A STUDY OF POLYVINYLPYRROLIDONE

AS A DETOXIFYING AGENT

by

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A thesis submitted to the Faculty of Graduate Studies
and Research in partial fulfillment of the requirements
for the Degree of Master of Science.

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ABSTRACT

After the in vivo administration of a drug, the drug exists in two forms: a portion which is bound to tissue and plasma proteins and a portion which is free or unbound. Binding of a drug decreases the concentration of drug available for diffusion across membranes to the site of action. In states of drug overdose plasma protein binding of a drug approaches saturation and high concentrations of unbound drug result in exaggerated pharmacological or toxic effects.

An unexplored treatment of drug overdose states is the use of a binding agent which after intravenous administration would immediately increase the concentration of drug in the bound, non-diffusible, pharmacologically inert form. Such a binding agent should be of small enough molecular size so that it would rapidly distribute throughout the extra-cellular fluid but should not penetrate cells. The binding agent with the bound drug should then be rapidly filtered at the glomerulus. Renal tubular reabsorption of the bound drug should be minimal and urinary excretion of the drug should therefore be increased.

Some of the qualities of a non-specific binding agent are seen in the synthetic plasma volume expander polyvinylpyrrolidone (P.V.P.). P.V.P. has been reported to bind many drugs, is of low toxicity and is available in a low molecular weight of 10,000 (P.V.P.-10). The binding of common drugs by P.V.P.-10 and the toxicity, distribution and excretion of P.V.P.-10 are unknown. The use of P.V.P.-10 in the treatment of experimental intoxication with common drugs has not been studied.

In order to study the potential of P.V.P.-10 as a detoxifying agent, salicylate was chosen as a model toxic drug.

The results of our in vitro experiments using equilibrium dialysis and ultrafiltration techniques showed that P.V.P.-10 reversibly bound a significant quantity of salicylate. The binding of salicylate was proportional to the molecular weight of P.V.P., the concentration of P.V.P. and the concentration of salicylate. Hydrogen ion concentration from pH 4.0 to 8.0 did not influence the binding of salicylate by P.V.P.-10. The addition of P.V.P.-10 to human albumin did not give the expected additive binding effect. However P.V.P.-10 produced a large decrease in the concentration of salicylate in the "cellular" fluid of an in vitro system which simulated the three major fluid spaces in the in vivo system. In order to clarify the mechanism of binding by P.V.P. it was shown that each vinylpyrrolidone unit in the molecular chain of P.V.P. represented a potential binding site. Saturation of these binding sites was not observed at concentrations of salicylate far greater than those observed in salicylism.

After intravenous administration of P.V.P.-10 to rabbits, the P.V.P.-10 distributed into a volume corresponding to the calculated extracellular fluid. The P.V.P.-10 was rapidly excreted by the kidney with 95 percent recovery in the urine 12 hours after its administration. There was no gross evidence of toxicity of intravenously administered P.V.P.-10 in rabbits.

The treatment of experimental salicylate intoxication in rabbits with P.V.P.-10 was compared to control treatment with mannitol. The animals treated with P.V.P.-10 showed a greater decrease in the concentration of salicylate in the serum, a greater increase in the urinary excretion of salicylate and greater amelioration of some of the physiological abnormalities resulting from salicylism, than did the control animals.

INTRODUCTION

A. STATEMENT OF THE PROBLEM

The higher the concentration of a drug at the site of its action within cells or on cell surfaces, the greater the pharmacological and toxic effects of the drug. After oral administration of a drug, the concentration reached at the effector cells is dependent upon the amount of drug absorbed from the gastrointestinal tract into the vascular system as well as upon the ease of its transfer across the capillary membrane and its attachment to or penetration of effector cells and upon the rate of its degradation, inactivation and excretion. Most of these processes involve the passage of the drug across cell membranes. The rate at which a drug crosses these membranes is dependent upon many factors including the lipid solubility of the drug, the amount of the drug in the unionised state, the molecular size of the drug and the concentration of the drug in the unbound form.

Most drugs exist in the body in an unbound form and a portion which is bound to plasma and tissue protein. Albumin is the most important plasma protein in terms of capacity for and non-specificity of drug binding. Most of the albumin-bound form of the drug remains in the intravascular space and does not reach the cellular site of action and therefore may be considered pharmacologically inactive. The unbound portion of the drug diffuses across membranes and is responsible for the pharmacological and toxic effects of the drug.[1, 2] At low total concentrations of a drug which binds to albumin there is a high ratio of the number of available binding sites on the albumin

molecule to the number of molecules of drug. Therefore a high percentage of the total amount of drug will be present in the bound form. As the total concentration of the drug is increased the finite albumin binding sites are progressively saturated resulting in a greater proportion of drug being in the unbound form.

High serum concentrations of drug are observed in drug overdose. As a result, albumin binding capacity for the drug is exceeded and a high concentration of unbound diffusible drug is available for entry into cells to produce exaggerated pharmacological and toxic effects.

The usual methods of treatment of patients with drug overdose are directed at ameliorating the physiological abnormalities of the toxic state and increasing the excretion of the drug. In some states of drug intoxication specific drug inactivators have been used - dimercaprol for mercury, edathamil calcium sodium for lead, and desferrioxamine for iron. These inactivators combine chemically with the toxic agent and occupy a portion of the molecule which would otherwise exert a toxic effect. The use of these specific binding or chelating agents is limited to a small number of relatively infrequent drug intoxications. The more non-specific binding property of albumin has been utilised in the treatment of drug overdose by its addition to the dialysate in peritoneal dialysis for barbiturate intoxication.[3, 4] Albumin has also been added to donor blood in replacement transfusion in the treatment of hyperbilirubinaemia in order to increase the removal of bilirubin as the albumin-bound complex.[5] After intravenous administration of albumin its molecular size largely limits its distribution to the intravascular space. Therefore the quantity which can be safely administered is small, restricting its use as a non-specific binding agent in the treatment of drug overdose.

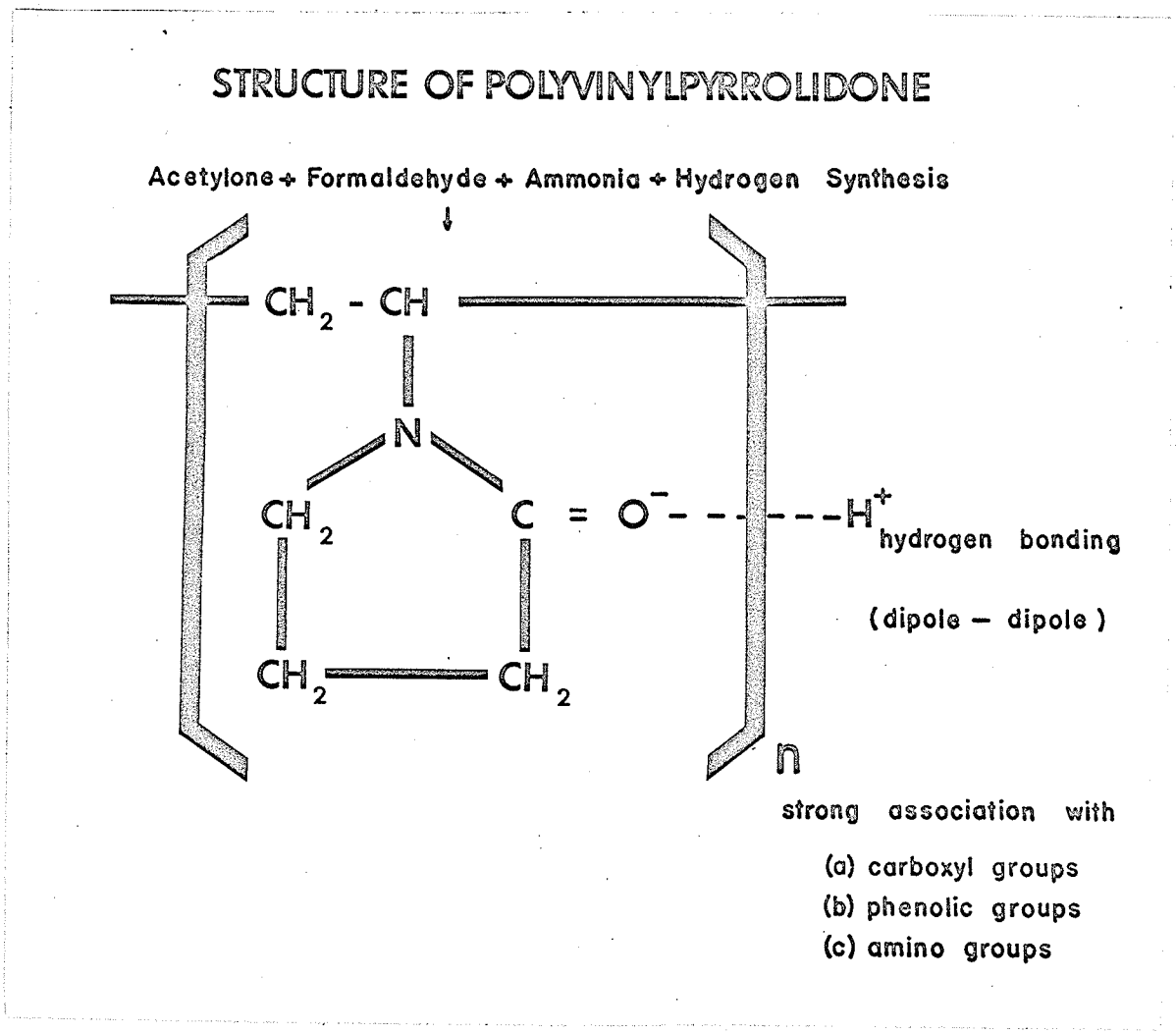
The use of other non-specific binding agents in the treatment of patients with drug intoxication has not been carefully studied. Such a binding substance should have certain qualities. It should have a high binding affinity for common drugs. After intravenous administration the binding substance should be of small enough molecular size so that it would rapidly distribute throughout the extracellular fluid space but be of large enough molecular size so that it would not accumulate in cells. Within the extracellular fluid the substance should bind a significant proportion of the toxic drug. This would decrease the capacity of the drug to diffuse into cells or attach to receptor sites. The small molecular size of the binding substance should allow it to be rapidly filtered at the glomerulus. This should result in high concentrations of the binding substance in the tubular urine. The binding substance should not be reabsorbed by the renal tubules and should thereby decrease any tubular reabsorption of the toxic drug. Enhanced urinary excretion of the drug should occur in the form of the drug-binding vehicle complex.

Some of these desired qualities of a non-specific binding agent are seen in the synthetic plasma volume expander polyvinylpyrrolidone (P.V.P.). P.V.P. has been reported to bind many drugs, is available in a wide range of molecular weights, has been used extensively in humans and is relatively non-toxic.

B. P.V.P. INTRODUCTION

In 1933 polyvinylpyrrolidone was produced by the controlled synthesis with acetylene, formaldehyde, ammonia and hydrogen under high temperature and pressure.[6] The structure of the resulting polymer is shown in Figure 1.

Figure 1


STRUCTURE OF POLYVINYLPYRROLIDONE
Acetylone + Formaldehyde + Ammonia + Hydrogen Synthesis
CH2 - CH
N
CH2
C = O⁻ - - - - - H⁺
hydrogen bonding
(dipole – dipole)
CH2
CH2
n
strong association with
(a) carboxyl groups
(b) phenolic groups
(c) amino groups

A comparatively crude large molecular weight preparation of P.V.P. in saline was used extensively in Germany during World War II as an emergency plasma volume expander.[7] By improved control of synthesis and further fractionation P.V.P. is now available in a wide range of molecular weights (10,000 to 360,000). The molecular weight of a specified P.V.P. preparation as designated by its mean, follows a nearly normal distribution curve with approximately 90% of the P.V.P. within 20% of the mean.

C. BINDING PROPERTIES OF P.V.P.

The binding potential of P.V.P. was first noted by Bennhold and Schubert in 1944.[8] Further investigations by Schubert[9] demonstrated that intravenous administration of P.V.P. decreased the mortality of animals given a lethal dose of the diazo dye, diamine red, and subsequently demonstrated by electrophoresis the in vitro binding of diamine red and some sulfonamides to P.V.P. In 1947 Pellerat and Murat[10] demonstrated by an in vitro binding experiment with a collodion ultrafilter that sodium salicylate is fixed by P.V.P. in proportion to the concentration of P.V.P. It has been known that iodine binds with a high affinity to P.V.P. The complex formation of iodine with P.V.P. has formed the basis for a colorimetric method for the quantitative analysis of P.V.P. A number of reports have been published more recently which state that P.V.P. "binds" a number of drugs, chemicals, dyes, toxins and viruses.[11-16] In the majority of these investigations, binding by P.V.P. was used as the explanation for observed results of therapeutic trials with little or no factual evidence to indicate binding per se.

Recently a group of investigators have studied the in vitro binding of

a number of drugs and chemicals to P.V.P. of 40,000 molecular weight.[17-22] Drugs which were observed to be bound by P.V.P. included sulfathiazole, chloramphenicol, sodium salicylate and phenobarbital. No binding of procaine, penicillin, cortisone, caffeine, theophylline, aminopyrine or citric acid by P.V.P. was demonstrated.

In the above studies the amount of drug bound by P.V.P. was shown to increase in direct proportion to the concentration of the drug and the concentration of P.V.P. The amount of drug bound was shown to have some relation to its structure. Higuchi and Kuromoto[18] list a series of drugs including structurally related compounds placing them in order of their decreasing binding affinity to P.V.P. - sulfathiazole, m-hydroxybenzoic acid, p-hydroxybenzoic acid, para-aminobenzoic acid, salicylic acid, phenobarbital, benzoic acid, sodium salicylate, mandelic acid and chloramphenicol. Spitzer and McDonald[23] have shown no change in binding of bromophenol blue with an increase in the molecular weight of P.V.P., while Scholtan[24] observed that the formation of the Congo-red-P.V.P. complex increased rapidly with an increase in molecular weight of P.V.P.

The complex formation between drug and P.V.P. has been demonstrated to be a reversible process and to obey the Law of Mass Action.[24] An increase in temperature from 4°C to 35.5°C was found to decrease the binding of the dye, bromophenol blue, to P.V.P.[23], whereas the binding of chlorbutanol to P.V.P. increased with an increase in temperature.[22] The only study on the effect of pH on binding by P.V.P. is described by Scholtan.[24] He observed no change in the binding of the dye, methyl orange, to P.V.P. over the pH range of 5.7 to 8.1.

Mechanisms for the binding property of P.V.P. have been postulated on the basis of factors observed to affect binding. Some investigators[19,23,24]

favour the theory that each acid amide group in each repeating vinylpyrrolidone unit of the polyvinylpyrrolidone chain is a potential binding site. (See Figure 1) Others[25] believe that the single terminal unsaturated double bond of the P.V.P. chain is the binding site.

D. DISTRIBUTION AND EXCRETION OF P.V.P.

P.V.P. has been investigated primarily for its use as a plasma volume expander. Since P.V.P. of molecular weight of 40,000 has been shown to possess the optimum qualities for a plasma volume expander, the distribution, excretion and toxicity of P.V.P. of this particular molecular weight have been studied in greatest detail.

After intravenous administration, P.V.P. distributes in a volume approaching that of the extracellular fluid at a rate inversely proportional to its molecular size.[26, 27] Similar observations have been made on the distribution of dextrans of various molecular weights.[28] P.V.P. is stored in the reticuloendothelial cells of the liver, spleen and kidney where it may remain for an indefinite period of time. The amount and duration of storage is directly proportional to the molecular weight of P.V.P.[25, 29] After intravenous administration, P.V.P. enters the bile in significant amounts, and as much as 13% of the total dose may be eliminated through the bowel.[25,27,30,31] After intravenous administration to the mother, P.V.P. also appears in milk[32] and in fetal cord blood.[32,33] There is no evidence that P.V.P. can be metabolised.[26] Orally administered P.V.P. is excreted through the intestinal tract with no evidence of absorption.[34]

The renal excretion of P.V.P. is dependent upon the permeability of the glomerular capillary membrane and the size and shape of the P.V.P. molecule.

The glomerular membrane is freely permeable to substances with molecular weights less than 10,000 with no appreciable permeability to those with weights greater than 70,000.[35] Campbell et al.[36] believe that this threshold does not apply to P.V.P. since according to their findings P.V.P. of molecular weight up to 100,000 was excreted through the kidneys of patients with normal renal function. Any differences between the passage of P.V.P. of different molecular weights through capillaries of the glomeruli and capillaries elsewhere may be explained by Pappenheimer's observation[37] that the capillaries of glomeruli contain more pores per unit of filtering surface than other capillaries, and that the glomerular pores are somewhat larger.

A number of investigators[26,38-41] have determined that after the intravenous administration of P.V.P. of molecular weight of 40,000 to man and several species of animals, 50 to 75 percent of the total dose is excreted in the urine in 24 hours. Scholtan[42] postulated that one of the reasons for the variability in the renal excretion of P.V.P. could be the variability in the configuration of the P.V.P. molecule. He stated that P.V.P. is a filiform molecule which in aqueous solution could assume varied forms, from stretched to coiled, thus affecting its filtration rate at the glomerulus. The renal excretion of P.V.P. has been shown to be inversely proportional to its molecular weight.[36,43,44] In dogs and cats Hecht[43] compared the renal clearance of P.V.P. of molecular weights of 4,600, 12,600, 16,400, and 30,000 in relation to the creatinine and inulin clearance. The clearances of P.V.P. of molecular weights of 4,600 and 12,600 expressed as a percentage of creatinine clearance, were 91 ± 2%, and 95 ± 2%, respectively. The clearances of P.V.P. of molecular weight of 16,400 and 30,000 were 80 ± 1.6%, and 67 ± 6%, respectively. One hour after intravenous administration of P.V.P. of each of the four molecular weights the approximate cumulative urinary excretion, expressed as percentage of total

dose, was 97%, 95%, 80% and 70% respectively. These results were supported by Richards[45], and were similar to those of Giebisch et al.[46] who determined the renal excretion and volume of distribution of dextran of various molecular weights. Intravenous administration of P.V.P. of molecular weight of 12,600 to man and several species of animals produced little or no change in circulating blood volume,[45] little or no osmotic diuresis,[47,48] no change in renal plasma flow or glomerular filtration rate,[48,49] and no change in water reabsorption by the renal tubules,[48] for the dosage employed.

E. TOXICITY OF P.V.P.

P.V.P. has been shown to be of low toxicity. It is non-antigenic in man[29] and in most animals. The dog shows a peculiar sensitivity to P.V.P. marked by hypotension which frequently progresses to death.[50-52] This effect can be prevented by prior small injections of P.V.P.[69] The intravenous LD_{50} of P.V.P. of molecular weight of 40,000 in rats is 12-15 g./kg., with death attributed to cardiovascular overloading. No deaths occurred in rats and guinea-pigs given an acute oral dose of 100 g./kg. of P.V.P.[34] After intravenous administration of P.V.P. of molecular weight of 12,000 to dogs there were no changes in haematocrit, slight transient increases in the white cell count and erythrocyte sedimentation rate, slight transient decreases in the concentrations of plasma protein and no changes in blood clotting factors.[45] P.V.P. of molecular weight of 40,000 produced greater and more sustained changes in each of the above measurements.[45] Reinhold et al.[53] did not demonstrate any change in liver function tests after the administration of P.V.P. to man. Hueper in 1957[54] reported P.V.P. to be

carcinogenic but in 1961, after further experiments,[55] retracted the claim with the explanation that the tumour formation originally observed was probably the result of impurities in the relatively crude P.V.P. preparation.

F. SUMMARY OF KNOWN AND UNKNOWN PROPERTIES OF P.V.P.

As a result of the review of the above studies of binding, distribution and excretion, and toxicity it was thought that P.V.P. showed potential as a non-specific binding substance and that P.V.P. of molecular weight of 10,000 (P.V.P.-10) would best satisfy the qualities necessary for optimum distribution and excretion for use in the treatment of states of drug overdose.

The reported studies of binding by P.V.P., its distribution and excretion, particularly in reference to P.V.P.-10, were too incomplete to allow a definite assessment of its use in overdose states. The effect of molecular weight of P.V.P. on its binding capacity required further study. The stability of binding by P.V.P.-10 in the range attainable in vivo, by the concentration of the toxic drug and by the pH within the range seen in serum, tissues, and urine in the toxic state also required further elucidation. The interaction between the binding by albumin and P.V.P.-10 in combination needed study. The distribution and rate of excretion of a large acute dose of P.V.P.-10 had not been studied. The efficacy of P.V.P.-10 in the treatment of states of experimental drug overdose had not been adequately investigated.

G. PURPOSE OF THE STUDY

The purpose of this investigation was to study in detail the capacity and stability of binding by P.V.P.-10, the excretion and distribution of P.V.P.-10, and the use of P.V.P.-10 in experimental drug overdose.

Salicylate was chosen as a model toxic drug for our investigation of the binding potential of P.V.P.-10 and the usefulness of this potential in experimental salicylism. This choice was made because of the high incidence of overdose by salicylate and the knowledge of the toxic state. There is also detailed knowledge of the pharmacology of salicylate specifically in regard to plasma protein binding and excretion.

METHODS AND MATERIALS

METHODS OF DETERMINATION OF SALICYLATE AND P.V.P.

Sodium salicylate was used throughout the study and salicylate concentrations were determined by the method of Trinder.[56]

P.V.P. of molecular weights of 10,000 (P.V.P.-10), 40,000 (P.V.P.-40), 160,000 (P.V.P.-160) and 360,000 (P.V.P.-360) was supplied by the General Aniline and Film Corporation, New York. The P.V.P.-10 for in vivo use was classified as pharmaceutical grade after pyrogen tests by the manufacturer. P.V.P. concentrations were determined by a modification of the iodine colorimetric method of Campbell and Hunter.[57] To measure the concentration of P.V.P. in serum or plasma, Campbell and Hunter used 0.1 N. calcium chloride to precipitate protein. When this concentration of calcium chloride was used in our laboratory many serum samples remained opalescent interfering with later spectrophotometric analysis. This effect was avoided by using 0.005 N. calcium chloride.

All binding studies were determined by an equilibrium dialysis technique and/or an ultrafiltration technique. In some studies both techniques could be used and verification of results could be provided.

EQUILIBRIUM DIALYSIS TECHNIQUE

Visking cellulose tubing was employed in a modified equilibrium dialysis technique.[58] All solutions to be dialysed and all dialysing solutions were prepared in saline and unless otherwise stated the pH was adjusted to 7.0 with a few drops of 0.1 N. NaOH. In all studies (unless otherwise stated) 10 ml. of the solution to be dialysed was placed in a prepared cellulose bag and suspended in 20 ml. of dialysing solution contained in a 50 ml. polypropylene tube. Dialysis was allowed to proceed for 6 hours at room temperature with constant stirring by a small Teflon coated magnetic stirring bar which had been placed in the polypropylene tube. It was established that with the highest concentration of salicylate employed in these studies equilibrium was reached at the end of six hours while P.V.P. of the lowest molecular weight (P.V.P.-10) did not penetrate through the cellulose membrane within that time.

In all dialysis studies the following measurements and calculations were made. At equilibrium, the concentration of salicylate was measured in a sample from inside the dialysis bag and expressed as the final concentration of total salicylate in the dialysant. The concentration of salicylate was measured in a sample from outside the dialysis bag and expressed as the final concentration of unbound salicylate in the dialysate. The final volume of the dialysant was measured to detect water shift. This volume could be measured with an error of $\pm$ 0.1 ml. It was found that the volume occupied by one gram of P.V.P. was 0.65 ml. The volume occupied by one gram of albumin was 0.75 ml.[59] In order to determine the volume of

water in the dialysant at equilibrium the volumes occupied by binding substances (P.V.P. or albumin) were subtracted from the measured final volume of the dialysant.[59] The final content of total salicylate in the dialysant was expressed as the product of the final concentration of total salicylate in the dialysant and the final volume of the dialysant. The final content of unbound salicylate in the dialysant was expressed as the product of the final concentration of unbound salicylate in the dialysate and the volume of water in the dialysant. The final content of bound salicylate in the dialysant was calculated by subtracting the final content of unbound salicylate in the dialysant from the final content of total salicylate in dialysant. The content of bound salicylate and the content of P.V.P. in the dialysant were expressed in millimoles (mM.). Binding could therefore be recorded as the ratio of mM. of salicylate bound per mM. of P.V.P.

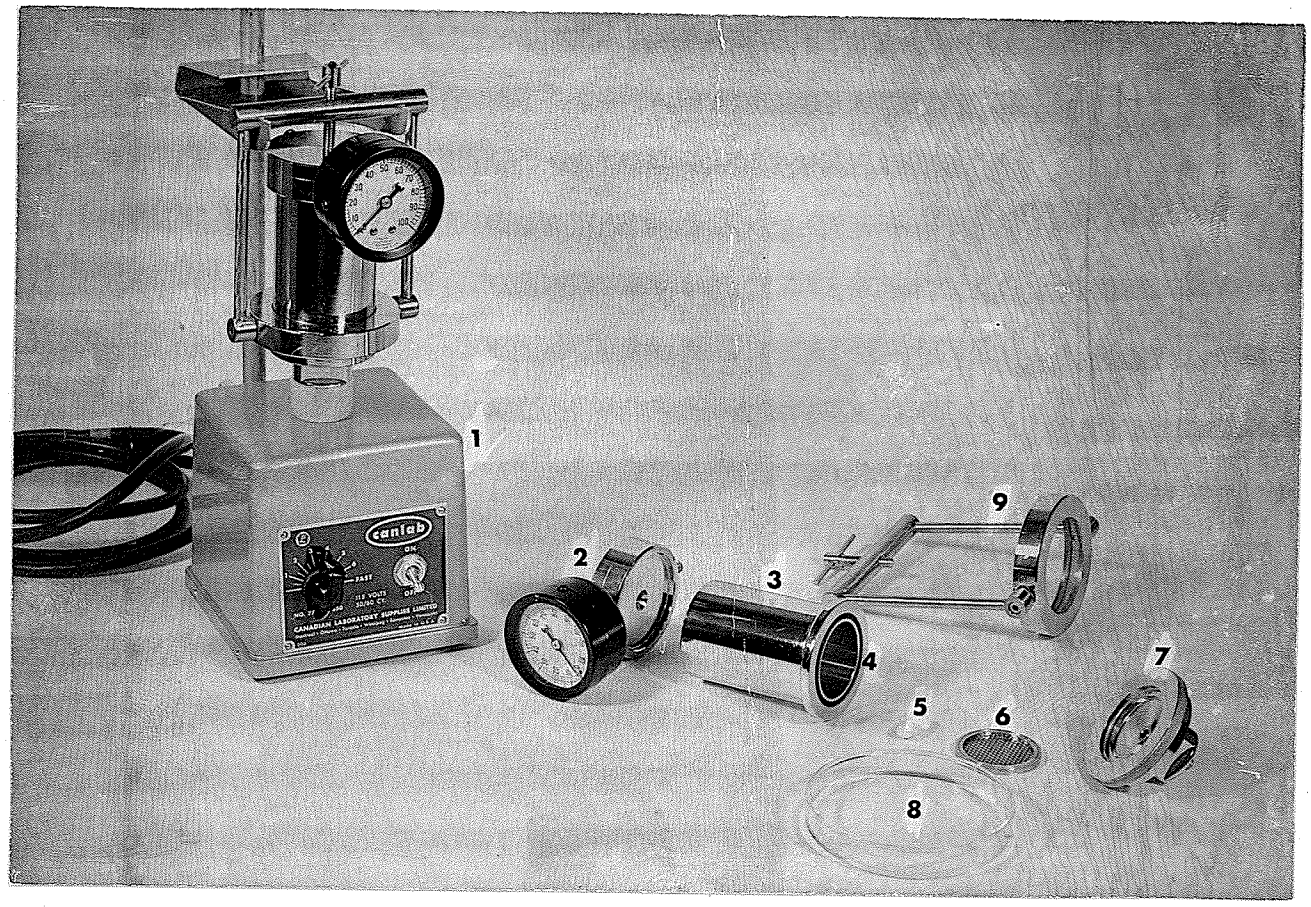
ULTRAFILTRATION TECHNIQUE

The ultrafiltration unit used (Figure 2) was a modification of that described by Linford.[60] Commercially available thick collodion type ultrafiltration membranes were used. (Schleicher and Schuell, Keene, New Hampshire). These membranes had a mean pore size of five millimicrons. It was established that these membranes would not allow the passage of P.V.P.-10 in the time necessary to complete ultrafiltration studies.

After instillation of the filtering solution and a Teflon coated magnetic stirring bar, each of the assembled ultrafiltration units was pressurised to 100 lb/square inch with 100% nitrogen and suspended over an individual magnetic stirrer. Ultrafiltration was allowed to proceed with constant stirring at room temperature. The first few drops of filtrate

Figure 2

ULTRAFILTRATION APPARATUS



The assembled ultrafiltration unit and its components. (1) magnetic stirrer (2) pressure gauge (3) cylinder (4) rubber O-rings (5) magnetic stirring bar (6) support screen (7) ultrafiltration membrane (8) collecting base (9) assembly bracket.

were discarded, then a 0.2 ml. sample was collected for analysis. This required a total of 15 minutes after which ultrafiltration was stopped. In order to minimize any concentrating effect on the binding substance in the filtering solution,[61] the volume of filtrate collected was never greater than five percent of the total volume of the filtering solution.

Ambard and Trautman[61] observed membrane retention of electrolytes in their ultrafiltration experiments. They also showed that membrane retention of electrolytes decreased as the concentration of the electrolytes increased. The membranes used in our experiments retained a small amount of salicylate. The amount of salicylate retained was determined by the ultrafiltration of a series of solutions of known concentrations of salicylate prepared in distilled water and in normal saline and by the measurement of the concentration of salicylate in the resulting filtrate. Results of binding experiments could then be corrected for any membrane retention phenomenon.

I. IN VITRO BINDING OF SODIUM SALICYLATE BY P.V.P.

A. Effect of Molecular Weight of P.V.P. and Concentration of Salicylate.

The solutions to be dialysed contained P.V.P.-10, P.V.P.-40 and P.V.P.-160 in concentrations of 0.01 mM./ml. (10 mg./ml., 40 mg./ml. and 160 mg./ml. respectively). P.V.P.-360 was prepared in a concentration of 0.001 mM./ml. (36 mg./ml.) since a higher concentration was too viscid for efficient dialysis. All P.V.P. solutions were prepared in normal saline. Ten ml. of solutions of P.V.P.-10, P.V.P.-40 and P.V.P.-160 were dialysed. Only five ml. of the P.V.P.-360 solution was dialysed to minimize the more significant hydrophilic effect of P.V.P. of this molecular weight. The dialysing solutions of sodium salicylate were prepared in concentrations of 100, 200, 400, 600, 800, 1200, 1600, 2400, 4800 and 7200 mg./100 ml. High concentrations of salicylate were used in an attempt to saturate binding. Solutions of

P.V.P. of each molecular weight were dialysed against solutions of salicylate of each concentration.

B. Effect of Concentration of P.V.P.-10 on the In Vitro Binding of Salicylate.

The solutions to be dialysed contained concentrations of P.V.P.-10 of 16, 32 and 64 mg./ml. (P.V.P.-10 content 160, 320 and 640 mg. respectively). The dialysing solutions contained concentrations of salicylate of 20, 40, 80, 100 and 160 mg./100 ml. P.V.P.-10 of each concentration was dialysed against salicylate of each concentration.

C. Effect of pH, Double Dialysis and Exhaustive Dialysis on the Stability of Binding of Salicylate by P.V.P.

(i) Effect of pH (dialysis)

The solution to be dialysed contained 32 mg./ml. of P.V.P.-10. The dialysing solutions contained 80 mg./100 ml. of salicylate. Aliquots of each solution were titrated with 0.1 N. NaOH or 0.1 N. HCl as required to adjust the pH to 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0. Solutions of P.V.P.-10 were dialysed against solutions of salicylate of the same pH. The pH of all solutions was measured again at the end of dialysis.

(ii) Effect of pH (ultrafiltration)

In order to verify the results of the dialysis studies of the effect of pH on binding, the procedure was repeated using P.V.P.-160 and the ultrafiltration method. A solution of P.V.P.-160 (16 mg./ml.) and salicylate (80 mg./100 ml.) was divided into five 50 ml. aliquots. These were adjusted to pH of 4.0, 5.0, 6.0, 7.0 and 8.0, respectively, with 0.1 N. NaOH, and ultrafiltered.

(iii) Effect of double dialysis

The solutions to be dialysed contained concentrations of P.V.P.-10 of 12.5, 25.0, 50.0, 100.0, and 150.0 mg./ml. The dialysing solutions contained salicylate, 180 mg./100 ml. Each P.V.P. solution was dialysed against each dialysing salicylate solution. At the end of the first dialysis, at which time equilibrium had been reached, the concentration of unbound salicylate in the dialysate was determined. Each dialysis bag was then resuspended in saline only and dialysed to equilibrium for a second time. The concentration of unbound salicylate in the dialysate was again determined.

(iv) Effect of exhaustive dialysis

Solutions of P.V.P.-10, P.V.P.-40, P.V.P.-160 and P.V.P.-360 were prepared in concentrations of 0.01 mM./ml. (10, 40, 160 and 360 mg./ml. respectively) with salicylate, 100 mg/100 ml. Each of these solutions was dialysed against continuously running tap water for 48 hours. The concentration of salicylate was determined in each dialysed sample at the end of dialysis.

D. Effect of Human Albumin and P.V.P.-10 on the <u>In Vitro</u> Binding of Salicylate.

The three solutions dialysed were P.V.P.-10, 32 mg./ml. (3.2 mM./ml.), human albumin, 50 mg./ml. (0.725 mM./ml.) and P.V.P.-10, 32 mg./ml. with human albumin, 50 mg./ml. The dialysing solutions were prepared in seven concentrations of salicylate of 10, 20, 40, 80, 100, 120 and 160 mg./100 ml. Each of the three solutions containing binding substances was dialysed against solutions of each concentration of salicylate.

E. Effect of P.V.P.-10 and Human Albumin on the *In Vitro* Binding of Salicylate in a Three-Compartment Dialysis System Simulating *In Vivo* Distribution

The following experiment was designed to simulate the distribution of albumin, P.V.P.-10 and salicylate in the intravascular, interstitial, and intracellular spaces.

The first solution to be dialysed, representing the intravascular space contained albumin, 50 mg./ml.; P.V.P.-10, 32 mg./ml. and salicylate, 80 mg./ml. Five ml. of this solution was placed into a small cellulose bag and this bag in turn was placed inside a larger cellulose bag containing 15 ml. of the second solution, representing the interstitial space. This solution contained P.V.P.-10, 32 mg./ml. and salicylate, 80 mg./ml., but no albumin. This system was placed into 30 ml. of a solution of salicylate, 80 mg./ml., representing the intracellular space. The concentration of salicylate and the volumes of the three compartments were measured when dialysis had reached equilibrium.

II. *IN VIVO* DISTRIBUTION AND EXCRETION OF P.V.P.-10 IN RABBITS

The solution of P.V.P.-10 used was prepared in 5 grams of glucose/100 ml. of distilled water in a concentration of 20 gm./100 ml. The pH of this solution was adjusted to 7.4 with 1N. NaOH. The solution was autoclaved for 15 minutes at 15 lbs. pressure (as recommended by the manufacturer).

The distribution and excretion of P.V.P.-10 was studied in a series of 10 unanaesthetised adult male rabbits ranging in weight from 2.3 to 4.8 kg. Twenty-four hours prior to the study each animal was placed in a separate cage and given lettuce and water to ensure adequate hydration.[62]

At the start of the study a Foley catheter (size 8 or 10) was inserted into the urinary bladder and the balloon inflated to three cc. Each rabbit was placed in a rabbit restraining box. The catheter was allowed to drain continuously into a graduated cylinder outside the box. One hour was allowed for the animal to adjust to this new environment. A scalp vein needle (size 23) was inserted into an ear vein and P.V.P.-10 in a total dose of 4 g./kg. was administered continuously for two hours using a Harvard pump. Thereafter a solution of 5 grams of glucose/100 ml. of distilled water was infused at a rate of 10 ml./kg./hr. for four hours. By this time the animals were taking food and fluid. Samples of urine, and samples of venous blood from the ear not being used for infusion were taken at intervals after the start of the P.V.P.-10 infusion, and until 24 hours. The concentration of P.V.P. was determined in each of these samples. In five animals these samples were collected until 48 hours. Stool was not collected for determination of excretion of P.V.P. by the gastrointestinal tract. The animals were killed and histological examination made of the kidney, liver and spleen.

III. COMPARISON OF THE EFFICACY OF TREATMENT OF EXPERIMENTAL SALICYLATE INTOXICATION IN RABBITS WITH THE INTRAVENOUS ADMINISTRATION OF P.V.P.-10 AND A CONTROL MANNITOL SOLUTION

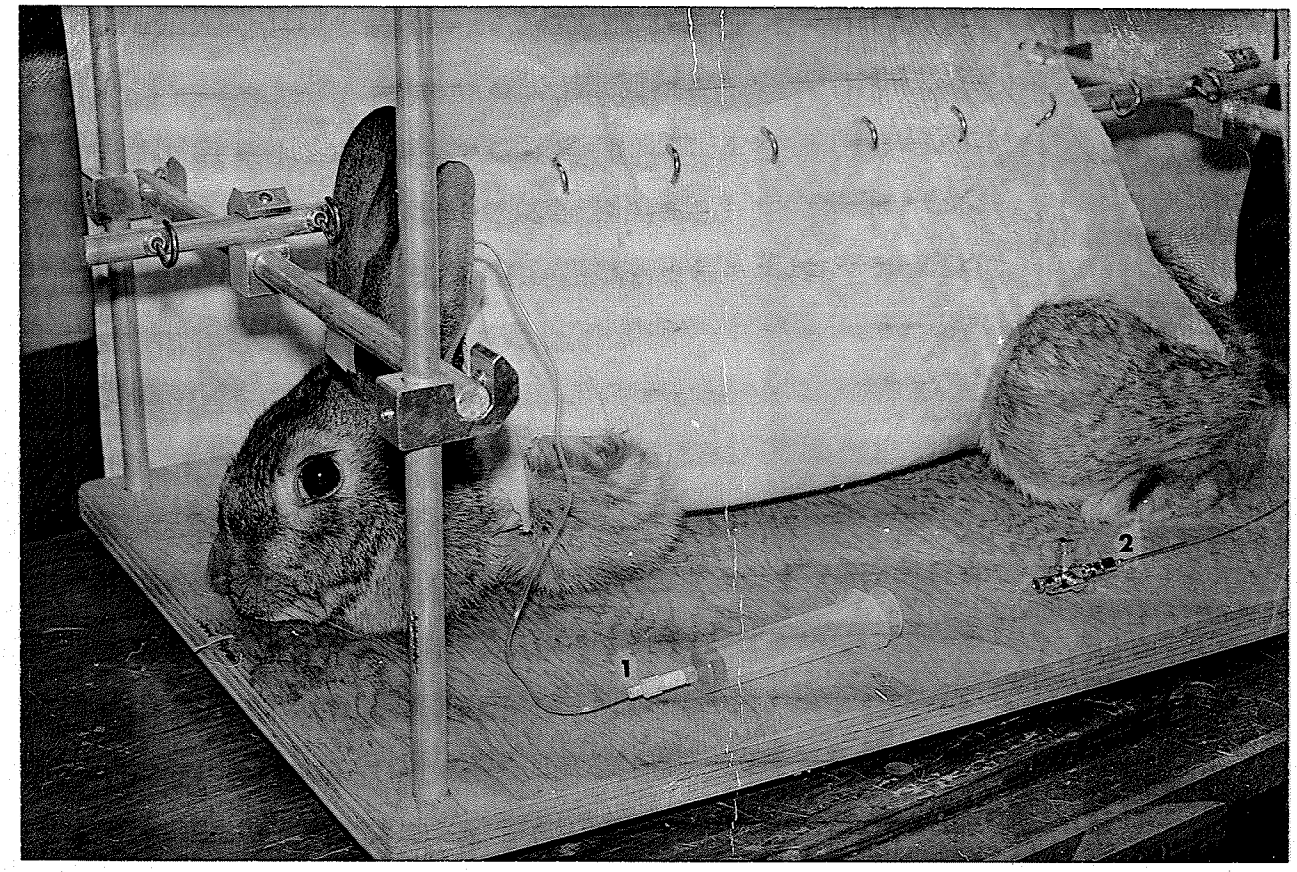
Adult male and female rabbits were randomly selected for treatment with P.V.P.-10 or mannitol. There were seven animals in the control group ranging in weight from 2.9 to 3.8 kg., and seven animals in the P.V.P.-10 treated group ranging in weight from 3.0 to 3.5 kg.

The preparation of each animal before P.V.P.-10 and mannitol treatment was identical. No general anaesthetic was used. Each rabbit was placed in an individual cage 24 hours prior to the experiment and given a diet of lettuce and water. This ensured adequate hydration and maintained the urine pH above 7.0 throughout the experiment. The blindfolded rabbit was held on its back while a Foley catheter (size 8 or 10) was inserted into the urinary bladder. A removable director inside the catheter was required when catheterising female rabbits. The catheter balloon was inflated to three ml. and the catheter was allowed to drain continuously into a graduated cylinder. After shaving the skin over the femoral artery the skin and deep tissues were infiltrated with 1 percent lidocaine (without adrenalin). A small cutdown was made and the proximal end of the femoral artery was cannulated with a small polyethylene tubing filled with saline and fitted with a three-way stopcock which was attached to a mercury manometer for the recording of blood pressure. Heparin was not used since it is known to interfere with salicylate binding. The rabbit was suspended in a canvas sling which allowed the rabbit's limbs to have contact with the ground (Figure 3). In this way restraint stress was avoided. A probe from a Telethermometer was inserted into the rectum. A scalp vein needle (size 23) was inserted into an ear vein for infusions.

During the study, glomerular filtration rate was measured using Cobalt 57 (Co^{57}) labelled vitamin B_{12}.[63, 64] This technique required pretreatment of the animal with unlabelled B_{12} (30 μg./kg.), intramuscularly, 12 hours and 1 hour prior to the study. A priming dose of Co^{57} labelled B_{12} was given intravenously, followed by a slow continuous infusion of the sustaining dose delivered by a Harvard pump. (Co^{57} 0.02 μc./kg. and stable B_{12} 30 μg./kg.

Figure 3

ANIMAL PREPARATION



Animal preparation used in all in vivo studies. (1) ear vein needle-cannula (2) femoral artery cannula. Not visualised - indwelling urinary bladder catheter.

were combined and diluted to six ml. in saline. Two ml. of this solution was used as the priming dose and the remaining four ml. for the sustaining dose was diluted to 10 ml. in saline and administered at a rate of 0.02 ml./minute). During the 45 minute period for equilibration of Co^{57} the animal also received a continuous intravenous infusion of saline at a rate of 10 ml./kg./hr., in order to ensure adequate hydration.

After the 45 minute equilibration period sodium salicylate in a dose of 250 mg./kg. (250 mg./ml. solution) was infused intravenously in 15 minutes. 45 minutes was allowed for distribution of the salicylate. Treatment was then started and continued for four hours. The animal treated with P.V.P.-10 received P.V.P.-10, 20 g./100 ml. in saline, pH 7.4, and the control group received mannitol, 2.75, 3.10 or 3.66 g./100 ml. (150, 175, 200 mM./100 ml.) in saline, pH 7.4. The dose of mannitol was varied in order to obtain similar urine outputs in all animals. The P.V.P.-10 and mannitol solutions were administered continuously, intravenously, at a rate of 10 ml./kg./hr. delivered by a Harvard pump.

Arterial blood and urine samples were taken before the administration of salicylate, before treatment with P.V.P.-10 or mannitol and periodically during treatment. Five ml. of arterial blood was collected anaerobically in a heparinised syringe. A three ml. aliquot was centrifuged and the supernatant plasma decanted. The concentration of total salicylate in the plasma was measured. One ml. of the plasma was counted in a Tricarb liquid scintillation counter for Co^{57} activity. The remaining two ml. of arterial blood was maintained in the anaerobic state and used for measurement of pCO_2 with a Beckman CO_2 electrode and for measurement of arterial pH with an

Astrup apparatus. Arterial pCO_2 was corrected for the measured body temperature of the rabbits.[65] After the collection of each urine sample the bladder was rinsed with 10 ml. of saline in order to ensure complete bladder emptying. Urine volumes were recorded and the pH and concentrations of salicylate were measured. One ml. of urine was counted for Co^{57} activity.

Equilibrium dialysis studies were used in three experiments to correct the plasma Co^{57} counts for the small degree of plasma protein binding of Co^{57} labelled B_{12}.[63,64] The binding of Co^{57} labelled B_{12} by P.V.P.-10 was studied using ultrafiltration and equilibrium dialysis techniques.

The results of the effect of treatment of experimental salicylate intoxication with P.V.P.-10 and mannitol were evaluated by calculating the mean and standard deviation for each set of observations in each group of rabbits and then comparing the difference between these means using the Student "t" test.[66]

The amount of P.V.P. excreted in the urine during the four hours of treatment was measured in four of the seven rabbits treated with P.V.P.-10.

RESULTS

METHODS OF DETERMINATION OF SALICYLATE AND P.V.P.

The Trinder method[56] was used for measurement of the concentration of salicylate in water, saline, solutions containing P.V.P. and albumin, urine and serum. The accuracy of this method was ± 0.5 mg. of salicylate/100 ml. of each of the fluids. Reproducibility was evident from consistent daily standards.

Using our modification of the method of Campbell and Hunter[57], we were able to achieve accuracy of ±0.1 mg. of P.V.P./ml. in serum and

±0.5 mg. of P.V.P./ml. in urine. Reproducibility was achieved if a 15 minute period was allowed for completion of the colour reaction.

MEMBRANE RETENTION OF SALICYLATE

The concentrations of salicylate in the filtrates after ultrafiltration of solutions of known concentrations of salicylate in distilled water and in saline are shown in Table I. The values given for the concentration of salicylate in the filtrate are the average of duplicate determinations. The concentration of salicylate in the filtrate increased in those filtering solutions prepared in saline. This is in agreement with the results of Ambard and Trautmann.[61] The concentration of salicylate in the filtrate showed a straight line relationship with the concentration of salicylate in the filtering solution (Figure 4). All solutions which were ultrafiltered were prepared in saline and the results of these binding studies were corrected using the graph shown in Figure 4. By plotting the measured concentration of unbound salicylate in the filtrate on the abscissa, the corrected concentration of unbound salicylate in the filtering solution could be read from the ordinate.

I. IN VITRO BINDING OF SODIUM SALICYLATE BY P.V.P.

A. Effect of Molecular Weight of P.V.P. and Concentration of Salicylate

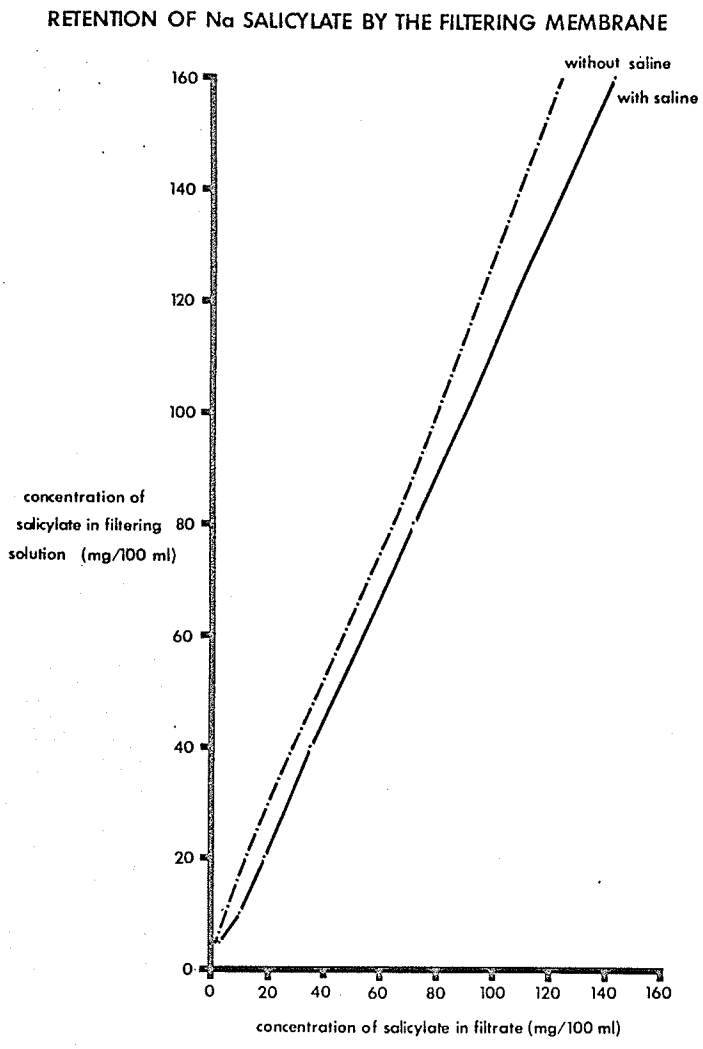
Tables IIa and IIb show the results of the equilibrium dialysis studies of the effects of the molecular weight of P.V.P. and the concentration of salicylate on the binding of salicylate by P.V.P. The concentrations of P.V.P. for P.V.P.-10, P.V.P.-40 and P.V.P.-160 were 10, 40 and 160 mg./ml., respectively (0.01 mM./ml.) and for P.V.P.-360 was 36 mg./ml. (0.001 mM./ml.)

Table I

RETENTION OF SODIUM SALICYLATE BY THE FILTERING MEMBRANE

Concentration of NaCl in filtering solution (g./100 ml.)	Concentration of total salicylate in filtering solution (mg./100 ml.)	Concentration of salicylate in filtrate (mg./100 ml.)
0	5	2.0
0	10	5.2
0	20	12.2
0	40	28.4
0	80	64.0
0	160	123.2
0.9	5	3.0
0.9	10	9.0
0.9	20	19.0
0.9	40	35.6
0.9	80	71.2
0.9	160	142.0

Figure 4



Data taken from Table I

Table II a

EFFECT OF THE MOLECULAR WEIGHT OF P.V.P. ON THE IN VITRO BINDING OF SODIUM SALICYLATE

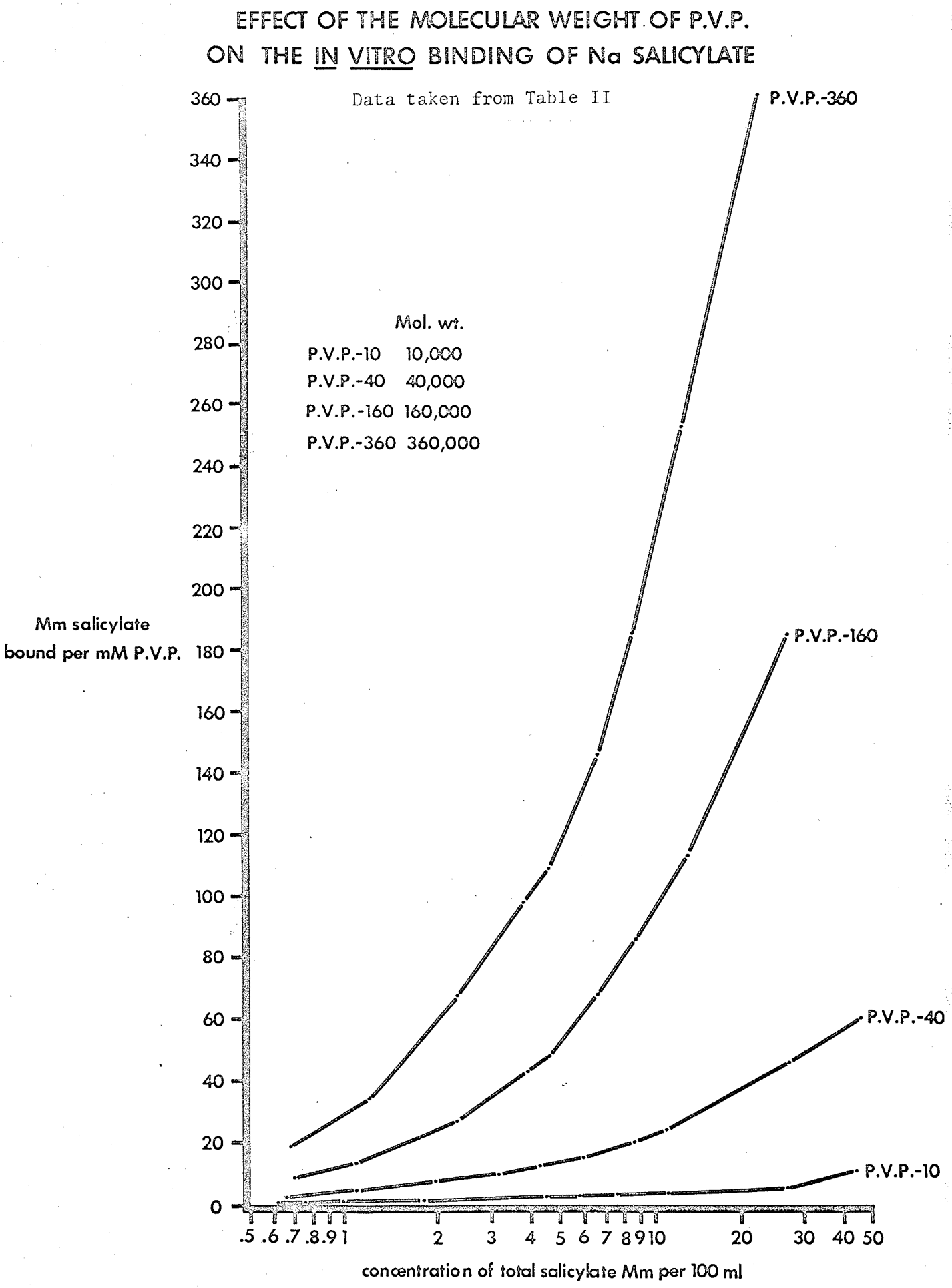
Final concentration of total salicylate in dialysant (mg./100 ml.)	Final concentration of unbound salicylate in dialysate (mg./100 ml.)	Final volume of dialysant (ml.)	Volume occupied by P.V.P. in dialysant (ml.)	Volume of water in dialysant (ml.)	Final content of total salicylate in dialysant (mg.)	Final content of unbound salicylate in dialysamt (mg.)	Final content of bound salicylate in dialysant (mg.)	mM salicylate bound per mM P.V.P.
I. P.V.P.-10 (10,000 molecular weight)								
98.0	91.5	11.0	0.06	10.9	10.80	10.0	0.8	0.5
160.0	148.0	11.0	0.06	10.9	17.6	16.2	1.4	0.9
300.0	291.0	11.2	0.06	10.9	33.6	31.9	1.7	1.7
530.0	515.0	11.3	0.06	10.9	60.0	56.5	3.5	2.2
723.0	717.0	11.5	0.06	10.9	83.1	78.7	4.4	2.8
930.0	921.0	11.6	0.06	11.0	108.0	103.1	4.9	3.1
1211.0	1195.0	11.6	0.06	11.0	142.0	136.4	5.6	3.5
1770.0	1750.0	11.4	0.06	10.9	201.0	192.2	8.8	5.5
4300.0	4223.0	11.2	0.06	10.9	483.0	465.4	17.6	11.0
6990.0	6830.0	10.9	0.06	10.8	762.0	739.6	22.4	14.0
II. P.V.P.-40 (40,000 molecular weight)								
105.0	79.5	14.0	0.24	13.7	14.7	10.9	3.8	2.4
179.0	124.0	14.2	0.24	13.9	25.4	17.2	8.2	5.1
365.0	282.0	13.8	0.24	13.5	50.4	38.5	11.9	7.7
510.0	398.0	13.0	0.24	12.7	66.4	50.9	15.5	9.7
684.0	544.0	13.2	0.24	12.9	90.4	70.6	19.8	12.4
943.0	775.0	13.7	0.24	13.4	129.0	104.0	25.0	15.6
1360.0	1150.0	14.2	0.24	13.9	193.0	161.0	32.0	20.0
1770.0	1530.0	14.7	0.24	14.4	260.0	221.3	38.7	24.2
4280.0	3850.0	14.8	0.24	14.5	634.0	560.4	73.6	46.0
7260.0	6700.0	14.9	0.24	14.6	1080.0	983.0	97.0	60.6

Table II b

EFFECT OF THE MOLECULAR WEIGHT OF P.V.P. ON THE IN VITRO BINDING OF SODIUM SALICYLATE

Final concentration of total salicylate in dialysant (mg./100 ml.)	Final concentration of unbound salicylate in dialysate (mg./100 ml.)	Final volume of dialysant (ml.)	Volume occupied by P.V.P. in dialysant (ml.)	Volume of water in dialysant (ml.)	Final content of total salicylate in dialysant (mg.)	Final content of unbound salicylate in dialysant (mg.)	Final content of bound salicylate in dialysant (mg.)	mM salicylate bound per mM P.V.P.
III. P.V.P.-160 (160,000 molecular weight)								
111.0	47.8	21.0	0.96	20.0	23.3	9.6	13.7	8.6
175.0	75.0	21.4	0.96	20.4	37.5	15.3	22.2	13.9
372.0	175.0	19.8	0.96	18.8	73.7	33.0	40.7	26.7
620.0	277.0	19.3	0.96	18.3	119.6	50.8	68.8	43.0
726.0	380.0	21.1	0.96	20.1	153.1	76.5	76.5	47.8
1040.0	559.0	21.3	0.96	20.3	221.7	113.5	108.2	67.7
1382.0	785.0	21.4	0.96	20.4	296.0	160.5	135.5	84.8
2030.0	1255.0	21.5	0.96	20.5	436.0	257.0	179.5	112.1
4250.0	3000.0	21.3	0.96	20.3	906.0	610.0	296.0	185.0
7265.0	5900.0	21.4	0.96	20.4	1551.0	1183.0	368.0	230.0
IV. P.V.P.-360 (360,000 molecular weight)								
108.0	85.7	6.4	0.10	6.3	6.9	5.4	1.5	19.0
192.0	153.0	6.7	0.10	6.6	12.9	10.1	2.7	34.0
378.0	303.0	6.7	0.10	6.6	25.3	19.9	5.4	67.0
610.0	500.0	6.2	0.10	6.1	37.8	30.5	7.3	98.0
730.0	626.0	6.7	0.10	6.6	49.9	41.3	8.6	108.0
1060.0	924.0	6.4	0.10	6.3	69.8	58.2	11.6	145.0
1385.0	1175.0	6.5	0.10	6.4	90.0	75.2	14.8	185.0
1960.0	1655.0	6.6	0.10	6.5	129.2	109.0	20.2	252.0
3450.0	3080.0	6.7	0.10	6.6	231.0	203.2	28.8	360.0
7290.0	6630.0	6.4	0.10	6.3	466.0	424.0	42.0	526.0

Figure 5


EFFECT OF THE MOLECULAR WEIGHT OF P.V.P.
ON THE IN VITRO BINDING OF Na SALICYLATE
Data taken from Table II
Mol. wt.
P.V.P.-10 10,000
P.V.P.-40 40,000
P.V.P.-160 160,000
P.V.P.-360 360,000
P.V.P.-360
P.V.P.-160
P.V.P.-40
P.V.P.-10
Mm salicylate bound per mM P.V.P.
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360
.5 .6 .7 .8 .9 1 2 3 4 5 6 7 8 9 10 20 30 40 50
concentration of total salicylate Mm per 100 ml

The amount of salicylate bound by P.V.P. of each molecular weight increased with increases in the concentration of salicylate. The amount of salicylate bound for any given concentration of salicylate increased with increases in the molecular weight of P.V.P. The final concentration of total salicylate in the dialysant was converted to mM./100 ml. (100 mg./100 ml. equals 6.25 mEq./100 ml.) and plotted against the calculated mM. of salicylate bound per mM. of P.V.P. This curvilinear relationship is shown in Figure 5.

B. Effect of Concentration of P.V.P.-10 on the In Vitro Binding of Salicylate

Table III shows the results of equilibrium dialysis studies of the effect of the concentration of P.V.P.-10 and the concentration of salicylate on the binding of salicylate by P.V.P.-10. In vitro, a substantial amount of salicylate was bound by P.V.P.-10. The binding of salicylate by P.V.P.-10 increased with increases in the concentration of P.V.P.-10. For any given concentration of P.V.P.-10 the binding of salicylate increased with increases in the salicylate concentration. The final content of total salicylate in the dialysant was plotted against the final content of bound salicylate in the dialysant for each concentration of P.V.P.-10. This curvilinear relationship is shown in Figure 6.

C. Effect of pH, Double Dialysis and Exhaustive Dialysis on the Stability of Binding of Salicylate by P.V.P.-10

(i) Effect of pH (dialysis)

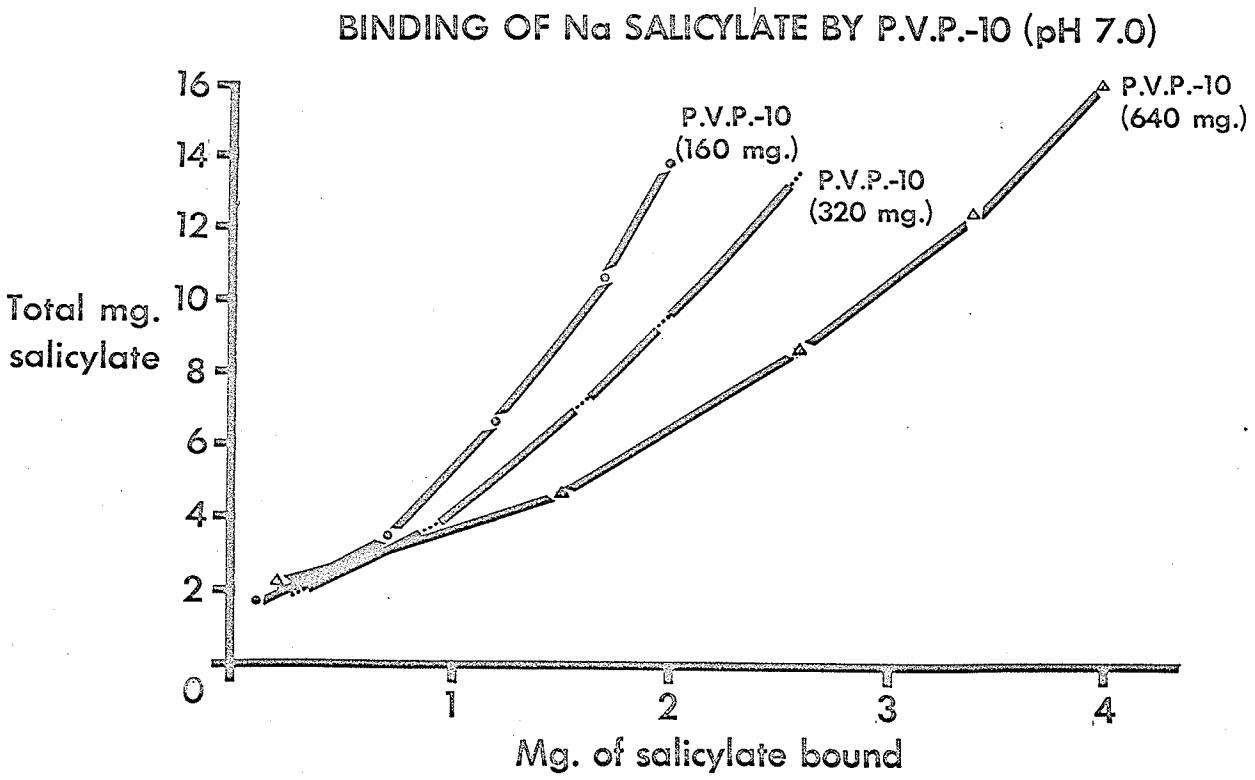
The pH at the end of each dialysis varied by only 0.1 pH units from the initial pH. It was observed that 0.5 unit increments in pH from 4.0 to 8.0 had no effect on the amount of salicylate bound by P.V.P.-10 when P.V.P.-10 (32 mg./ml.) was dialysed against salicylate (80 mg./100 ml.).

Table I-II

EFFECT OF CONCENTRATION OF P.V.P.-10 ON THE IN VITRO BINDING OF SODIUM SALICYLATE

Content of P.V.P. in dialysant (mg.)	Final concentration of total salicylate in dialysant (mg./100 ml.)	Final concentration of unbound salicylate in dialysate (mg./100 ml.)	Final volume of dialysant (ml.)	Volume occupied by P.V.P. in dialysant	Volume of water in dialysant (ml.)	Final content of total salicylate in dialysant (mg.)	Final content of unbound salicylate in dialysant (mg.)	Final content of bound salicylate in dialysant (mg.)
640	114.0	93.5	14.2	0.4	13.8	16.0	12.9	4.1
640	88.5	65.2	14.2	0.4	13.8	12.4	9.0	3.4
640	60.6	43.4	14.2	0.4	13.8	8.6	6.0	2.6
640	32.4	22.4	14.2	0.4	13.8	4.6	3.1	1.5
640	14.1	13.0	14.2	0.4	13.8	2.0	1.8	0.2
320	124.0	102.5	11.7	0.2	11.5	14.5	11.8	2.7
320	81.2	65.2	11.7	0.2	11.5	9.5	7.5	2.0
320	60.7	47.8	11.7	0.2	11.5	7.1	5.5	1.6
320	31.6	24.2	11.7	0.2	11.5	3.7	2.8	0.9
320	16.2	13.9	11.7	0.2	11.5	1.9	1.6	0.3
160	127.0	110.0	10.6	0.1	10.5	13.5	11.6	1.9
160	94.0	84.7	10.6	0.1	10.5	10.6	8.9	1.7
160	62.2	51.4	10.6	0.1	10.5	6.6	5.4	1.2
160	33.0	26.6	10.6	0.1	10.5	3.5	2.8	0.7
160	16.0	15.2	10.6	0.1	10.5	1.7	1.6	0.1

Figure 6



Data taken from Table III

The binding of 0.29 ± 0.01 mM. of salicylate per mM. of P.V.P.-10 was similar to the results of other dialyses with these particular P.V.P.-10 and salicylate concentrations.

(ii) Effect of pH (ultrafiltration)

The pH at the end of each ultrafiltration varied by only ± 0.1 pH units from the initial pH. The observed concentrations of salicylate in the filtrates were corrected for the membrane retention of salicylate according to Figure 4. It was observed that 1.0 unit increments in pH from 4.0 to 8.0 had no effect on the binding, when the combination of P.V.P.-160 (16 mg./ml.) and salicylate (80 mg./100 ml.) was ultrafiltered. The binding of 6.25 ± 0.01 mM. of salicylate per mM. of P.V.P.-160 was similar to the results of other ultrafiltrations with these particular P.V.P.-160 and salicylate concentrations.

(iii) Effect of double dialysis

By definition, equilibrium is reached with the dialysis technique when the concentration of unbound salicylate in the dialysant is equal to that in the dialysate. Table IV shows the results of the experiments with double dialysis. The concentrations of unbound salicylate in the dialysant after the first dialysis are shown. If binding of salicylate by P.V.P. were irreversible only this unbound salicylate should distribute freely in the second dialysis. The "expected" concentrations of unbound salicylate in the dialysis after second dialysis, assuming irreversibility of binding, are shown. The observed concentrations of unbound salicylate in the dialysate were greater than the "expected" values showing that the binding of salicylate by P.V.P. is reversible under these particular circumstances.

Table IV

EFFECT OF DOUBLE DIALYSIS ON THE STABILITY OF BINDING OF SODIUM SALICYLATE TO P.V.P.-10

Initial concentration of salicylate in the dialyzing solution (mg./100 ml.)	Concentration of P.V.P.-10 in dialysant (mg./ml.)	Concentration of unbound salicylate in dialysant after first dialysis (mg./100 ml.)	Expected concentration of unbound salicylate in dialysate after second dialysis (mg./100 ml.)	Observed concentration of unbound salicylate in dialysate after second dialysis (mg./100 ml.)
180	150	27.6	9.2	19.6
180	100	33.4	11.1	19.8
180	50	43.6	14.5	20.2
180	25	49.6	16.5	20.4
180	12.5	54.8	18.3	20.5
180	0	59.8	19.9	19.8

(iv) Effect of exhaustive dialysis

The exhaustive dialysis experiment was completed to estimate the degree of reversibility of binding of salicylate by P.V.P.-10. In a duplicate study, 48 hours after exhaustive dialysis of a solution of P.V.P.-10 and salicylate, no salicylate was detected in the P.V.P. solution.

D. Effect of Human Albumin and P.V.P.-10 on the In Vitro Binding of Salicylate

This experiment was designed to demonstrate the in vitro interaction of the binding properties of P.V.P. and albumin in an attempt to simulate in vivo conditions as they might occur in the intravascular space. The results are shown in Table V. The binding of salicylate to human albumin, P.V.P.-10 and human albumin with P.V.P.-10, increased as the concentration of salicylate increased. In Figure 7, the mM. of salicylate bound per mM. of binding substance was plotted against the final concentration of total salicylate in the dialysant. This comparison showed that the in vitro binding of salicylate by the combination of P.V.P.-10 and albumin was much less than for albumin alone. However, these results are expressed as the amount of salicylate bound per mM. of binding substance. The P.V.P.-10 with albumin solution contained many more millimoles than did albumin alone. If binding is expressed as the percentage of salicylate in the dialysant which is bound, there is very little difference between binding by albumin or the albumin with P.V.P.-10 (Figure 8).

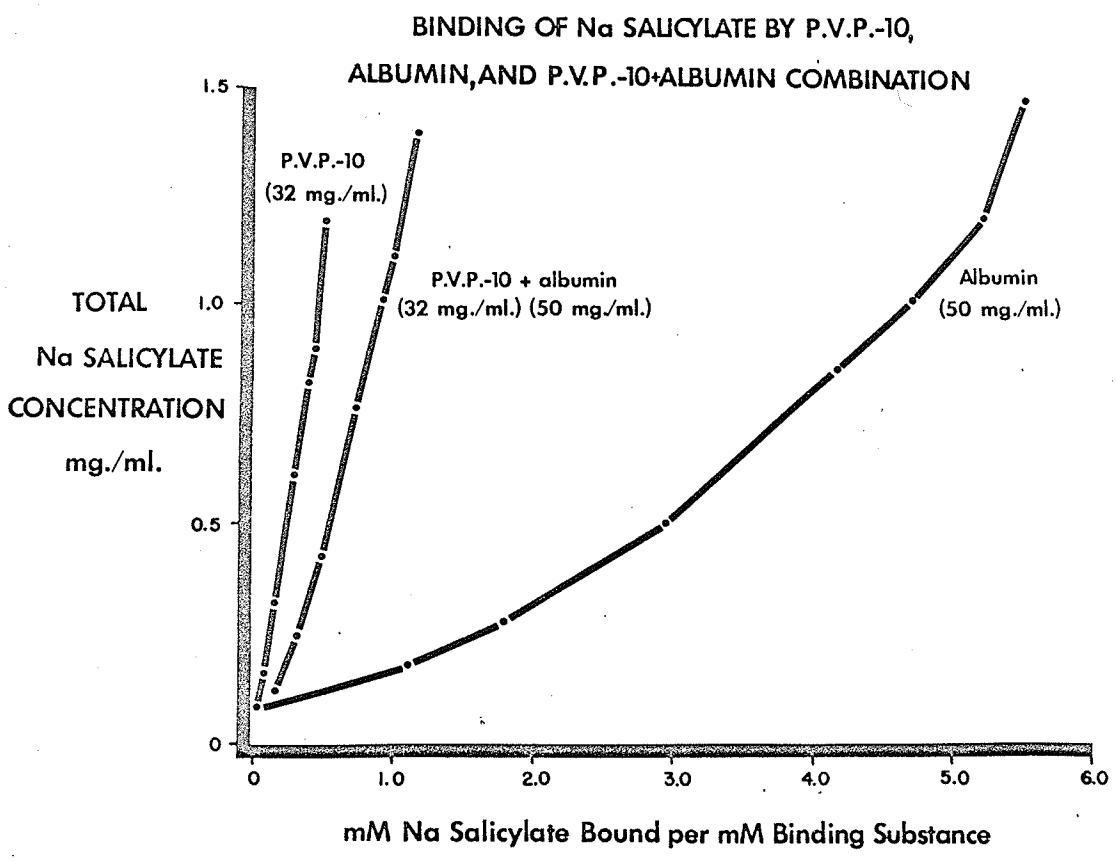
E. Effect of P.V.P.-10 and Human Albumin on the In Vitro Binding of Salicylate in a Three Compartment Dialysis System Simulating an In Vivo Distribution

Table V

IN VITRO BINDING OF SODIUM SALICYLATE BY HUMAN ALBUMIN AND P.V.P.-IO

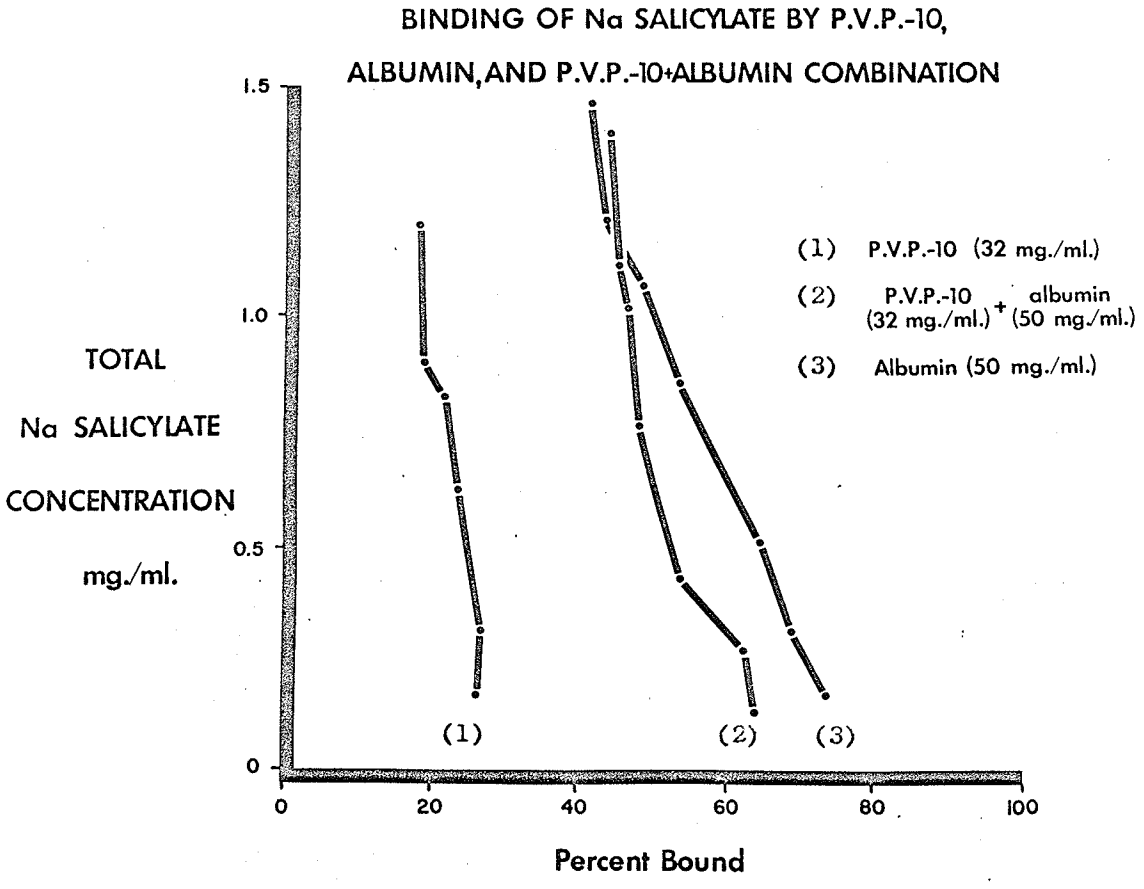
Final concentration of total salicylate in the dialysant (mg./100 ml.)	Final concentration of unbound salicylate in dialysate (mg./100 ml.)	Final volume of dialysant (ml.)	Volume occupied by the binding substance in dialysant (ml.)	Volume of water in dialysant (ml.)	Final content of Total salicylate in dialysant (mg.)	Final content of unbound salicylate in dialysant (mg.)	Final content of bound salicylate in dialysant (mg.)	mM. salicylate bound per mM. binding substance
I. EFFECT OF P.V.P.-10 (32 mg./[illegible] ml.)								
119.0	99.0	11.70	0.20	11.50	13.92	11.38	2.54	0.49
89.0	73.5	11.70	0.20	11.50	11.41	9.30	2.12	0.42
82.0	66.0	11.70	0.20	11.50	9.59	7.59	2.00	0.39
61.0	48.5	11.70	0.20	11.50	7.14	5.58	1.56	0.31
32.0	25.0	11.70	0.20	11.50	3.74	2.87	0.87	0.17
16.5	14.0	11.70	0.20	11.50	1.93	1.61	0.32	0.06
9.0	9.0	11.70	0.20	11.50	1.05	1.03	0.02	0.00
II. EFFECT OF HUMAN ALBUMIN (50 mg./ml.)								
147.5	90.0	10.60	0.38	10.22	15.63	9.20	6.44	5.54
120.0	64.5	10.60	0.38	10.22	12.72	6.59	6.13	5.28
106.5	56.5	10.60	0.38	10.22	11.29	5.77	5.51	4.76
85.5	41.0	10.60	0.38	10.22	9.06	4.19	4.87	4.21
50.0	18.0	10.60	0.38	10.22	5.30	1.84	3.46	2.98
28.5	9.0	10.60	0.38	10.22	3.02	0.92	2.10	1.81
17.0	5.0	10.60	0.38	10.22	1.80	0.51	1.29	1.12
III. EFFECT OF P.V.P.-10 (32 mg./ml.) WITH HUMAN ALBUMIN (50 mg./ml.)								
140.0	85.0	12.30	0.58	11.72	17.22	9.96	7.26	1.16
110.5	62.5	12.30	0.58	11.72	13.59	7.32	6.21	1.00
100.5	56.0	12.30	0.58	11.72	12.36	6.56	5.80	0.92
75.5	41.0	12.30	0.58	11.72	9.29	4.80	4.48	0.71
42.5	20.5	12.30	0.58	11.72	5.23	2.40	2.82	0.45
26.0	10.5	12.30	0.58	11.72	3.20	1.23	1.97	0.31
12.5	5.0	12.30	0.58	11.72	1.54	0.59	0.95	0.15

Figure 7



Data taken from Table V

Figure 8



Data taken from Table V

Results are shown in Table VI. The three compartment system consisted of two Visking tubing dialysis bags, one within the other. The inner bag filled with a solution of albumin and P.V.P. in saline, represented the "intravascular" space. The outer bag, filled with P.V.P.-10 in saline, represented the "interstitial" space. The bags were placed in a solution containing saline, which represented the "intracellular" space. Each of the three fluid compartments contained initially 80 mg./100 ml. of salicylate. Salicylate was equally distributed through all three compartments. This combined volume represented the "total body water". P.V.P.-10 was equally distributed in the two dialysis bags. This combined volume represented the "extracellular fluid" space. At equilibrium the distribution of salicylate changed so that a greater concentration of salicylate was present in both the "intravascular" and "interstitial" spaces while the concentration of salicylate in the "intracellular" space was substantially decreased. Volume changes greater than the experimental error ± 0.1 ml. were not detected.

II. IN VIVO DISTRIBUTION AND EXCRETION OF P.V.P.-10 IN RABBITS

The concentrations of P.V.P.-10 in the serum of rabbits (n=10), after intravenous administration of 4.0 g./kg., are shown in Table VII. The mean ± S.D. of the concentration of P.V.P. in the serum for each sample period was calculated and plotted against time in Figure 9. A concentration of P.V.P.-10 in the serum of 22.1 ± 2.3 mg./ml. was reached at the end of the two-hour infusion period and rapidly decreased to 12.3 ± 3.7 mg./ml. one hour thereafter and to 4.7 ± 1.4 mg./ml. 4 hours after the end of the infusion. In 24 hours the serum concentration was 1.8 ± 0.5 mg./ml. and only a trace was detected at 72 hours.

Table VI

EFFECT OF ALBUMIN AND P.V.P.-10 ON THE IN VITRO BINDING OF SODIUM SALICYLATE IN A THREE COMPARTMENT SYSTEM SIMULATING AN IN VIVO DISTRIBUTION

	"Intravascular Space"	"Interstitial Space"	"Intracellular Space"
Binding Substance	Albumin (50 mg./ml.) P.V.P.-10 (32 mg./ml)	P.V.P.-10 (32 mg./ml.)	
Concentration of Na salicylate before dialysis (mg./100 ml.)	80.0	80.0	80.0
Concentration of Na salicylate after dialysis (mg./100 ml.)	95.3	86.0	46.2

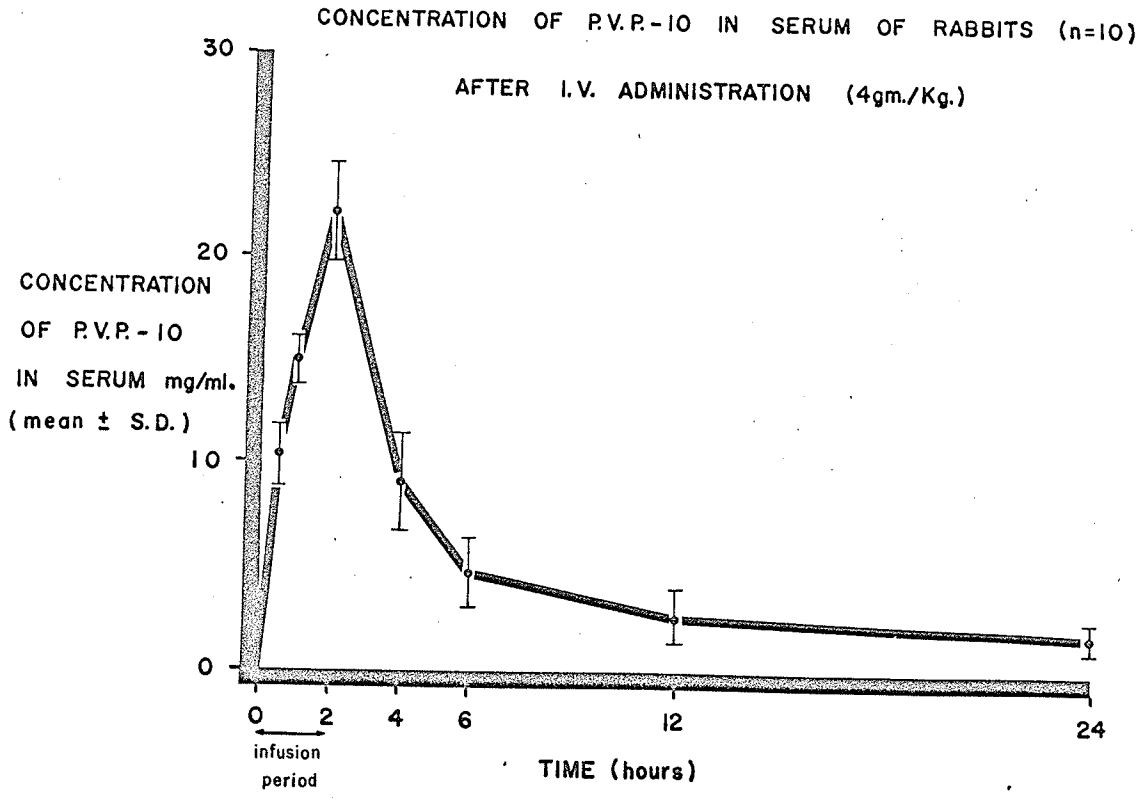
Table VII

CONCENTRATION OF P.V.P.-10 (mg./ml.) IN SERUM OF RABBITS (n = 10)

AFTER INTRAVENOUS ADMINISTRATION (4 g./kg.)

P.V.P.-10 infusion	Time (hours)	Animal Number										Mean ± S.D. (mg./ml.)
		1	2	3	4	5	6	7	8	9	10	
started	0											
	1/2	10.2	12.2	10.1	10.5	10.1	9.6	8.2	9.7	11.9	11.1	9.36 ± 1.6
	1	15.0	14.8	12.6	15.9	12.6	13.5	14.8	16.8	15.9	16.8	14.8 ± 1.4
	1 1/2	17.7	15.7	16.6	18.6	18.3	18.9	18.9	18.5	17.2	18.3	17.4 ± 1.2
completed	2	22.8	14.8	16.9	21.7	21.6	23.8	24.2	20.6	23.6	23.6	22.1 ± 2.3
	2 1/2	20.0	12.6	12.1	19.6	16.2	22.3	20.6	11.7	14.3	21.2	17.0 ± 4.1
	3	15.6	10.0	8.4	--	11.7	19.2	14.4	7.2	9.3	14.7	12.3 ± 3.7
	4	9.1	9.5	7.0	17.3	7.0	11.6	10.6	6.8	8.4	11.2	9.0 ± 1.8
	5	7.5	7.5	--	14.8	5.2	8.1	5.6	4.1	6.5	8.9	7.5 ± 3.1
	6	5.7	7.2	4.0	10.3	3.9	4.2	3.9	3.0	4.1	6.8	4.7 ± 1.4
	12	--	6.2	2.3	6.5	2.3	--	2.1	2.1	3.0	4.1	2.6 ± 0.8
	24	1.7	4.6	1.2	3.4	1.8	2.7	1.4	1.8	2.4	3.4	1.8 ± 0.5
	48	1.0	2.6	--	--	--	1.1	1.0	0.5	0.9	1.1	1.0 ± 0.3
	72	0.8	2.2	--	--	--	0.4	--	0.2	0.4	0.7	0.7 ± 0.4

Figure 9



Data taken from Table VII

The cumulative urinary excretion of the administered P.V.P.-10 was calculated and the results are shown in Table VIII. The mean ± S.D. of the cumulative excretion of P.V.P.-10 in the urine for each sample period was calculated and plotted against time in Figure 10. By the end of the two-hour infusion period 62.7 ± 4.2% of the total administered dose was excreted in the urine. At 6 hours and 24 hours after the start of infusion 89.1 ± 1.9% and 96.5 ± 1.1% respectively was excreted. In five rabbits followed for 48 hours 98.8 ± 0.3% was excreted.

The distribution of P.V.P.-10 was calculated knowing the amount of administered dose, the serum concentration and the amount of urinary excretion of P.V.P.-10 at any given time. The extracellular fluid volume (E.C.F.) was estimated as 18% of body weight in kg.[67] The calculated volume of distribution of P.V.P.-10 approached that of the E.C.F., varying from approximately 14% to 17% of body weight.

The fluid input-output during the two-hour P.V.P.-10 infusion period for the 10 rabbits was measured to determine fluid balance. These observations are shown in Table IX. Positive fluid balance was observed in five animals with a mean of 0.66 ml./kg./hr. and negative fluid balance was observed in five animals with a mean of 0.67 ml./kg./hr. There was therefore no apparent osmotic diuresis in spite of the high filtered load. This is in agreement with the observations of others.[47, 48]

The animals were closely observed during the study and no untoward effects were noted. All animals took food and fluid well throughout the experimental period. When the animals were killed the bowel showed no signs of haemorrhage or infarction. These changes can occur as a result of restraint stress.

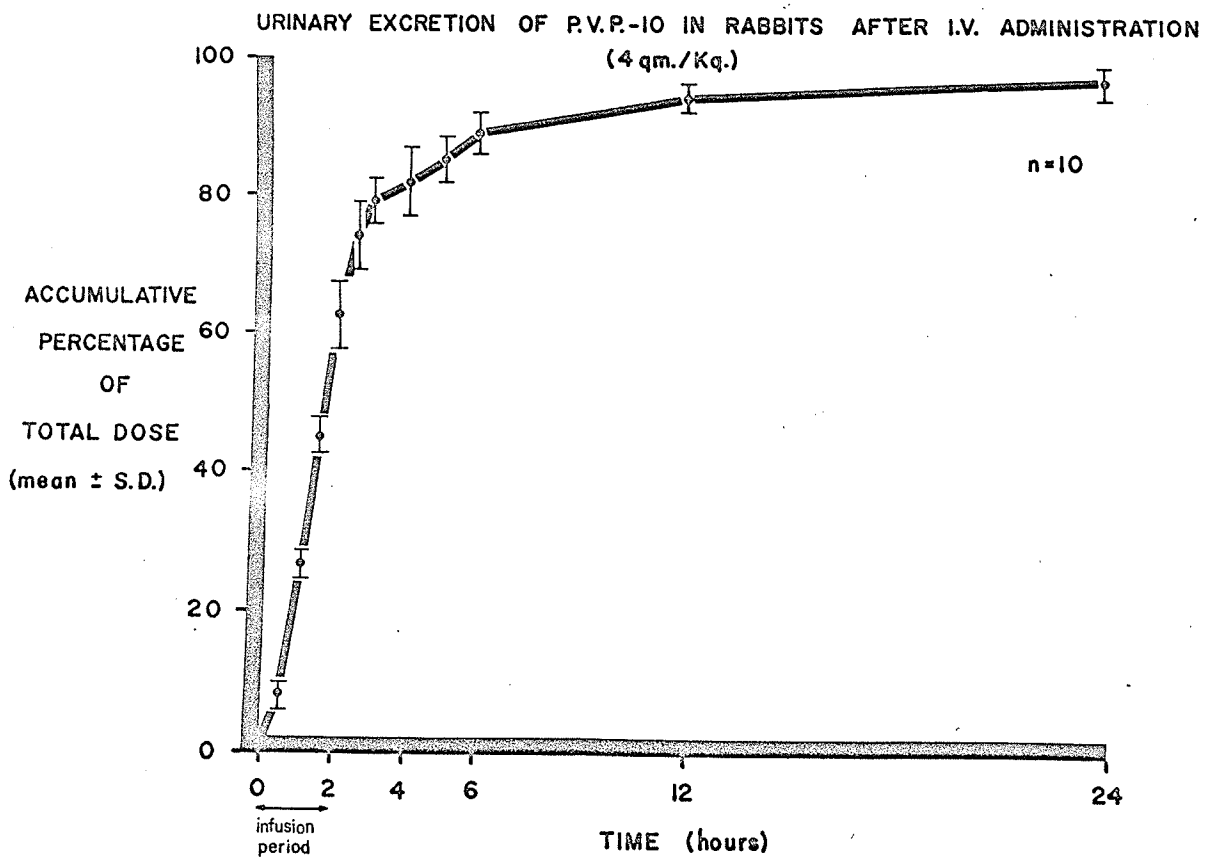
Table VIII

CUMULATIVE PERCENTAGE OF TOTAL ADMINISTERED DOSE OF P.V.P.-10

EXCRETED IN URINE OF RABBITS (n = 10)

P.V.P.-10 infusion	Time (hours)	Animal Numbers 1	2	3	4	5	6	7	8	9	10	Mean ± S.D. %
started	0											
	1/2	8.1	9.8	5.9	7.7	8.8	9.2	9.9	8.4	10.1	8.5	8.7 ± 1.4
	1	27.5	27.2	25.8	25.2	25.3	27.2	28.3	25.3	28.9	26.4	26.7 ± 1.3
	1 1/2	40.9	44.8	45.3	43.9	46.1	45.1	48.0	43.0	47.4	45.6	45.0 ± 2.0
completed	2	58.9	67.8	66.7	54.0	62.6	60.4	65.5	60.8	66.1	63.9	62.7 ± 4.2
	2 1/2	72.2	77.7	79.1	63.7	73.9	76.2	78.1	72.1	73.6	77.2	74.4 ± 4.5
	3	76.7	82.1	83.3	--	76.1	79.0	81.6	76.8	77.2	79.3	79.1 ± 2.6
	4	82.2	--	88.3	72.3	80.6	81.1	86.1	80.0	83.5	81.4	81.7 ± 4.5
	5	84.9	87.6	--	82.2	83.0	82.0	90.3	83.2	87.2	85.7	85.1 ± 2.8
	6	86.9	89.8	91.6	89.3	85.8	87.5	91.8	88.9	89.9	89.1	89.1 ± 1.9
	12	94.2	--	94.4	--	94.4	93.1	96.7	93.8	94.7	93.1	94.3 ± 1.1
	24	97.2	94.0	95.6	95.0	97.4	96.8	97.9	96.3	97.8	97.0	96.5 ± 1.1
	48	--	94.5	--	--	--	--	99.2	98.4	98.9	98.6	98.8 ± 0.3

Figure 10



Data taken from Table VIII

Table IX

EFFECT OF INTRAVENOUS ADMINISTRATION OF P.V.P.-10

ON FLUID BALANCE IN RABBITS (n = 10)

Animal Number	Body Weight (kg)	Intravenous Input (ml./2 hrs.)	Urine Output (ml./2 hrs.)	Intravenous Input (ml./kg./hr.)	Urine Output (ml./kg./hr.)	Positive Balance (ml./kg./hr.)	Negative Balance (ml./kg./hr.)
1	3.3	132	156	2.00	2.36		0.36
2	3.0	120	98	2.00	1.63	0.37	
3	2.3	92	82	2.00	1.78	0.22	
4	4.8	192	103	2.00	1.08	0.92	
5	2.6	104	140	2.00	2.70		0.70
6	2.8	112	50	2.00	0.90	1.10	
7	2.8	112	135	2.00	2.41		0.41
8	2.4	96	50	2.00	1.04	0.70	
9	3.1	124	214	2.00	3.45		1.45
10	3.0	120	146	2.00	2.43		0.43
					Total (ml./kg./hr.)	3.3	3.35
					Mean (ml./kg./hr.)	0.66	0.67

The histological examinations of the liver, spleen and kidney were performed by Dr. J. Hoogstraten, Chief of Pathology, Winnipeg Children's Hospital. No abnormality was noted in the sections of liver and spleen. In the kidney, very scattered changes, isolated and shown in the photomicrograph (Figure 11), were observed. The observed swelling of tubular cells seemed to be limited to the loop of Henle. These changes were found to be identical with the tubular changes observed in the reversible osmotic nephrosis produced by a number of substances,[68] and were similar to those observed by others after the administration of P.V.P.[25,29] The changes have been found to resolve with no evidence of cell death.[25,29]

III. COMPARISON OF THE EFFICACY OF TREATMENT OF EXPERIMENTAL SALICYLATE INTOXIGATION IN RABBITS WITH THE INTRAVENOUS ADMINISTRATION OF P.V.P.-10 AND A CONTROL MANNITOL SOLUTION

The results of the experiments studying the effects of treatment with P.V.P.-10 and mannitol are shown in Tables X, XI, XII, And XIII, and Figure 12. Comparisons between the results of treatment in the two groups of animals were made by comparing the means of measurements made in the seven animals which received P.V.P.-10 with those in the seven animals which received mannitol.

The concentrations of total salicylate in the plasma are shown in Table X and Figure 12. There was no significant difference between the concentrations of total salicylate in the plasma in the periods before treatment. After the start of treatment the animals treated with P.V.P.-10 showed concentrations of total salicylate in the plasma which were significantly lower than those of the animals treated with mannitol. The significance of this difference increased from the first to the fourth hour of treatment ($p < 0.01$ and $p < 0.001$, respectively),

Figure 11

PHOTOMICROGRAPH OF RABBIT KIDNEY AFTER

INTRAVENOUS ADMINISTRATION OF P.V.P.-10



Swelling or renal tubular cells is observed
in the centre of the photomicrograph

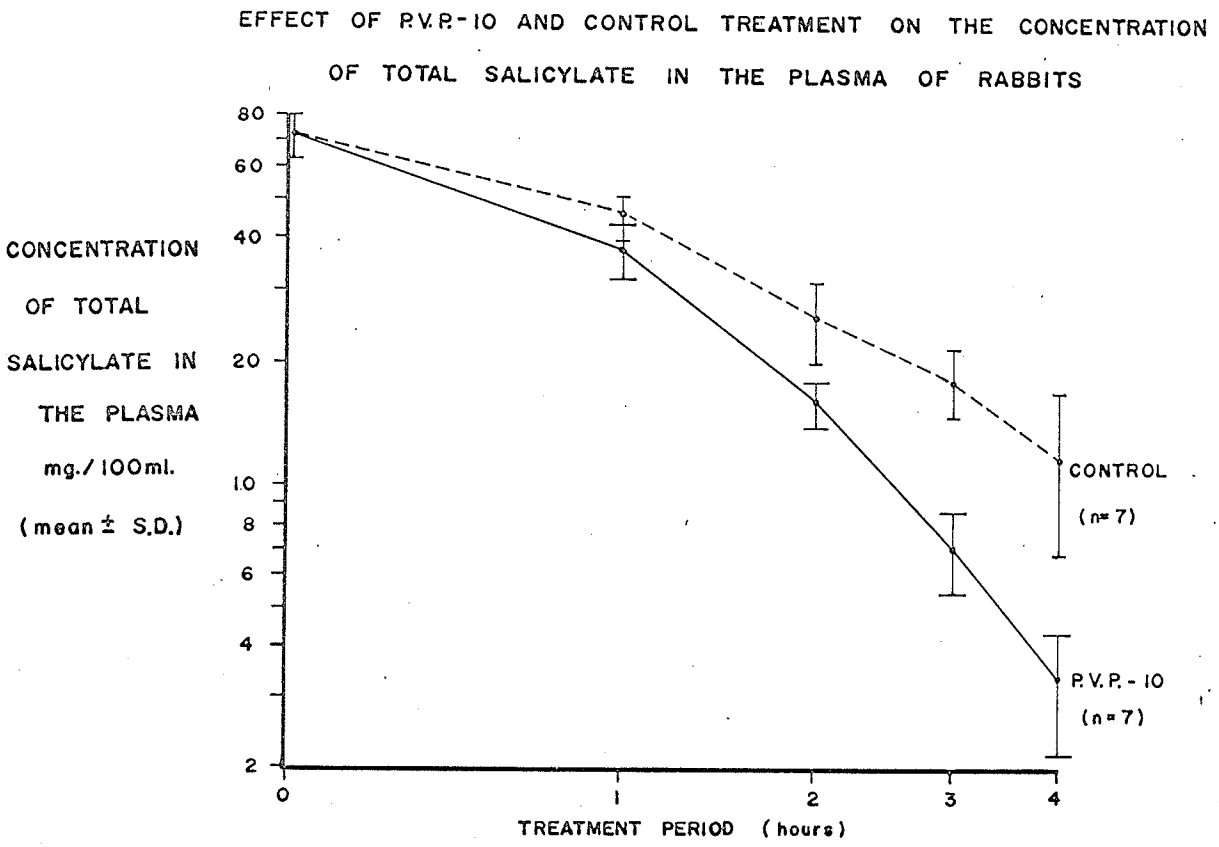
Table X

EFFECT OF TREATMENT ON THE CONCENTRATION OF TOTAL SALICYLATE IN THE PLASMA OF RABBITS

(n= 7 for each group)
.(control = mannitol treatment)

Animal Number	Concentration of total salicylate in plasma (mg./100 ml.)									
	Before treatment		After 1 hour of treatment		After 2 hours of treatment		After 3 hours of treatment		After 4 hours of treatment	
	P.V.P.-10	Control	P.V.P.-10	Control	P.V.P.-10	Control	P.V.P.-10	Control	P.V.P.-10	Control
1	68.0	76.0	34.5	52.5	18.5	32.0	6.5	26.0	4.5	11.5
2	82.0	77.5	42.0	53.5	16.0	32.5	8.5	19.5	2.5	15.0
3	69.0	74.5	38.0	50.0	14.0	36.5	6.0	30.0	3.5	22.0
4	62.0	71.5	33.0	42.5	13.5	20.0	7.5	13.0	4.0	8.5
5	82.5	69.0	48.0	40.0	21.0	24.0	9.5	15.0	4.0	8.0
6	69.0	71.0	39.5	47.0	20.5	21.0	6.5	12.5	3.0	8.0
7	72.0	69.0	30.0	43.0	10.0	22.0	6.0	13.0	2.0	8.5
Mean ± S.D.	72.1 ± 7.5	72.6 ± 3.4	37.9 ±6.3	47.0 ±5.4	16.2 ±4.0	27.0 ±6.6	7.2 ±1.4	18.4 ± 7.0	3.4 ± 0.9	11.6 ± 5.2
Student "t" test	No difference		$p < 0.01$		$p < 0.005$		$p < 0.001$		$p < 0.001$	

Figure 12



Data taken from Table X

with a difference in concentration of salicylate in the plasma of 8.2 mg./100 ml. at the end of the fourth hour.

The rates of urine flow and the urinary excretion of salicylate during the four hours of treatment are shown in Table XI. There was no significant difference between the rates of urine flow. There was more salicylate excreted in the urine of the animals treated with P.V.P.-10 ($p < 0.001$). This represented 3.5 mg./kg. more salicylate excreted in the urine of animals treated with P.V.P.-10 than in control animals. Urinary salicylate excretion was also assessed by calculating the total amount of salicylate excreted in the urine at the end of the four hours of treatment and expressing this as the percentage of the total administered dose. The animals treated with P.V.P.-10 excreted 94.7 ± 6.6 percent, significantly more than the control animals which excreted 82.0 ± 7.8 percent ($p < 0.01$) of the total administered dose.

The rate of urinary excretion of salicylate increases with an increase in the urine flow, urine pH, glomerular filtration rate (G.F.R.) and concentration of total salicylate in the plasma.[68] In Table XII the rate of urine flow, urine pH and G.F.R. in the two groups of rabbits, are shown. The mean of the urine pH and G.F.R. were calculated from the mean of two measurements taken during each period on each animal. In the second hour of treatment there was no significant difference between the rate of urine flow, urine pH or G.F.R. At the midpoint of this hour of treatment the concentrations of total salicylate in the plasma were significantly higher in the control group than in the group treated with P.V.P.-10 (37.0 ± 6.1 mg./100 ml. and 27.4 ± 7.0 mg./100 ml., respectively ($p < 0.02$). During the second hour of treatment the urinary excretion of salicylate in the animals treated with P.V.P.-10 was significantly greater than in the control animals (59.4 ± 9.1 mg./kg. and 49.1 ± 7.4 mg./kg., respectively ($p < 0.05$).

Table XI

EFFECT OF TREATMENT ON THE RATE OF URINE FLOW AND THE URINARY EXCRETION OF SALICYLATE DURING 4 HOURS OF TREATMENT.

(control = mannitol treatment)

	Urine Flow (ml./kg./hr.)		Salicylate excreted in urine (mg./kg.)	
Animal Number	P.V.P. - 10	Control	P.V.P. - 10	Control
1	12.9	15.4	152	129
2	11.5	8.0	171	128
3	12.6	11.2	200	176
4	9.4	15.8	177	113
5	14.6	15.1	173	140
6	16.4	14.7	205	184
7	10.2	9.8	193	161
Mean $\pm$ S.D.	12.5$\pm$2.2	12.9$\pm$2.7	182$\pm$19	147$\pm$27
Student "t" test	No difference		$p < 0.001$	

Table XII

EFFECT OF TREATMENT ON THE RATE OF URINE FLOW, URINE pH, and G.F.R.

(control = mannitol treatment)

	Urine Flow (ml./kg./hr.)		Urine pH (pH units)		G.F.R. ml./min.	
	P.V.P.-10	Control	P.V.P.-10	Control	P.V.P.-10	Control
Before salicylate			8.4 ±0.1	8.4 ±0.2	9.7 ±1.2	9.7 ±1.1
After 1 hour of treatment	9.4 ±3.7	6.3 ±2.1	8.2 ±0.2	8.3 ±0.2	10.0 ± 2.6	8.1 ±1.7
After 2 hours of treatment	12.8 ±3.1	13.9 ±4.9	8.0 ±0.1	8.0 ±0.1	8.4 ±2.1	8.3 ±2.8
After 3 hours of treatment	12.0 ±5.2	14.1 ±5.4	7.9 ±0.3	7.6 ±0.3	6.7 ±1.6	5.0 ±1.7
After 4 hours of treatment	11.5 ±3.5	12.6 ±7.3	7.5 ±0.3	7.4 ±0.3	5.2 ±2.7	3.0 ±1.6

In the serum samples studied by the equilibrium dialysis technique the degree of binding of Co^{57} labelled B_{12} by serum protein was small, (less than 5%) and constant throughout the experiment. G.F.R. calculations did not include a correction for this binding phenomenon since we were most concerned with changes rather than absolute values of G.F.R. No binding of Co^{57} labelled B_{12} by P.V.P. was observed using the equilibrium dialysis technique.

A comparison of the effect of treatment with P.V.P.-10 and mannitol during the entire four hours of treatment was also assessed by measuring mean arterial blood pressure, body temperature, G.F.R., arterial pH and arterial pCO_2. There was no difference between the mean arterial blood pressure of the two groups (110 $\pm$ 10 mm. Hg.) throughout the experiments. After the administration of salicylate there was an increase in the body temperature of one to three degrees centigrade in both groups of rabbits. After the first hour of treatment the temperature returned to normal. After the administration of salicylate there was usually an initial increase in the arterial pH (0.01 to 0.1 pH units). Thereafter a gradual decrease to normal (pH 7.42) or just below normal was observed. No significant difference in these changes in arterial pH could be shown in the two groups of rabbits. There was no difference in the G.F.R. before salicylate was given. The difference between the G.F.R. at the end of four hours of treatment was not significant ($p < 0.10 > 0.05$). The results of the effects of treatment with P.V.P.-10 and mannitol on the arterial pCO_2 are shown in Table XIII. There was no difference between the arterial pCO_2 values before treatment. The maximum decrease in arterial pCO_2 during treatment was significantly less in the group treated with P.V.P.-10 ($p < 0.001$).

In four of the seven rabbits treated with P.V.P.-10 the urinary excretion of P.V.P. at the end of four hours of treatment was 89.0 $\pm$ 10.0 percent of the total administered dose.

Table XIII

EFFECT OF TREATMENT ON ARTERIAL pCO_2.

(control = mannitol treatment)

	Arterial pCO_2 before treatment (mm Hg.)		Maximum decrease in arterial pCO_2 (mm Hg.)	
Animal Number	P.V.P.-10	Control	P.V.P.-10	Control
1	29.1	30.5	5.9	13.9
2	34.7	29.1	10.4	12.5
3	31.3	36.8	6.0	18.7
4	35.6	36.6	5.2	13.5
5	34.0	36.3	14.4	15.2
6	30.2	30.4	4.9	13.2
7	35.8	31.5	4.6	12.9
Mean $\pm$ S.D.	33.0$\pm$2.7	33.0$\pm$3.6	7.3$\pm$3.6	14.3$\pm$2.1
Student "t" test	No difference		$p < 0.001$	

DISCUSSION

To be useful as a detoxifying agent in salicylate intoxication, low molecular weight P.V.P. should fulfill four major criteria. It should bind substantial quantities of salicylate. After intravenous administration it should distribute into the extracellular fluid to exert this binding property. It should be rapidly filtered at the glomerulus to exert its binding property in the tubular urine to decrease reabsorption of the salicylate. It should be non-toxic.

Using equilibrium dialysis and ultrafiltration techniques, we studied several factors which could affect the binding of salicylate by P.V.P.-10 in an *in vivo* system. Our studies showed that P.V.P.-10 bound substantial quantities of salicylate *in vitro*. We were able to show that binding of salicylate by P.V.P. increased with both an increase in the concentration of salicylate and of P.V.P. For example a solution of 32 mg./ml. of P.V.P.-10, a concentration attainable in vivo in serum, was shown to bind approximately 20 mg./100 ml. of salicylate. At least 10 times this quantity of salicylate was shown to be bound at concentrations of salicylate and P.V.P.-10 attainable *in vivo* in urine. It was also shown that the binding capacity of P.V.P.-10 was not saturated even at a concentration of salicylate of 4000 mg./100 ml., a concentration far greater than that observed in states of salicylate overdose. *In vivo*, both salicylate and P.V.P.-10 are chiefly excreted in the urine and since urine pH may vary from approximately 4 to 8 it was necessary to establish the effect of hydrogen ion concentration on the binding equilibrium. The majority of the studies on the binding of properties of P.V.P. has been carried out at neutral pH or "physiological" pH of 7.4. We were able to show

that hydrogen ion concentration within the range studied did not influence the in vitro binding of salicylate by P.V.P. Theoretically an irreversible bond between salicylate and P.V.P. would be desirable, however, it is a reversible process as shown in our in vitro exhaustive dialysis and double dialysis studies. The in vitro binding of salicylate by the combination of albumin and P.V.P.-10 was studied since the in vivo administration of P.V.P.-10 would bring the binding sites of albumin into competition with the binding equilibrium of salicylate and P.V.P.-10. In vitro synergism of binding was not observed with the combination of the two binding agents. These results were not discouraging since in vivo, P.V.P.-10 distributed beyond the influence of albumin into the proportionately larger interstitial space and the tubular urine to exert its binding property. This distribution was simulated in our in vitro three-compartment dialysis experiment which showed that significant quantities of salicylate might be expected to be bound to P.V.P. in the interstitial space in vivo. This speculation is based on an in vitro system which did not include tissue protein binding as a factor which could influence the distribution of salicylate, with or without the presence of P.V.P.-10. At present, there is little knowledge of the significance of the binding of salicylate by tissue proteins, in fact tissue protein binding of all drugs has not been adequately studied. Confirmation of the effect of P.V.P.-10 on salicylate redistribution in vivo has been impeded by the practical problem of obtaining adequate samples from the various body fluids.

Our ultrafiltration experiments showed that after ultrafiltration of a salicylate solution devoid of any binding substance the concentration of salicylate in the filtrate was less than the concentration of salicylate in the original

filtering solution. It was shown that this difference decreased with increases in the salicylate concentration. We interpreted this observed effect to be due to retention of the salicylate by the ultrafiltration membrane. We were unable to corroborate or fully understand the significance of membrane retention until we discovered the careful ultrafiltration work of Ambard and Trautmann.[61] Some discrepancies between drug binding reports using ultrafiltration techniques may be explained by this phenomenon.

To determine the relationship of the binding potential of P.V.P. to its molecular weight with the hope of elucidating a mechanism of binding, two important methods were used. First, the comparison of binding of salicylate was made between equimolar concentrations of the different molecular weights of P.V.P. Second, an attempt was made to saturate potential binding sites of P.V.P. by using high concentrations of salicylate. From the results of these two experiments we agree with others[18,19,23,24] who believe that the acid amide group of each repeating vinylpyrrolidone unit in the molecular chain is a potential binding site. It has been suggested by Higuchi et al.[18] that the mutual interaction of amido groups of the polymer is highly restricted. This is believed to result in "exposure of dipolar bonds with strong affinity for any acidic hydrogens capable of forming hydrogen bonds". They observed that carboxyl groups (as in salicylate), phenolic groups and amino groups can all lead to definite association. Table XIV shows the total number of potential binding sites for P.V.P.-10, -40, -160 and -360, assuming each vinylpyrrolidone unit in the molecule to be a potential binding site. The actual number of binding sites occupied by salicylate at a concentration of salicylate of 10 mM./100 ml. was derived from Figure 5. It can be seen that the binding of salicylate increased in almost direct proportion to the increase in molecular weight of P.V.P. since the approximate ratio of

occupied to potential binding sites is nearly equal for equimolar concentrations of four different molecular weights. This agrees with the results of Scholtan[24] on the binding of Congo red by P.V.P. of different molecular weights, however Spitzer et al.[23] showed no change in the binding of Bromophenol blue by P.V.P. of increasing molecular weight. We believe Spitzer's conclusion was the consequence of comparing the binding by equiweight concentrations of P.V.P., a given solution of which would contain an equal number of potential binding sites irrespective of the molecular weight. Fine et al.[25] observed that less than one atom of iodine was bound by each molecule of P.V.P.-40 and concluded that the unsaturated terminal double bond on each P.V.P. molecule was the only binding site. We question this conclusion since it is possible that the low concentration of iodine used was insufficient to saturate more than one binding site. In our studies the ratio of occupied to potential binding sites decreased with increases in the concentration of salicylate but complete saturation (ratio 1:1) was not observed even at the highest concentration of salicylate employed in this study. In Table XIV the slight decrease in the ratio of occupied to potential binding sites with increasing molecular weight of P.V.P. could be explained by the increasing inaccuracy of the manufacturer's mean molecular weight with increase in the molecular weight of the P.V.P. or by a diminishing excess of salicylate molecules in proportion to the increasing number of binding sites with increase in molecular weight of P.V.P.

The distribution and excretion of a small intravenous dose of low molecular weight P.V.P. has been investigated[43,45] but it was necessary to study the distribution and excretion of a large intravenous dose of P.V.P.-10 since this was employed in our experimental salicylate intoxication experiments. The rate of urinary excretion of the relatively large administered dose of

Table XIV

POSTULATED NUMBER OF POTENTIAL BINDING SITES OF P.V.P.

OCCUPIED BY SODIUM SALICYLATE
(SALICYLATE CONCENTRATION 10 mM/100 ml)

P.V.P.	MOLECULAR WEIGHT	POTENTIAL BINDING SITES	BINDING SITES OCCUPIED	APPROXIMATE RATIO OF OCCUPIED TO POTENTIAL BINDING SITES
10	10,000	90	5.3	1:17
40	40,000	360	23.0	1:16
160	160,000	1440	96.0	1:15
360	360,000	3240	234.0	1:14

P.V.P.-10 was very rapid with approximately 63 percent excreted by the end of the infusion. The slight lag in excretion rate over infusion rate resulted in accumulation of the P.V.P.-10 as evidenced by the increasing concentrations of P.V.P.-10 in the serum up to the end of the infusion. According to the calculated volume of distribution, the accumulated P.V.P.-10 rapidly diffused into a volume approaching the theoretical extracellular fluid volume. The binding potential of the P.V.P.-10 could thus extend beyond the intravascular limits of the binding properties of albumin. The rapid renal clearance of P.V.P.-10 (90 percent of the administered dose four hours after the completion of the infusion), provided high urine concentrations of P.V.P.-10. In rabbits intoxicated with salicylate the high concentration of P.V.P.-10 in tubular urine should bind salicylate and prevent tubular reabsorption. Although the milligram concentration of P.V.P.-10 in the urine was high, the milliosmolar concentration was very low because of the large particle size. It was therefore not surprising that an osmotic diuresis was not observed in our experiments or in the studies of other investigators.[47,48] The observed rapid distribution and excretion of a large intravenous dose of P.V.P.-10 is in keeping with the results of Hecht[43] and Richards[45] for smaller doses of P.V.P. of nearly similar molecular weights. Failure to achieve 100 percent recovery of the administered dose of P.V.P.-10 could be explained by the experimental error of the quantitative determination of P.V.P. in the urine. It is possible that some of the P.V.P.-10 was excreted in the stool, as shown by others[25,27,30,31]. This was not assessed in our study. Trace quantities of P.V.P.-10 detected in the serum after 72 hours probably represented the very small proportion of larger molecular weight P.V.P. present in P.V.P.-10.

There were no observed acute toxic effects after the intravenous

administration of large doses of P.V.P.-10 to rabbits. Because of the wider distribution and more rapid excretion of P.V.P.-10, cardiovascular overloading as can occur with P.V.P. of larger molecular weight, did not present a problem. The main "toxicity" of P.V.P. which has curtailed its use as a plasma volume expander in North America has been the observed storage phenomenon in reticuloendothelial cells. However, reticuloendothelial storage of P.V.P. of lower molecular weight has not been observed[25,29]. We were not able to show histological evidence of storage of P.V.P.-10 in the liver or spleen of rabbits. The scattered renal tubular changes which we observed were similar to those demonstrated in "osmotic nephrosis".[68] Whether the changes we observed were due to absorption of P.V.P.-10 by the tubular epithelium or to some external influence of the P.V.P.-10 on the tubular cells is not known. Although we have not conducted chronic experiments to determine the course of this altered morphology it has been shown by others[25,29] to be completely reversible with no evidence of cell death. P.V.P. of 12,600 molecular weight is commercially available in Germany and has been administered to many humans with no reported untoward effects.

In order to assess the efficiency of P.V.P.-10 in the treatment of experimental salicylate intoxication, it was necessary to control the factors affecting the distribution and excretion of salicylate. After absorption, salicylate is avidly bound by serum albumin[70] in which form it is pharmacologically inactive since it cannot diffuse to the cellular site of action. However at serum concentrations of salicylate observed in severe salicylism and in our experiments, albumin binding is near saturation and approximately 90 percent of the total amount of salicylate is unbound[71] and capable of diffusing into the total body water.[72] It is this large unbound

portion of salicylate that is responsible for the direct stimulation of the respiratory centre producing respiratory alkalosis. The direct inhibitory effect of diffusable salicylate on cellular metabolism usually leads to a metabolic acidosis[73,74] and the signs of severe salicylate intoxication. The rate of urinary excretion of unbound salicylate is influenced by glomerular filtration rate (G.F.R.), rate of proximal renal tubular secretion of salicylate, the renal tubular reabsorption of salicylate and the filtered load of salicylate determined by the concentration of unbound salicylate in the serum. Excretion by metabolic degradation is limited.[75,76]

Any condition resulting in decreased G.F.R. will decrease salicylate clearance. Salicylism can result in oliguria[77] and damage to renal tubular epithelium[78] but objective assessment of renal function in severe salicylate overdose has not been studied. For this reason G.F.R. was measured and comparisons of renal excretion of salicylate between the treated and control groups were made only at a period when G.F.R. was the same in both groups of animals. Urine flow and urine pH were controlled since the renal tubular reabsorption of salicylate is decreased by an increase in both the rate of urine flow and the urine pH.[79,80] Salicylate clearance can be doubled by increasing urine flow eightfold.[81] Water diuresis increases salicylate clearance to a lesser degree than does an osmotic diuresis. Water diuresis increases salicylate clearance to a lesser degree. Adequate urine flow was maintained in our animals with the aid of a mannitol solution in the control group. There is a negligible increase in salicylate clearance with an increase in urine pH up to 7.0; however a fourfold increase in salicylate clearance is produced by increasing the urine pH from 7.0 to 8.0.[82-85] The increased renal clearance of salicylate with increasing urine alkalinity has been explained on the basis of decreased renal tubular reabsorption of ionized salicylate.

However since the pK of salicylate is about 3.0, any further increase in the intratubular concentration of ionized salicylate with the change of pH from 7.0 to 8.0 would be negligible.[86,87] The explanation may in part lie in the observed increase in active tubular secretion when the urine pH is greater than 7.4.[82] Because of the important effect of pH on salicylate excretion, urine was maintained at or above pH 8.0 in all our salicylate intoxication experiments. It was shown that during the second hour of treatment the group of animals treated with P.V.P.-10 excreted significantly more salicylate in the urine while there was no difference in the G.F.R., rate of urine flow and urine pH between the two groups. The ooncentration of salicylate in the serum in the control animals was higher than in the animals treated with P.V.P.-10 during this second hour of treatment. This condition would enhance salicylate excretion in the control group, since more unbound salicylate would be filtered at the glomerulus. However, the animals treated with P.V.P.-10 excreted significantly more salicylate during the second hour of treatment and throughout the entire treatment period, in spite of the lower filtered load of salicylate in these animals.

Two of the physiological abnormalities of salicylate intoxication observed in our experiments were a marked decrease in the arterial pCO_2 and a progressive deterioration of the G.F.R. Both of these effects were much less marked in the animals treated with P.V.P.-10. It is unlikely that the P.V.P. was acting as a respiratory depressant since this effect has never been reported. We attribute this amelioration to inactivation of salicylate by the increased binding to P.V.P.-10. However, these effects can also be explained by the observed lower concentrations of salicylate in the serum secondary to increased urinary excretion of salicylate. An arenal animal preparation would

be required in an attempt to separate these two possible explanations.

In vivo serum binding studies were not done because of the prohibitively large samples required for meaningful ultrafiltration studies. Equilibrium dialysis by our technique was unsuitable since it includes an inherent dilution error. However, large urine samples were ultrafiltered and it was shown that binding was identical to that in the in vitro studies of saline solutions containing concentrations of P.V.P.-10 and salicylate similar to those found in the urine. The increased urinary excretion of salicylate in the P.V.P.-10 treated animals could have been due to glomerular filtration of a greater load of salicylate in the form of a salicylate P.V.P. complex, to decreased tubular reabsorption of the salicylate bound by the high concentrations of P.V.P.-10 in the tubular urine or both. Although there was a significant difference in the concentration of salicylate in the serum and urinary excretion of salicylate between the two groups of animals, both groups did well probably because the renal clearance of salicylate in rabbits is five times the G.F.R. when the urine pH is greater than 7.0[87]. This marked effect of pH on the tubular secretion of salicylates is not observed in most animals or in man.[82]

On the basis of these preliminary experiments we believe that the non-specific binding properties of low molecular weight P.V.P. offers a direct and unexplored approach in the treatment of drug overdose states.

CONCLUSIONS

The following conclusions were drawn from our assessment of the binding properties of polyvinylpyrrolidone (P.V.P.) for salicylate, the distribution and excretion of P.V.P.-10 and the efficacy of P.V.P.-10 in the treatment of experimental salicylate overdose.

In vitro;- 1) P.V.P.-10 bound a significant quantity of salicylate.

2) Binding of salicylate by P.V.P. was a reversible process as determined by exhaustive and double dialysis techniques.

3) Binding of salicylate by P.V.P. was proportional to the molecular weight of P.V.P., the concentration of P.V.P. and the concentration of salicylate.

4) Binding of salicylate by P.V.P. was not influenced by hydrogen ion concentration between pH 4.0 and 8.0.

5) Binding synergism was not observed with the combination of P.V.P.-10 and human albumin; however, P.V.P.-10 increased the "extracellular" concentration of salicylate in an in vitro system simulating the three major fluid spaces in the in vivo system.

6) Each repeating vinylpyrrolidone unit in the molecular chain of P.V.P. represented a potential binding site.

In vivo;- 1) P.V.P.-10 administered intravenously to rabbits distributed rapidly into a volume similar to the estimated extracellular fluid volume.

2) Urinary excretion of P.V.P.-10 in rabbits was exceedingly rapid with approximately 95 percent recovery of the administered dose in the urine in 12 hours.

3) The renal excretion of P.V.P.-10 in rabbits with the dose employed did not produce a measurable osmotic diuresis.

4) There was no gross evidence of toxicity of intravenously administered P.V.P.-10 in rabbits in the dose of 4 g./kg. Histological examination of the spleen and liver in these animals did not show evidence of reticuloendothelial storage of P.V.P.-10. Histological changes observed in the renal tubular epithelium were minor in nature and resembled the reversible microscopic changes shown in osmotic nephrosis. In our preparations these observed changes could not be attributed to cellular accumulation of P.V.P.-10.

5) A comparison of treatment of experimental salicylate overdose in rabbits was made between intravenously administered P.V.P.-10 and a control mannitol solution. The animals treated with P.V.P.-10 showed a more rapid fall in the concentration of salicylate in the serum, and a more rapid urinary excretion of salicylate. The animals treated with P.V.P.-10 showed a more rapid recovery from salicylism than the control animals.

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