distilled water. The levels of the solutions in the glass tubes were adjusted to heights a few centimeters below the levels predicted from preliminary experiments, and the sacs were immersed in distilled water from the same source used to make the solutions. The readings of

* From the Departments of Surgery and Pharmacology, the University of Western Ontario, and the Department of Surgery, the University of Toronto.

the higher of two columns for each concentration, at equilibrium, are plotted graphically in Fig. 1, after correction for capillarity and conversion to mm./Hg. from the measured specific gravity of each solution, but without correction for dilution by the small influx of water which occurred in each case. The temperature was approximately 20° C. As shown in this graph, the concentrations of this PVA required to furnish osmotic pressures of 30 and 40 mm./Hg., the range of osmotic pressure of the plasma proteins, were 2.8 and 3.8% respectively. The average molecular (particle) weight, assuming that the curve may be extrapolated to lower concentrations as indicated, was estimated at 17,000 by the standard formula.[4]

ESTIMATION OF PVA IN BLOOD AND URINE

Because the polyvinyl alcohols are inert chemically, the following nephelometric method was devised by one of us (L.W.), for their estimation. A correction for the presence of proteins is involved, and the standards should lie near the expected value of the unknowns. An amount of plasma expected to contain an amount of PVA equivalent to 0.5 c.c. of 1% solution, is pipetted into a colorimeter tube and diluted to 2 c.c. with water. One-half c.c. of a standard 1% solution is added to another tube and a sufficient amount of plasma containing no PVA, but with a protein content very near that of the unknown, is added, and the mixture diluted to 2 c.c. A blank, containing 2 c.c. water, is also

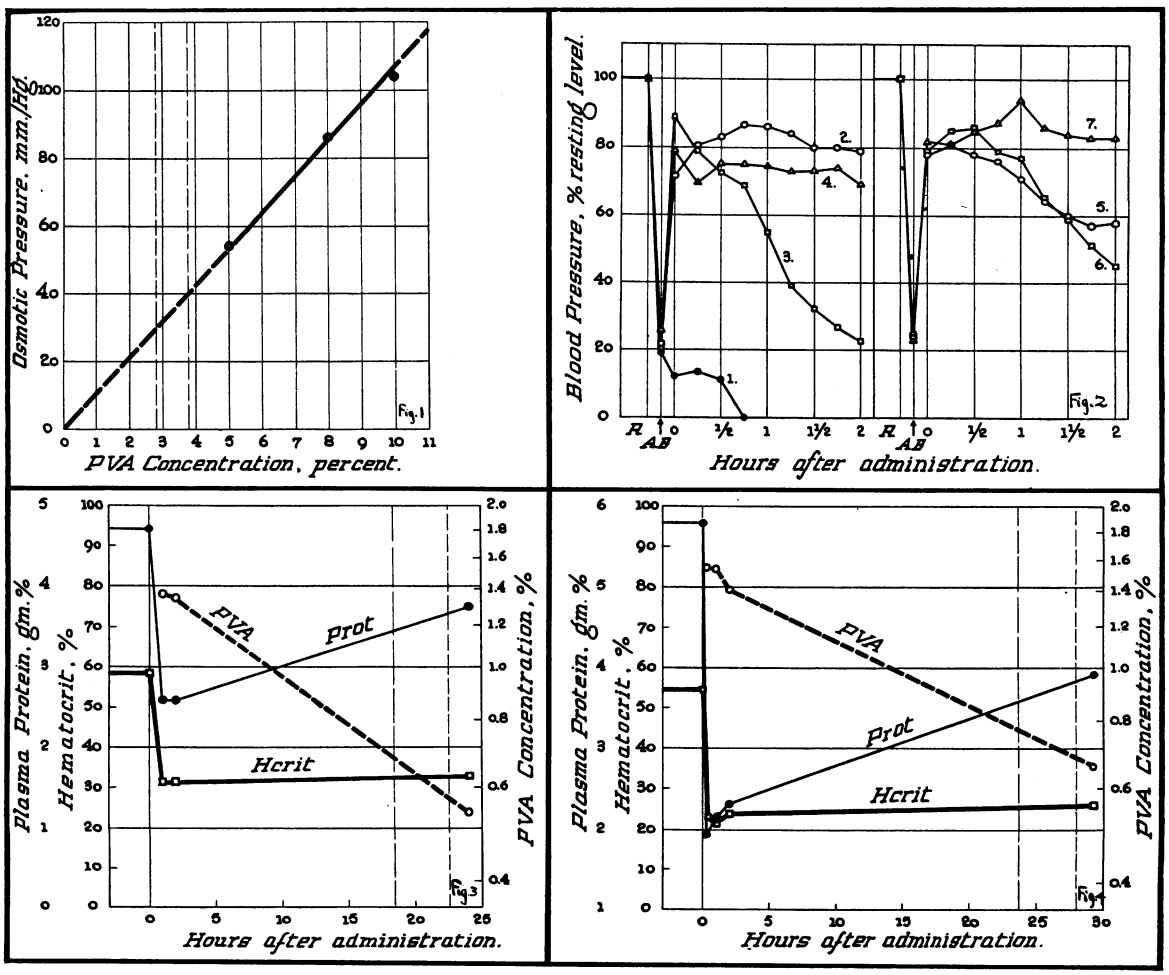

Fig. 1.—Oncotic pressure of PVA type RH623. The vertical broken lines indicate the concentrations for 30 and 40 mm./Hg. respectively, the oncotic pressure of the plasma proteins. **Fig. 2.**—Average blood pressure, as percentage of the resting level (R), after bleeding (AB), and at quarter-hour intervals after completion of treatment by various agents. The numbered curves are identified in the text. **Fig. 3.**—Graph of the hæmatocrit, plasma protein and PVA concentrations in Experiment 31. Type RH623. The two broken lines indicate the estimated times at which (1) the PVA concentration has fallen to half its original value, and (2) the plasma proteins have half-regenerated. **Fig. 4.**—Graph of the hæmatocrit, plasma protein and PVA concentrations in Experiment 26. Type RH393. The two broken lines indicate the times at which (1) the PVA concentration has fallen to half its original value, and (2) the plasma proteins have half-regenerated.

set up. To each tube, 2 c.c. 2% gum ghatti, and 6 c.c. 22.2% sodium sulphate solution are added. The readings were then made as usual on an Evelyn colorimeter, using filter 520. The PVA content in urine was estimated similarly.

A colorimetric method, based on the staining of PVA by dilute iodine, was also tried but was unsatisfactory and not applicable to all grades of PVA.

SUPPORT OF THE BLOOD PRESSURE AFTER HÆMORRHAGE

The following experiments were used to compare the supportive effect on the blood pressure of several grades of PVA with other materials, after severe hæmorrhage. They are similar to the experiments of Buttle, Kekwick and Schweitzer,[5] except that these workers used cats, and replaced 5/6th of the volume of blood removed; in our experiments on dogs, ½ of the blood removed was replaced by various agents including citrated dog blood, citrated plasma, gum acacia,[6] and three grades of PVA. Each agent was tested in from 2 to 5 experiments.

The animals were rendered quiet by giving morphine sulphate subcutaneously, usually in doses of ½ grain, although additional doses totalling up to 2 grains were required by a few large dogs. The femoral arteries and veins were isolated in each thigh after procain infiltration. The blood pressure was recorded by direct cannulation of a femoral artery. The resting level was ascertained, and the pressure observed during the bleeding and treatment, and at quarter-

TABLE I.
TREATMENT OF ACUTE HÆMORRHAGE BY VARIOUS AGENTS

Experiment No.	Weight (b.w.) kgm.	Bled		Treatment		Hæmatocrit			Plasma protein			Survived or died
		c.c.	% b.w.	c.c.	% b.w.	Resting %	A	B	Resting gm. %	A	B	
	(1) Control experiments											
8	6.3	295	4.67	0								S
9	5.8	288	4.98	0								S
10	13.5	690	5.13	0								D
11	6.3	325	5.12	0								D
19	6.7	263	3.95	132	1.98							D
	(2) Citrated blood transfusion											
12	8.6	458	5.3	230	2.66	50.3	..	89.0	4.92	..	95.0	S
16	10.8	450	4.2	252	2.34	...	...	..	...	...	...	S
17	17.6	935	5.3	505	2.87	48.8	81.0	..	5.67	87.0	..	S
	(3) Citrated plasma											
18	7.0	348	5.0	174	2.5	53.2	60.0	71.0	5.38	96.0	77.0	D
20	7.6	400	5.3	200	2.64	..	..	..	3.57	85.0	89.0	D
25	7.8	488	4.97	240	2.44	44.0	62.0	69.0	4.63	111.0	102.0	S
	(4) Gum Acacia in Ringer's Solution (6%)											
21	18.2	977	5.4	492	2.7	56.0	48.0	51.0	4.11	46.0	52.0	S
24	15.5	892	5.77	446	2.88	57.5	51.0	55.0	5.89	44.0	48.0	S
	(5) PVA type RH349 in Ringer's (2½, 2, 3, 2½, and 4% respectively).											
13	19.7	1128	5.7	550	2.79	48.7	50.5	59.5	4.31	65.0	65.0	S
14	21.2	1140	5.4	570	2.69	52.0	45.0	49.0	5.12	44.0	53.0	D
15	24.8	1318	5.3	655	2.64	47.5	49.0	48.0	5.66	44.0	48.0	S
22	24.4	1330	5.47	660	2.70	43.5	55.0	58.0	5.64	56.5	58.0	D
23x	10.0	553	5.5	278	2.77	64.5	40.0	..	5.89	35.0	36.0	D
	(6) PVA type RH393 in Ringer's (5, 3, 2, 4, and 6% respectively).											
26	12.9	643	5.0	322	2.5	54.3	42.0	44.0	5.73	34.0	40.0	S
27	10.3	533	5.17	266	2.58	49.3	53.0	64.0	5.32	42.0	53.0	S
28x	7.7	313	4.05	156	2.02	28.8	55.0	..	5.17	55.0	..	D
29x	8.85	467	5.3	234	2.65	46.0	42.0	..	4.53	34.5	..	D
30	6.15	308	5.0	154	2.5	47.5	42.0	46.0	4.41	34.0	44.0	S
	(7) PVA type RH623 in Ringer's (5, 6, 4, and 5% respectively).											
31	5.4	278	5.2	139	2.6	58.5	53.0	53.5	4.72	55.0	55.0	S
32	9.1	469	5.15	235	2.6	48.7	45.0	52.5	5.38	37.0	43.0	S
35	18.8	820	4.4	410	2.2	48.2	50.0	51.0	..	..	..	D
38	7.4	336	4.5	168	2.3	49.0	45.0	62.5	4.63	40.0	52.0	S

NOTE:—Values A for hæmatocrit and plasma protein were 5 to 30 minutes after conclusion of treatment, and values B were 105 to 120 minutes after treatment, and are expressed in % of the resting level. Experiment numbers followed by "x" indicate that the animal was being used a second time, after recovery from a previous experiment.

hour intervals for (usually) two hours after completion of the treatment. At this time the animal was replaced in its cage and observed at intervals for several days. Blood samples for hæmatocrit, total protein, and PVA content were drawn (1) in the resting state; (2) soon after treatment; (3) 1 to 2 hours after treatment; and (4) after one or more days in a few cases. The total protein content of the plasma was estimated nephelometrically, and the PVA content as described above.

Each dog was bled rapidly from an arterial cannula; 5 to 7 minutes were usually required to adjust the blood pressure to levels less than 30 mm./Hg. and without tendency to recover spontaneously. Treatment was begun 2 to 3 minutes after completion of the bleeding. Control experiments were carried out to determine the blood loss required to be invariably lethal without treatment. One animal which died during the beginning of treatment is included in the control series.

The observations made in these experiments are listed in Table I, and the graph Fig. 2. The blood pressures have been converted to percentages of the resting levels in this graph, in order to avoid the wide spread of resting levels encountered, and also because the percentage reduction of the blood pressure is a better criterion of the shocking effect of hæmorrhage than the numerical value.[7] The administration of PVA solutions resulted in no depression of the blood pressure in any case.

It will be noted that PVA RH623, in 4 to 6% solution (curve 7), maintained the blood pressure as well (on the average), as did whole citrated blood (curve 2). The other grades of PVA (curves 5 and 6) produced good immediate responses, but failed to maintain the blood pressure during the second hour. The acacia solution (curve 4) resulted in a well maintained, although somewhat lower, level of blood pressure. Plasma (curve 3) resulted in poor maintenance of the pressure, and early death of two animals; this unexpected effect may be explainable by the high citrate content of the plasma.

In Fig. 3 is given a graphic representation of the PVA content of the blood, the hæmatocrit, and the plasma protein level, in one animal in the above series, treated with PVA RH623 (experiment 31). It will be noted (1) that the hæmatocrit is nearly constant, indicating no great change in blood volume, from the time of treatment to the following day, and (2) that the rate of loss of the PVA is such that a 50% loss has occurred in about 18½ hours, while the plasma proteins have regenerated 50% at 22½ hours. Fig. 4 is a similar graph in an animal given PVA RH393 (experiment 26). It will be seen that again the hæmatocrit was maintained at a nearly constant level; the half-life of the PVA was 23¾ hours; while the proteins had half-regenerated at 28 hours. In these two graphs the PVA concentration is plotted on a logarithmic scale, inasmuch as its rate of loss is probably nearly percentile.

Experiments Regarding Possible Toxicity

Hueper, Landsberg, and Eskridge[8] have reported certain "toxic" manifestations in rabbits and dogs after the administration of a PVA solution. They reported that after a single injection of from 6 to 15 c.c. of 5% solution of PVA grade RH391 in 1% saline, in these animals, there were no untoward acute reactions,

Table II.

Average Deviations from Resting Level Observed in Acute Experiments

	Samples B	*Samples C*
*Red cell count.....A.....	-1.4%	+6.1%
*Hæmoglobin.....A.....	-6.4	+3.8
*Hæmatocrit.....A.....	-8.2	+8.6
*White cell count.....A.....	-3.0	+63.5
Platelet count.....	-11.0	-30.0
Sedimentation rate.....	+220.0	+84.0
Clotting time.....	+4.0	-4.0
Total protein.....A.....	-5.5	-5.3
Glucose.....A.....	+1.4	-47.0
Creatinin.....A.....	-4.3	+14.8
Albumin.....A.....	-1.5	-10.3
Cholesterol.....A.....	-15.0	-1.4
Chlorides.....	0	-0.8
Phosphorus.....A.....	-2.5	
Sodium.....	+1.3	+0.7
Potassium.....A.....	-1.9	-2.2
PVA content %.....	*0.42*	*0.24*
Average of items marked A.....	-4.4 %	
Average of 4 starred items.....	-4.75%	

but there was a reduction of the red cell count and the hæmoglobin content of the blood, occurring within three minutes of the injection and present for twenty-four hours or more.

Repeated injections, the same authors report, produced similar but more pronounced blood changes; the animals became less alert and lost "considerable" weight. Total amounts of type 391 of from 14 to 63.5 gm. were given over periods of from 5 to 23 days in eight dogs and resulted in the death of three. At autopsy, the spleen was found enlarged, and two had fluid in the peritoneal, pleural, and pericardial cavi-

ties, with generalized œdema. There was "marked alteration" of the plasma protein and non-protein nitrogen levels. The blood viscosity rose to levels about 9.4 and 10.7, and the plasma viscosity rose as high as 6.4. Coagulation of the blood was prolonged, and the sedimentation rate increased.

Other authors have found no evidence of toxicity in their experiments on similar materials. Stierlen[9] stated that there was no demonstrable toxicity from the injection of 6 c.c./kgm. of a 35% PVA solution, except for a reduction in the red cell count, which he attributed to dilution; and Jorns[10] suggested its intravenous use.

TABLE III.
EFFECTS OF REPEATED DOSES OF PVA 623.

Date	Weight kgm.	Treatment and appearance	Blood				Urine
			Hæmatocrit %	PVA content gm. %	Total protein gm. %	NPN mgm. %	PVA content gm. %
		Dog D.					
7/19	17.4	90 c.c. 10% solution	48.3	0	3.5	41.0	0
7/20		100 c.c.					
7/21	17.05	90 c.c.					
7/22	16.95	110 c.c. Appeared well	45.2	0.52	3.7	42.4	0.28
7/23		105 c.c.					
7/24	16.4	100 c.c.					
7/25	16.75	105 c.c. Appeared well	38.4	0.83	3.0	43.3	0.44
		3 minutes after injection	*33.1*	*1.39*	*2.7*		
		5 hours after injection	*32.5*	*1.01*	*2.9*		*0.77*
7/26		*23 hours after injection*	*36.6*	*0.88*	*2.9*		*1.28*
7/29	15.8	No treatment	43.0	0.78	3.6	45.2	0.26
8/11	17.2	Appeared well. Autopsied					
		Dog E.					
7/19	9.6	90 c.c. 10% solution	46.1	0	4.6	38.6	0
7/20		90 c.c.					
7/21	9.6	90 c.c. Appeared well					
7/22	10.0	110 c.c. Large hæmatoma in thigh, and moderate direct loss of blood during sampling	26.0	0.87	4.5	30.7	0.47
7/23		110 c.c. Large hæmatoma in opposite thigh.					
7/24	10.1	105 c.c.					
7/25	10.25	105 c.c. Hæmatomas smaller	16.3	1.67	4.1	35.8	0.52
7/28	10.25	No treatment. Appeared well. Autopsied.					

The following experiments resemble those of Hueper *et al.*, except that in the present series (1) larger doses of the PVA were usually given, and (2) PVA grade RH623 was used. It must be noted that this grade of PVA differs markedly from the grade 391 used by Hueper *et al.* Its molecule is much smaller, as indicated by its viscosity in solution (4% solutions, at 20° C., have viscosity 5 for RH623, 55 for RH391).

Dogs were used for both acute and chronic experiments. The acute group, experiments 33, 34, 36, and 37, were without general anæsthesia, morphine sulphate gr. ¾ being given to dogs 33 and 37, and local infiltration with procain made directly from blood flowing from a vein. Thirty c.c. of PVA solution was then given intravenously, and similar samples, with replacement of blood, were taken at 13 to 66 minutes (samples B), and again, without replacement, at 22 to 23 hours (samples C). The chemical estimations were carried out in an Evelyn colorimeter. The observations are listed as average deviations in per cent, from the resting levels, in Table II.

It will be noted in this table that there was an average depression, at the first sampling, of the ten items (marked A) of 4.4% from the resting levels. The expected depression of these items due to the dilution by the injected fluid, was 3.3%, calculated from the blood volumes of

the dogs as 1/13 of their body weight, and without consideration of possible osmotic effects of the 8% solution. The net depression after this correction was thus 1.1%, which is within the limits of error of the estimations in this small series, or which may be due to the influx of fluid into the blood stream resulting from the administration of a hypertonic solution. The average depressions of the four items (starred in Table II) which were found by Hueper *et al.*, depressed 21 and 24% in their two series, were 4.75% in the present series. This figure also is insignificant in view of the considerations outlined immediately above. Therefore it may be concluded that there is no demonstrable acute effect after injection of PVA 623 solutions, such as was described for PVA 391 by Hueper *et al.*

The findings at the second sampling were distorted by the occurrence of a pyæmia in one dog; hence no discussion of the changes at this time is warranted, although the findings are given in Table II.

Two additional dogs were given repeated doses of PVA type RH623. The procedure and findings are given in Table III. One of these, dog E, suffered large losses of blood into hæmatomata of the thighs, due to needling with a large calibre needle, and also lost an unknown amount of blood externally on one occasion; this must be considered in relation to the findings in this animal.

Both dogs appeared healthy throughout the course of injections; one gained a little weight, the other lost weight, but other dogs in the laboratory were also observed to lose weight during the same period of hot weather. The only abnormality observed was that the animals shook their heads and licked their lips, during or just after the injection. The blood non-protein nitrogen remained essentially constant. In dog D the hæmatocrit and total protein fell slightly during the injections, but recovered considerably in the 4 days after completing the injections. Dog E showed a slight protein fall, and a larger hæmatocrit fall, explainable on the basis of the hæmorrhage aforementioned.

Thus, no serious changes, such as described by Hueper *et al.*, were observed. This difference appears to us entirely explainable by the physical differences between the two materials. Type RH623 is gradually excreted in the urine (see Table IV) and no large amount accumulates in the circulation. Because there was no general depression accompanying the small blood changes

TABLE IV.
PATHOLOGICAL FINDINGS

Dog No.	Weight kgms.	Material given		Weight, gm.			Other organs examined	Abnormalities found. Remarks
		Type	Dose gm.	Liver	Kidneys	Spleen		
1	27.5	PVA: 349	50.5	720	108	65	Suprarenals, heart, aorta, lung, pancreas.	No abnormalities. Preliminary experiment.
13	19.7	PVA: 349	13.7	...	...	...	Suprarenals,	Kidneys showed red cells in a few capsular spaces, and blood casts in some convoluted tubules.
14	21.2	PVA: 349	11.4	420	115	40	Heart.	Tissues autolyzed, not suitable for microscopic examination.
15	24.8	PVA: 349	19.6	923	160	67	....	Liver showed Kupffer cells slightly enlarged, not vacuolated; spleen showed slight siderosis.
21	18.2	Acacia	29.5	771	107	56	....	Kidney showed a few red cells in capsular spaces, and granular debris in proximal tubules.
24	15.5	Acacia	26.8	350	69	35	Suprarenals.	No abnormalities.
32	9.1	PVA: 623	14.1	390	51	19	....	Liver showed an occasional storage globule in Kupffer cells.
35	18.8	PVA: 623	16.4	200	44	26	....	No abnormalities.
B	13.0	PVA: 349	30.0	670	60	30	Heart, suprarenals, aorta, lung, lymph node.	Liver showed a faint milky blue colour with Lugol's solution in gross. Pregnant. No abnormalities on section.
D	17.2	PVA: 623	70.0	477	93	50	Heart.	No sections available: faint blue colour on liver with Lugol's in gross.
E	10.2	PVA: 623	70.0	320	54	53	Heart, aorta, lung.	Doubtful bluish colour in subpelvic connective tissue, kidney, with Lugol's in gross. Splenic pulp cellular, with a few multinucleated cells. No foam cells. Pregnant.

observed, it may be that these changes are entirely physiological, due to the introduction of an effective blood substitute into the circulation, and displacing in part the normal elements.

EXPERIMENTS RE ANTIGENICITY AND PYROGENICITY

Although the chemical nature of PVA does not suggest that it is antigenic, sensitization of six guinea pigs was attempted. First doses of 1, 10, and 100 mgm. were given, and second doses of 1 and 10 mgm. were given 20 days (3 animals) and 30 days (3 animals) later. There was no fall of blood pressure upon giving the second dose, nor was any other stigma of anaphylactic shock noted.

Tests made upon two batches of commercially prepared material[11] by the intravenous injection of 30 c.c. 4% solution of PVA RH623 in rabbits weighing about 2 kgm., produced no febrile reaction, the animals being replaced into stock without appearing abnormal in any way.

PATHOLOGICAL STUDIES

The animals listed in Table IV were necropsied, immediately after electrocution, by Dr. John Fisher (Professor of Pathology, University of Western Ontario), and the sections of the tissues listed were studied by Dr. George Shanks (Pathologist, Toronto Western Hospital). Brief statements are given in this Table as to the findings, and Dr. Shanks states in summary as follows: "There are no appearances in these (sections) which would suggest that the viscera and arteries of these animals had been damaged, or that there was storage of foreign material."

ADMINISTRATION IN HUMANS

Solutions[11] of 4% PVA RH623 were given to four human subjects, in amounts of 125, 420, 430 and 480 c.c. respectively. There were no untoward subjective or objective effects noted. Dilution of the blood occurred, and persisted appreciably for 24 hours, after the three larger doses. These cases are to be reported in more detail elsewhere.

DISCUSSION

The studies described indicate that PVA RH623 is a suitable colloid for a plasma substitute. Its essential physical properties closely resemble those of the plasma proteins, and it appears innocuous to the recipient. In tests of its effectiveness after acute hæmorrhage in dogs, it maintained the blood pressure as well as did similar blood transfusions. It is lost from the blood stream in dogs at about the same rate as the plasma proteins regenerate. It did not appear antigenic. These features, if further confirmed, make it appear nearly ideal as an inert plasma substitute.

It must be noted that inert substitutes have certain inherent disadvantages. They do not promote wound healing, nor act as foodstuffs, as a protein might. They do not contribute to hæmostasis by clotting; large amounts would be expected to impede clotting, by diluting the fibrinogen. Their non-protein nature is also an advantage however, in lessening the likelihood of anaphylactic reactions. They obviously do not provide antibodies and erythrocytes, and hence are valueless in anæmias and in infections, except to improve the peripheral circulation.

If limited to its proper field of usefulness, however, this type of PVA would appear very desirable as a first treatment for hæmorrhage or shock, assuming that hæmostasis can be obtained or that direct bleeding is not a prominent feature. Its small cost, relative ease of preparation, and stability in storage, would permit keeping it available for emergency use, in amounts larger than can be economically kept in a blood or plasma bank.

SUMMARY

1. Several members of the polyvinyl alcohol series were examined as colloids for a blood or plasma substitute.

2. Of these, grade RH623 appeared effective in the treatment of acute hæmorrhage in dogs, and also seemed innocuous to the recipient.

3. The further testing of this material is suggested, preliminary to clinical use in shock and hæmorrhage, as its properties closely resemble those of the ideal (inert) blood substitute.

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11. These solutions were very kindly prepared and tested by Mr. T. H. Gewecke, of Baxter Laboratories of Canada.

170 St. George St., Toronto 5.