Effect of Polyvinylpyrrolidone (PVP) on Mouse Liver Function.* (21167)

JACK FROMMER.† (Introduced by J. Walter Wilson.)

From the Department of Biology, Brown University, Providence, R. I.

Reticulo-endothelial changes occur when large amounts of polyvinylpyrrolidone (PVP) are administered to animals(1-3). Although structural changes due to PVP administration are well established, there is a dearth of information on functional changes. Reinhold *et al.*(4) concluded that PVP seemed to be without ill effects on the liver after they performed a battery of liver function tests on surgical patients given either one or 2 liters of PVP intravenously. No studies were made, however, on non-surgical patients and with the use of larger quantities of PVP. It is the purpose of this paper to report on the use of the bromsulphalein-retention test to determine whether changes in mouse liver function occur after administration of PVP.

Materials and methods. Casals and Olitsky (5) investigated 6 liver function tests applicable to mice and found that retention of bromsulphalein (BSP) was the most sensitive and accurate indicator of liver damage brought about by chemical poisoning, bacterial infection, starvation, and injection of mouse-liver autolysate. In this laboratory their BSP-retention test has been modified as a micro-method which is convenient for use with mice. It allows the use of tail blood rather than heart blood, and requires only 0.03 cc of blood for each test. Some advantages of the micro-method are as follows: the necessity for heart puncture is eliminated; fatalities are nil; daily determinations can be made on the same animal; the BSP level in the blood of a single animal can be determined at several short intervals after injection of the dye to show the changes which occur in the blood BSP level; the small amount of bleeding does not cause a significant change in the condition of the animals due to blood depletion; and anesthesia is unnecessary. The animals used were albino mice of both sexes from the inbred strain BUA maintained at the biological laboratory of Brown University. They were all between 60 and 90 days of age at the time the experiments were begun and were maintained on a diet of Purina Laboratory Chow. The BSP-retention test as routinely used is as follows: 0.125 mg BSP in 0.05 cc physiological saline per g of body weight is injected intraperitoneally. After 20 minutes the mouse is bled from the tail. The blood is drawn into a 0.03 cc micropipette. This blood is mixed with 0.1 cc of a 2% solution of potassium oxalate in saline; thus, the plasma fraction of the blood is diluted approximately 7 times. The mixture is centrifuged at 3000 r.p.m. for 10 minutes and the diluted plasma removed with a capillary pipette. Into each of 2 small test tubes is placed 0.04 cc of this diluted plasma and 0.10 cc of physiological saline. To one tube 0.05 cc of a 10% solution of sodium hydroxide is added to bring out the color of the dye. To the other tube 0.05 cc of a 10% solution of HCl is added to provide a blank. With a capillary pipette these solutions are transferred to very thin cuvettes made from 5 mm glass tubing. An adapter is used for the cuvettes, and readings are made in a Coleman Jr. spectrophotometer at 580 mμ. The values read are compared with a standard curve prepared with known dilutions of BSP and expressed in milligrams per 100 cc of plasma. Values read are multiplied by 7 because of the original dilution of the plasma with oxalate-saline. Standard solutions are treated like the centrifugate in the above procedure. A group of normal mice was used to establish a base line for what might be considered a normal BSP-retention. Another group of mice was injected subcutaneously with a standard dose of 0.1 cc of a 40% solution of CCl_4 in sesame oil which produces a typical central necrosis of the liver. BSP-retention was determined 48 hours later when liver injury was at a maximum (Table I).

* This work comprises portion of thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Brown University.

† Schenley Laboratories Fellow in Biology.

Present address: Department of Anatomy, Tufts College Medical School, Boston, Mass.

TABLE I. Bromsulphalein-Retention in Uninjected Control Mice and in Mice Injected with Carbon Tetrachloride.

Treatment	No. of mice	Bromsulphalein in plasma in mg/100 cc Avg	Range
Controls	35	3.2	2.0- 4.8
Carbon tetrachloride	16	22.6	6.2-41.8

Several groups of mice were injected intravenously, intraperitoneally, or subcutaneously with solutions of PVP which varied in respect to concentration and K-value. The K-value represents the viscosity of the solution from which the average molecular weight of the PVP may be calculated. 1) The K-20 material is a low molecular weight preparation supplied by Schenley Laboratories in powder form. It was used to prepare a 20% solution of PVP in 0.9% NaCl. 2) The K-30 material was supplied in 2 forms by Schenley Laboratories: (A) PVP (MACROSE) was contained in infusion bottles as a sterile solution containing 3.5% by weight of PVP, plus inorganic salts. (B) A powder form from which a 10% solution was prepared in 0.9% NaCl. 3) The K-71 material is a high molecular weight PVP prepared by chemical fractionation by Dr. W. O. Ney of Arnold, Hoffman and Co., Providence, R. I. It was used as a 5% solution in distilled water.

Mice of one group were given intravenous tail vein injections of 0.5 cc of 20% PVP with a K-value of 20 which represents an average molecular weight of approximately 20,000. This volume, given to the mice in a single dose, corresponds approximately to a one-liter infusion in an adult human. The animals of this group were subjected to the BSP-retention test at different times throughout the 3 days following the PVP administration (Table II). Six mice were injected with 0.5 cc of a 20% solution of PVP with a K-value of 20 on alternate days for 15 days. Due to the difficulties encountered in making a long series of tail vein injections, each mouse received 3 or 4 injections intravenously and the remainder intraperitoneally. These animals received a total of 4.0 cc. The BSP-retention test was performed one, 3, 7, 14, and 30 days after the last injection (Table III). Three groups of mice were injected with K-30 PVP, the material which is prepared as a 3.5% solution for human infusions. It has an average molecular weight of approximately 40,000. The mice of one of these groups were injected intravenously with 0.5 cc of a 10% solution given in a single dose. They were then subjected to the BSP-retention test at different times ranging from 0-24 hours, as shown in Table II. The

TABLE II. Bromsulphalein-Retention in Mice Injected Intravenously with a Single Injection (0.5 cc) of Polyvinylpyrrolidone.

K-value	PVP (%)	No. of mice	Hr after inj. of PVP	Bromsulphalein in plasma in mg/100 cc Avg	Range
20	20	6	I*	2.4	1.6-3.2
		6	1	2.2	1.8-2.6
		6	2	3.1	2.0-3.8
		6	4	3.2	1.8-4.6
		6	6	3.5	2.2-4.4
		4	24	3.2	2.8-3.6
		4	72	3.3	2.6-4.2
30	10	6	I	2.9	2.0-4.2
		6	1	3.4	2.8-4.6
		6	2	4.0	2.2-4.8
		4	6	3.2	2.2-4.0
		8	24	3.0	2.0-3.8

* I = Immediately.

TABLE III. Bromsulphalein-Retention in Mice Injected with a Series of 0.5 cc Injections of Polyvinylpyrrolidone.

K-value	PVP (%)	Total inj. (cc)	No. of mice	Days after final inj. of PVP	Bromsulphalein in plasma in mg/100 cc Avg	Range
20	20	4	6	1	2.4	1.8-3.6
			6	3	1.8	1.4-2.4
			6	7	2.9	2.4-3.6
			6	14	3.0	2.4-3.8
			6	30	2.4	1.8-3.6
30	10	5	6	1	2.2	1.2-3.6
			6	3	2.5	1.6-3.6
			6	7	3.2	2.2-4.2
			6	14	3.0	2.2-4.0
			6	28	2.3	1.6-3.8
			4	38	2.6	1.6-4.0
			4	59	2.6	2.0-3.6
30	3.5	10	3	1	2.9	2.6-3.4
			6	17	2.7	1.8-4.0
			6	28	2.9	1.6-4.4
			6	49	2.8	2.0-4.2
71	5	5	5	2	3.3	2.2-4.2
			5	10	3.0	1.8-4.2
			4	30	2.8	1.6-3.8

2 other groups receiving the K-30 PVP preparation were given a series of 0.5 cc intraperitoneal injections totalling 5.0 cc of a 10% solution in one group, and 10.0 cc of a 3.5% solution in the other group. The BSP-retention test was performed on the mice of the first group one, 3, 7, 14, 28, 38, and 59 days after the last injection. The second group was subjected to the test one, 17, 28, and 49 days after the last injection (Table III). Five mice were given a series of 0.5 cc subcutaneous injections totalling 5.0 cc of a 5% solution of PVP with a K-value of 71. The average molecular weight of this highly polymerized material is approximately 125,000. These mice were subjected to the BSP-retention test at 2, 10, and 30 days after the last injection (Table III).

Results. Table I gives the average BSP-retention for untreated controls and for carbon tetrachloride-injected mice, and also shows the range within each group. The group of 35 control animals in which no PVP was administered had an average BSP-retention of 3.2 mg per 100 cc of plasma. The average value for the 16 animals injected with CCl_4 was 22.6 mg per 100 cc of plasma. Since none of the untreated controls showed retention levels above 5 mg and none of the CCl_4 injected animals showed levels below 6 mg, it would seem that readings above 6 mg per 100 cc of plasma could be considered abnormal, while those below 6 mg could be considered within the normal range.

The average and range of the BSP-retention levels after different intervals for the mice given a single intravenous injection of PVP are presented in Table II. The same information for the mice heavily laden with a series of PVP injections is presented in Table III. For all groups of PVP-injected animals the BSP-retention levels are not significantly affected.

Discussion. The early experiments in this investigation were undertaken to demonstrate that the micro-method for BSP-retention was a reliable indicator of liver function and that the presence of circulating PVP did not alter the rate of dye-removal from the blood stream. It was also necessary to determine whether the presence of PVP in the reticulo-endothelial cells would hinder the clearance of BSP from the blood. The micro-method devised for determining BSP levels is a reliable test for poorly functioning livers damaged by CCl_4 (Table I). Experiments in which the dosage and the molecular size of the PVP were varied were designed to detect any dysfunction which might occur in livers which were known to exhibit slight to extensive changes in the reticulo-endothelial cells. The data for all groups of PVP-injected mice show that the BSP-retention levels were not increased. One may conclude from this that the injection of PVP, even in massive doses, does not impair liver function as it is measured by the BSP-retention test.

Summary. A micro-method utilizing blood from tail vessels has been devised for determining the BSP-retention of mice. This method has been shown to be a reliable test for altered function of livers damaged by CCl_4. When applied to mice at varied intervals after PVP administration, the BSP-retention values were invariably within the normal range. Increasing the dosage and the molecular size of the PVP did not increase the dye-retention. The results indicate that PVP, as used in this investigation, does not alter the mechanism of BSP-removal from the blood of normal animals, and it does not cause impaired liver function, either immediate or deferred, as determined by the BSP-retention test.

The author wishes to express his appreciation to Dr. J. Walter Wilson for his helpful comments and suggestions.

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Received May 26, 1954. P.S.E.B.M., 1954, v86.