Special Communication

POLYVINYL PYRROLIDONE AS PLASMA EXPANDER

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Loss of effective blood volume, the primary cause of hemorrhage and traumatic shock, which if carried out beyond a certain critical point, leads to circulatory failure. This is treated most effectively by fresh whole blood or lacking this, by fluids known as plasma expanders or extenders. In most instances, if the blood volume is promptly restored, even though its composition be somewhat abnormal, a reversal of most or all of the deleterious effects of hemorrhagic shock takes place. The supportive effects of fresh whole blood are remarkably superior to those of any other available mixture. The red blood corpuscles are of importance not only for their oxygen transport and buffering capacities, but also because of their space occupying function. The osmotic pressure exerted by the plasma proteins is also an important property of whole blood. A plasma expander is used, as an alternative to whole blood or plasma, to restore the circulating blood volume by preventing loss of water into extravascular space. The basic concept of control of water balance by the colloidal osmotic pressure exerted across the capillary walls, due to Starling has not been changed by recent work

The need for a plasma substitute arose from the adverse features of using whole blood or pooled plasma in the treatment of blood loss or shock. Among the adverse features are limited availability, a brief storage period, the transmission of disease, the blood typing reactions and virus transmission etc. (Thompson 1960). The inadequacies of low molecular weight crystalloids such as saline and glucose have been well recognized. In fact many of the substances such as high polymers, plant as well as animal colloids, which are cheap, stable and can be sterilized, when tried also cause unfavourable side effects, such as histamine release, the clumping of erythrocytes and plasma proteins, troublesome storage phenomenon, and hemostatic defects (Thompson 1960).

The use of solutions of hemoglobin in saline has been shown to be superior to dog plasma in supporting the circulation following moderate hemorrhage in dogs (Hamilton et al., 1947). However, its rapid conversion to methemoglobin and disappearance from the circulation, interference with renal function, storage in liver and spleen, production of increased ESR and other toxic effects have prevented its extensive clinical use (Thompson, 1960; Gropper et al., 1952).

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The use of human globin extracted from erythrocytes as a plasma substitute has been suggested by Strumia (1952). Gropper et al. (1952) have reviewed its use in 108 patients without reactions. In the dog, however, globin prepared from dog blood has been found to produce respiratory distress and symptoms of histamine release (Hamilton et al., 1947). It is much less effective than saline in supporting the circulation of dogs following hemorrhage (Hamilton et al., 1947; Vars et al., 1952). Globin has been discarded as a plasma substitute and finds no clinical use today.

The use of gelatin as a plasma substitute in dogs utilizing BVI (Vars et al., 1952) was found to be superior to plasma and polyvinyl pyrrolidone (PVP) but inferior to dextran and albumin. In a study of survival following hemorrhage and replacement in dogs (Govier and Colovos, 1952) gelatin appeared superior to dextran and PVP. In this study it was found that if gelatin was used as the replacement fluid, the hemoglobin concentration could be reduced to 20% of control with a survival of greater than 50%. Gropper et al. (1952) in an extensive review of clinical reports concluded that gelatin is equivalent to plasma in the expansion of blood volume and survival following hemorrhage but it is to be noted that there is some controversy as to its superiority to normal saline in burn shock.

Polyvinyl pyrrolidone (PVP) was developed as a plasma expander in the early 1930s and was first used medically during the second world war as the colloidal component of the plasma substitute periston (Hecht and Weese, 1943) to treat haemorrhagic and traumatic shocks. In addition to being a colloid, PVP also binds to various macromolecules, such as dyes (Bennhold and Schubert, 1944; Bennhold et al., 1950; Scholtan, 1953) and toxins (Dieckhoff and Kunstler, 1943; Bovet et al., 1947; Schubert, 1948) whereby it comes close to what could be termed a "synthetic protein". In more recent years, PVP has been used clinically and experimentally to measure glomerular permeability (Scholtan, 1959; Hecht and Scholtan, 1959; Hardwicks et al., 1968; Hulme and Hardwicke, 1968; Ariss et al., 1969), the permeability of the gastrointestinal tract to circulating macromolecules (Gordon, 1959; Jarnum, 1961; Fell et al., 1969; Hardy, 1969; Clarke and Hardy, 1969; Clarke and Hardy, 1969a) and the capillary transfer of macromolecules from the intravascular space (Vogler and Strocker, 1964). In vitro PVP proved to be a useful extracellular cryophylactic agent in the preservation of various vertebrate cells (Parsidsky and Richards, 1962; Parsidsky and Richards, 1963; Ashwood-Smith and Warby, 1971; Ashwood-Smith et al., 1972; Damjanovic and Thomas, 1974).

PVP is synthesized from acetylene, ammonia and formaldehyde under pressure. It is hygroscopic and accordingly very easily soluble in water. The solubility in alcohols, including glycerine is over 25%. It is insoluble in deeithyl ether, dioxane acetone, benzene, carbon tetrachloride and strong bases. Chemically it is mostly neutral and cannot be broken down by enzymes of the animals organism. The viscosity is strongly dependent on the molecular weight to the PVP fractions. By giving the K-value it is used for a more precise specification of type of the individual PVP fractions. This proved to be expedient as no exact molecular weight can be given for colloidal PVP solutions because of the great dispersion of the polymerization level.

A 3.5% solution of the molecular weight 25,000-56,000 provides the usual plasma expander (Hillery 1970). PVP with a molecular weight of 15,000 or less undergoes rapid renal excretion, but there is a sharp cut off at 35,000 to 40,000 (Gropper et al., 1952). Urinary excretion in patients has been estimated to be about 40% in 24 hours with a total excretion of not more than 60%. The efficiency of PVP as determined by BVI seems to be very low in dogs ranking less than plasma and oxypolygelatin and being superior only to saline (Vars et al., 1952). PVP has been found to be inferior to gelatin and dextran in volume maintenance in bled rabbits. In terms of survival it is inferior to gelatin and oxypolygelatin and superior to dextran (Govier and Colovos, 1952).

After the intravenous administration of PVP experience confirms that polymers with a molecular weight below 20,000 are completely eliminated through the kidneys. An excretion of 85 to 90% of injected dose takes place within the first three days. In children, the excretion of PVP 12,600 occurs so fast that after 6 hours 95% of the injected was detected in the urine. The remaining 10-15% which pass temporarily from the blood stream into the lymph into tissues leaves the human or animal organism within a few days. A longer retention or storage of PVP less than 20,000 is not detected with normal renal function. PVP behaves in this order of magnitude in the same way as low molecular weight dextran, which likewise leaves the body within 4-6 days (Wessel et al., 1971).

The frequently mentioned osmotic nephrosis i.e. hydropic swelling of the tubular epithelia is found in all highly molecular compounds and moreover also in low molecular osmotically effective compounds such as glucose, saccharose etc. The 'osmotic nephrosis' has no considerable pathological significance according to Zollinger (1966), if it does not join previously existing renal damage adding complications. Although

the histological changes of 'osmotic nephrosis' are induced by mannit, the observation that transplant kidneys pretreated with mannit resume their function earlier than unpretreated ones, supports the fact that the 'osmotic nephrosis' has no considerable pathological value.

While the reports on the renal threshold concerning the molecular weight vary in the various authors between 25,000-60,000 all authors agree that polymers with molecular weight below 20,000 are completely eliminated through the healthy kidneys.

The higher polymerized PVP of molecular weight greater than 30,000 is eliminated after a delay and partly retained for weeks to months. In animal experiments PVP polymer with a molecular weight of about 55,000 only 60% is eliminated in 72 hours, and indeed only 21% of polymers with a molecular weight of 120,000 is eliminated. Only 10% is eliminated with a molecular weight of 500,000 (Ravin et al., 1952). These values were ascertained by radioactive marking of PVP with 131 and the PVP 500,000 is retained at least eight months. Gartner et al (1969) gave information on the elimination mechanism in the kidneys. According to them the basal membrane of the capillary convolution of glomerula allow PVP molecules upto 250,000 to pass through.

The post glomerular capillaries of rats are also permeable for longer molecules upto molecular weight 650,000 while they however do not pass into the ultra filterate and are eliminated like the low molecular fractions,but retained again via the lymph into the venous blood. While the problem of storage has no relevance for the quickly eliminated low molecular PVP, the question of place and kind of storage in the organism is raised in the fractions over 30,000 molecular weight in which a retention of PVP takes place over weeks or months.

There are series of histological and electron microscopic studies in quantitative observations of PVP storage. According to Bargmann (1946) a hydrophobic swelling of the reticular cells of the red pulp in the spleen occurred in rats which had received over 5 days a total dose of 3.5 gm/kg PVP 38,000 iv. The vacuolation of the cells proceeded with increased dosages from the organ periphery into the deeper areas of the spleen. The lymph follicles of the spleen were poor in storage cells. Besides the spleen it was mostly the kupffer's cells of the liver which store PVP.

Husselmann (1952; cited by Wessel et al., 1971) reported 15 post mortem cases in which upto 3100 ml of a 4% PVP 40,000 was injected 3-4 months before death. In almost all cases PVP of high molecular fraction had accumulated in the liver, spleen, pleural induration, fresh grannulating tissues and lymphatic nodes. The author on the basis of staining PVP accumulations with congo red concluded that the hydro soluble PVP has bound to proteins. The PVP wanders into the interstice areas within increased permeability of the capillaries and has absorbed here by histiocytes.

In animal experiments (Fresen and Weese, 1952) on rabbits and dogs there was PVP storage, after i.v. injection of PVP 35,000 in the reticular cells of the spleen lymphatic nodes and the bone marrow. After 15 injections of 2 g/kg PVP 35,000 histiocytes of the supra renal cortex and pituitary gland as well as alveolar cells of the lungs participated. In PVP 50,000 more over an inflation of the epithelial cells of the main part of nephron was detected in the kidneys. Hueper (1961) found in addition in rats which were injected with a 25% solution of PVP 60,000 i.p. that there were PVP containing pseudoxanthoma cells in the lungs, heart, aorta, pancreas, plexus chorioideus and uterus. The finding of a regeneration of plasma cells in the storage areas, are of histological peculiarity. These findings however, could not be confirmed in other studies with, higher molecular PVP.

In the mouse 15 to 16% of injected PVP can be found in the caracass after 58 days. Most of this in is the liver, muscles and bones (Steele et al., 1952). Fractions of molecular weight 110,000 to 120,000 are stored for years although fractions of molecular weight 40,000 or below are excreted within a few days (Ravin et al., 1952). The severity of this prolonged storage has been demonstrated by studies of radiochromic phosphate captured by the reticuloendothelial system (RES) in rabbits given 350 mg/kg of PVP weekly for 12 weeks. Six weeks after the end of this procedure the rate of uptake of the chromic phosphate is 59% of control (Weikel and Lusky, 1956) .Rabbits given 16 injections of 350 mg/kg of PVP show splenomegaly with foam cells constituting one third to one half of the spleen and other storage phenomenon in lymph nodes, bone marrow, adrenal medulla, lungs and blood vessels persisting in the RES and endothelium for more than 4 weeks. This causes thrombosis and penetration of capillary walls with formation of granulomatous consolidation of the lung parenchyma (Hartman 1952). Hartman (1951) indicated that German army pathologists found damage to liver and kidneys in soldiers to whom PVP had been administered and advised against further use. Gropper et al. (1952) note that PVP has the capacity to bind certain molecules and reduce their absorption or excretion.

There are reports on storage of PVP 38,000 in the RES in rats (Fresen and Weese, 1952). With repeated or large intravenous injections,

varying amounts are taken up by the reticuloendothelial cells, the endothelium of blood vessels and particularly the parenchymal cells of liver and kidney. PVP storage of molecular weight 40,000 in man was described by Schallock (1943) in ten post mortem cases in adults. According to Schoen (1949) 11 injections of 60 ml PVP 38,000 each led to a swelling of Kupffer's cells in the liver. The spleen showed a severe hemosiderosis of the red pulp, at the same time showing numerous reticulum cells besides hemosiderin vacuoles with stored PVP. Schoen saw in the lymphatic nodes a strong proliferation of the reticuloendothelial elements with foamy cytoplasm and vacuolar inclusions. The lymphatic nodes revealed many giant cells of a foreign body type. Jeckeln (1952) confirmed in 16 infants that besides the RES in the spleen, lymphatic nodes and liver PVP (MW 40,000) is taken up by all activated mesenchymal cells. This applies mostly to proliferative inflammations, which produce a formation of histiocytes. All histiocytory elements in the inflamed areas and granulatory tissues store higher molecular PVP. In a casuistic on infants who had received PVP 40,000, Husselmann (1952; as cited by Wessel et al., 1971) reported that after 2 years he still found PVP storage in the Kupffer's cells in the liver. Stenger and Muller (1946) carried out clinical followup studies in 47 small children who two years previously had received PVP 40,000 as infants. These followup studies showed no harm to the organs which could be determined by clinical or blood chemical methods.

The quantitative data on PVP accumulation has been supplemented by electron microscopic findings which give information on how PVP was absorbed by the cells and in which cells organelle it was stored. The escape from the capillaries or the passage into the tissue respectively can take place while the PVP directly passes the pores of the basal membrane or is actively transported through the endothelial cells by the mechanism of the pinocytosis or cytopempsis (Winne 1965). Winne mentions the membrane diffusion as a further possibility of transportation, which is especially important for small molecules. Of these valid possibilities for the transportation of substances into the tissues membrane diffusion and pinocytosis are considered. For the absorption of PVP in the storage cells phagolysosomes which are then changed into lysosomes are to be considered an intracellular storage place of PVP (Weissmann 1965). Huebner (1962) and Miller (1958) also support the storage in phagolysosomes. The original assumption that PVP and also other highly molecular substances are stored in mitochondria (Gusek and Linder, 1960; Traenckner, 1954; Gabler, 1960) has proved to be false.

The polyvinyl storage disease only occurred if highly molecular PVP 50,000 was injected in for years or decades. In the group of people concerned there are mostly patients with impaired hormone function in whom a hormone substitution therapy continued for some years is essential for survival as for example in diabetcs insipidus, morbus addison, diabetes mellitus etc. The PVP which was known as a cause of the thesaurismosis, served in the injected hormone preparations as a vehicle whereby especially the ambatic function with retard effect should be made use of. The added PVP was never, as already mentioned, low molecular PVP, but high molecular PVP capable of storage.

According to Cabanne et al. (1966) after a period of latency of about 6-14 years during which the patients have received daily injections of PVP upto a total dose of 3,000 gPVP, a second stage of dermatological syndrome with papulous changes on arms and breast occurred. Other patients developed syndromes which mostly affected the knee finger and hip joints, and lead in these places to swelling and pains in which the syndrome resembles rheumatic polyarthritis. Simultaneously there is storage in the lymphatic nodes, liver and spleen with hepatosplenomegaly. In the last stage the storage of organ manifestation, there are pulmonary symptoms with gradually increasing dyspnoea which increase to severe respiratory insufficiency.

Cabanne et al. (1966) stress that the so called PVP disease only occurs after at least 10 years application of quantities of 2500g of PVP and when PVP with a molecular weight of more than 30,000 is used. With the PVP (12,600) used as a plasma expander, in which only 35g of PVP would be applied with a 500 ml infusion solution, symptoms of the PVP disease would never be able to occur as on one hand the amount did not suffice by far and on the other, the low molecular PVP would be quickly eliminated and not stored.

Reactions to PVP manifested by increased ESR (Thrower and Campbell, 1951) and hypotension and death in dogs are frequent. Pretreatment with 35 ml/kg does not prevent dogs, from reacting to 350 ml/kg. Graded doses of 8-210 ml/kg administered in 2-14 injections does decrease the response to larger doses. Pretreatment with massive doses gives almost complete protection against later injections. Dialyzed PVP does not cause hypotension in tolerant dogs but the gross external signs appear. The response is characterized by vasodilation and hemoconcentration by increased permeability (Marshall and Hanna, 1957). PVP administered intraperitoneally is 100% absorbed in the rabbit within 8 hours and 90% absorbed in the dog within 9 hours. Although ESR increased 11 fold in rabbits and 5 fold in dogs, there were no other manifestations of an anaphylactoid reaction and this method of administration has been proposed

for patients, in cases where intravenous infusion would not be possible (Narat et al., 1952).

Halpern and Briot (1953) demonstrated an increase in plasma histamine from less than 20 mcg/L to above 350 mcg/L following administration of PVP in dogs. The increase in plasma histamine persisted for greater than 30 minutes and was accompanied by a decrease in blood pressure to 20 to 60 mm Hg. They also noted a large increase in the volume of gastric secretion and the concentration of free acids, itching, edema, shock, hemoconcentration and refractoriness for 2-3 days following the reaction. Administration of 1-18 mg/kg of PVP to dogs results in variable responses, but severe hypotension, hemoconcentration and skin flushing invariably follow the intravenous administration of 105 mg/kg (Perlmutt et al., 1953). The small molecular weight fraction (20,500) is active but the very large molecular weight fraction (560,000) is inactive in producing these responses (Perlmutt et al., 1953). The so-called 'canine reaction' is thought to be species specific. However, it is highly probable that the dog is not unique but only more sensitive. High molecular weight (1,000,000) PVP is antigenic in man causing specific antibody formation (Maurer 1956, 1957). This does not necessarily indicate that clinical PVP is antigenic and anaphylaxis probably plays no part in the reactions to PVP seen in the dog.

There are many reports on PVP in the German literature for the most part on its clinical applications. Schallock (1943) after studying storage in 52 animals and 102 autopsies, found that the greater the relative amount of infused PVP, the greater the danger of pathological reactions. Muller (1946) reported that the appearance of the spleen and lymph nodes after PVP injections was comparable to that in the storage disease, while the liver and bone marrow showed only slight changes. Schoen (1949) noted accumulation of PVP after injection in liver, spleen, lymph nodes and lungs. German army pathologists found damage in both liver and kidneys from PVP and late in World War II advised use of lypholyzed plasma. Nelson and Lusky (1951) describe their findings in rabbits receiving 6 injections of 10 ml/kg of 3.5% solution of PVP in distilled water over a period of 30 days. Grossly the spleen showed slight enlargement. Microscopically the principal lesion was foam cell storage, noted mostly in the spleen. Tissues involved to a less extent were lymph nodes, bone marrow, adrenal medulla, liver, lungs and thymus.

In another study fifty mice were given seven injections of 3.5% PVP in isotonic sodium chloride solution, 1.05%mg/kg over a three weeks period. The material was well tolerated and the animals remained in good condition. Foam cell formation was noted after the third injection in the lymph nodes after the seventh injection retention was well marked in liver, kidneys, adrenals, bone marrow, lungs and blood vessel. The material is granules and vacuoles. No actual necrosis was found in the liver, but in the kidney the tubular epithelium frequently desquamated to form cell casts. The changes in the blood vessels are perhaps the most significant, as most of the changes described by Hueper (1939) resulting from the use of methylcellulose and polyvinyl alcohol are seen, that is swelling and vacudization of the endothelium with palisade formation, mural thrombus formation and rupture.

Although solutions of PVP have been used extensively in the past, especially in continental Europe, as plasma expander, but concern about its possible connection with cancer has led to its greatly reduced use in clinical practice (Ashwood-Smith 1971). Reports have also appeared on its induction of liver lesions etc., as discussed earlier. Such adverse biological effects of PVP have mostly been associated with the high molecular weight fractions always present in samples used in plasma expanders. In a study on the cryoprotective action of PVP it was found that the molecular weight spread of the commercially available samples was very much greater than the manufacturers for the chi of Mcw 40,000 the speed Mw was 20,000 80,000 claim (Ash wood-Smith 1971) and higher molecular weight fractions of such samples are that uncleared by the kidney. Experiments with three pure samples of PVP indicated molecular weight distribution extends from 1000 daltons to values greater than 150,000 dalton (Ashwood-Smith 1971). The evidence for this statement is unequivocal and is based on gel exclusion chromatography on 'sephadex' columns (both G 100 and G 200).

It seems quite obvious that injections of PVP or administration by other routes (PVP solutions, are used in a variety of proprietary preparations to delay liberation of active drugs such as hormones, antibiotics and sulpha drugs), would result in a substantial retention with the lysosomes of the cells of the RES and thus might well be implicated or associated with cancer induction (Allison 1958).

In general macro molecular substances such as PVP which have osmotic pressure and hydrophilic properties comparable with those of blood plasma and relatively inert chemically, are not metabolized in the body and therefore produce storage phenomena either acute, chronic or both and is rarely used as a plasma expander. Since such substances are inert and are not metabolized their elimination from the body and chronic retention in the tissues seems to depend

upon their stability and molecular size. The difficulties regarding molecular size have been partially, if not principally, due to the average molecular size rather than to the actual size. In other words it is necessary to obtain the molecular size needed for osmotic pressure and hydrophilic properties and then produce that size through synthesis in the case of PVP, thus eliminating the molecular too small to be effective and those so large that excretion is too slow and chronic retention becomes a possibility.

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