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## Effect of Polyvinylpyrrolidone on Reticulo-Endothelial Storage. (19465)

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Polyvinylpyrrolidone (PVP), a macromolecular substance first synthesized in Germany during World War II, has been used in Europe as a plasma substitute, reportedly with good results. Abstracts of the extensive European literature on PVP have been presented in a monograph(1) issued by the General Aniline and Film Corporation. Recently the possible use of PVP as "plasma expander" has been considered in this country. Hence biologic properties of PVP deserve continued investigation. Reports on storage of PVP in tissues are contradictory. Earlier negative findings were obtained on dogs and man (Korth and Heinlein(2)). On the other hand, Bargmann(3) observed storage in splenic macrophages of dogs and rats, and Barfuss and Eichler(4) noted transitory storage in rabbits, guinea pigs, and rats. Ammon and Müller(5) found evidence of PVP storage in rabbits, but not in infants treated with PVP, whereas Schoen(6) reported positive findings in such infants. Equivocal results were reported by Riedel and Zipf(7), and by Pellerat *et al.* (cit. 1, p. 50). Recently Nelson and Lusky(8) found in rabbits injected with PVP slight splenic enlargement, and presence of foam cells in spleen, lymph nodes, bone marrow, and other tissues. Bennhold, Schubert, and associates(9-12) reported an interesting property of PVP, namely its ability to promote renal excretion of dyes that are otherwise not excreted at all, or are excreted through the bile. Schubert(12) also found that PVP was capable of "washing out" dyes from tissues with subsequent renal excretion of the dye.

The present investigation is concerned with the effect of PVP on reticuloendothelial storage of dyes in mice. Since previous work (13-15) had shown that the extent of reticuloendothelial storage in mice may vary according to the strain, animals of several inbred strains were utilized for this study.

*Materials and methods.* Male and female mice, 3 to 6 months old, of strains C57 Black, dba, C3H, and CBA were used. The animals were free of tumors and any other obvious disease. They were kept on *ad libitum* diet of Rockland mouse pellets and water. The dyes used were 1) Erie Fast Rubin Conc. (EFR),* described in a previous communication(15), and 2) Chlorazol Black E (CBE). As a rule, 0.5 ml of 0.5% solutions of dye was injected subcutaneously 2 to 3 times weekly. This dosage was well tolerated by the animals, except in some instances where it was necessary to diminish the quantity of EFR. Controls and experimental animals received equal amounts of dye. PVP† was used as 3.5% solution in physiologic saline, of which 0.5 ml was injected daily subcutaneously, or intraperitoneally in some of the earlier experiments. The animals were weighed at least once weekly, and at the end of the experiments. They were sacrificed by decapitation, permit-

* This dye was obtained through cooperation of the National Aniline Division, Allied Chemical and Dye Corp.

† Mr. D. Witwer, General Aniline & Film Corp., furnished a generous supply of PVP. All experiments were carried out with a single lot (GA-PVP-118).

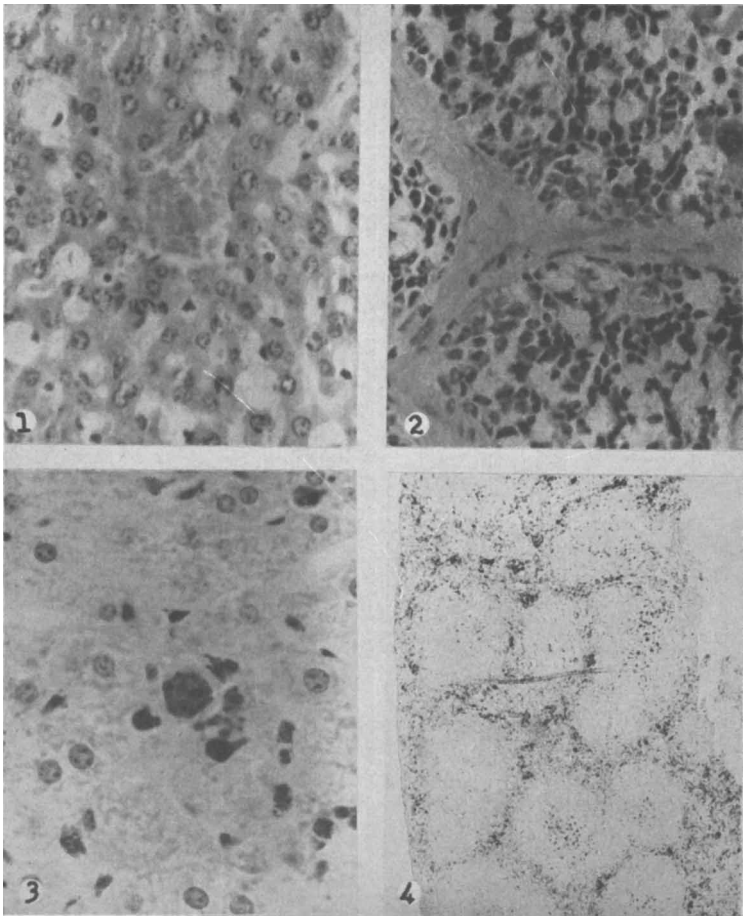

FIG. 1. Liver, C3H male, 18 injections of PVP. Note vacuolation and distention of Kupffer cells. H & E. × 300.

FIG. 2. Spleen. Same animal as Fig. 1. Extensive foam cell accumulation. H & E. × 300.

FIG. 3. Liver, C57 Black male (C-88). Control injected with EFR. Coarse granular dye storage in Kupffer cells. H & E. × 300.

FIG. 4. Spleen, C57 Black male (C-109). Control injected with CBE. Heavy perifollicular dye storage. Unstained. × 30.

ting collection of blood. Autopsies were done, the spleen weighed, and liver, spleen, kidney, lung, and skin fixed in formalin. Sections of these organs were stained with hematoxylin and eosin, and in experiments involving administration of dyes, also unstained sections were prepared in order to permit closer study of dye deposits. In some experiments the method of Chalkley(16) was used for quantitative estimation of dye storage. Details of this technic will be given later. During and after periods of injection of the dye the urine was watched for its appearance daily, and after sacrifice of the animals the coloration of the serum was recorded.

*Results.* In preliminary experiments, from 12 to 24 daily intraperitoneal or subcutaneous injections of 3.5% PVP were given to 45 C3H, 40 C57 Black, and 20 dba mice of both sexes. The animals showed no untoward effects from this treatment and continued to gain weight. They were sacrificed within from one to 3 days after the last injection of PVP. Moderate enlargement of the spleen was found, with the enlargement most pronounced in strain C3H and least in C57 Black. Tissue sections revealed considerable vacuolation and "ballooning" of Kupffer cells

in the liver (Fig. 1), and formation of similar vacuolated macrophages in the perifollicular areas of the spleen (Fig. 2), and in the reticulum of lymph nodes. The changes increased with dosage of PVP. They were most marked in C3H mice, while C57 Black animals presented them only after a minimum of 18 injections of PVP. The substance responsible for the appearance of the foam cells is presumably PVP or a derivative of it. Direct histologic identification of PVP is rendered difficult by its ready solubility in water and alcohol, which cause it to disappear from fixed and sectioned tissue. In accord with findings of Nelson and Lusky(8) imprints of fresh tissue stained with Gram's iodine showed a deep brown color of cells presumably containing PVP. Attempts to find such iodophilic material in tissue sections were unsuccessful in our hands.

Having thus found presumptive evidence for storage of PVP in reticulo-endothelial tissues of the mouse, the effect of PVP on storage of dyes was tested in three experimental arrangements: (a) simultaneous administration of dye and PVP; (b) administration of PVP after dye injection; (c) administration of PVP prior to injection of dye.

A. Experimental data and gross findings resulting from *simultaneous administration of PVP and EFR* are given in Table I. Histologic findings in the control group were identical with those made in the course of earlier studies(15). There was heavy storage of dye, mostly in form of coarse granules, in the Kupffer cells of the liver (Fig. 3), in perifollicular macrophages and littoral cells of the spleen (Fig. 4), and variable degrees of histiocytic storage in skin and lymph nodes.

TABLE I. Simultaneous Administration of PVP and Dye (.5 ml of .5% EFR).

| | Strain C57 Black | | | |
|---|---|---|---|---|
| | Exp. C-69 | | Exp. C-78 | |
| | Control group | Exp. group | Control group | Exp. group |
| No. of animals | 5 | 5 | 7 | 7 |
| Sex | ♀ | ♀ | ♂ | ♂ |
| Solvent of dye | Saline | PVP | Saline | PVP |
| Route of inj. | i.p. | i.p. | i.p. | i.p. |
| No. of inj. | 7 | 7 | 5 | 5 |
| Color of urine | Yellow | Red | Yellow | Red |
| " " serum | " | Purple | " | Purple |

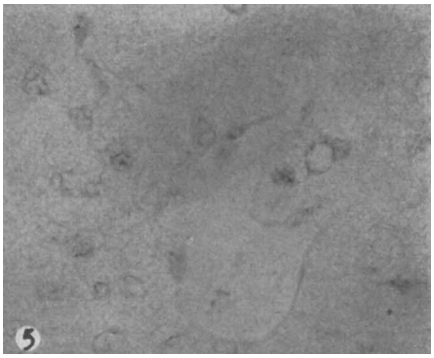

FIG. 5. Liver, C57 Black male (C-78). Exp. animal injected with EFR dissolved in PVP. Note absence of granular storage, and presence of "dye-vacuoles." Unstained. × 250.

The dye deposits were readily visible in hematoxylin-eosin stained slides. In the kidney only occasionally a faint staining of collecting tubules was noted; this was visible only in unstained preparations. In contrast, animals injected with dye dissolved in PVP showed hardly any dye deposits in hematoxylin-eosin stained sections of liver and spleen, while unstained sections revealed macrophages containing a pale red rim of dye around vacuoles apparently produced by intake of PVP (Fig. 5). On the other hand, the renal cortex presented grossly a reddish stain which on microscopic examination was found to be due to dye deposits in the tubular epithelial lining. This is obviously a corollary of the dye excretion noted in the urine of the PVP injected animals.

B. Experimental procedures and gross findings pertaining to the *administration of PVP after the injection of dyes* are summarized in Table II. In experiments C-88 and C-97 control animals were divided into 2 groups; one receiving no treatment after the initial dye injections, the other receiving subcutaneous injections of 0.5 ml of physiologic saline parallel with injections of the same quantity of PVP given to the experimental group. Since animals of the 2 groups showed identical findings, they were combined as one group, and later experiments did not include saline-injected controls. The gross findings in this type of experiment were quite similar to those obtained when dye and PVP were given simultaneously. This applied also when

TABLE II. Administration of PVP after Dye Storage.

| No. | Strain | Sex | Group | No. of animals | Dye and dosage (ml) | No. of PVP inj. | Color of serum | Dye storage |
|---|---|---|---|---|---|---|---|---|
| C– 88 | C57 Black | ♂ | C* | 11 | EFR 9× .5 | 0 | Y† | 3+ |
| | | | Exp. | 6 | | 12 | P‡ | 1+ |
| 97 | C3H | ♂ | C | 11 | " 9× .5 | 0 | Y | 2+ |
| | | | Exp. | 6 | | 12 | P | 1+ |
| 110 | C57 Black | ♂ & ♀ | C | 6 | " 3× .5 | 0 | Y | 3+ |
| | | | Exp. | 7 | +3× .3 | 18 | Deep P | 1+ |
| 116 | CBA | ♂ | C | 9 | " 5× .5 | 0 | Y to pink | 2+ |
| | | | Exp. | 9 | +4× .25 | 15 | Deep P | ± |
| 118 | " | ♂ | C | 10 | CBE 3×1 | 0 | Y | 1+ |
| | | | Exp. | 10 | | 15 | Y | ± |

* C = control. † Y = yellow. ‡ P = purple.

TABLE III. Effect of PVP on Dye Storage.

| No. | Strain | Group | No. of animals | Total storage Range | Total storage Mean | Total storage ± S.D. | Coarse granules Range | Coarse granules Mean | Coarse granules ± S.D. |
|---|---|---|---|---|---|---|---|---|---|
| C–88 | C57 Black | EFR | 11 | 22–40 | 32.6* | 4.3 | 16–31 | 22.5† | 4.1 |
| | | " +PVP | 6 | 19–34 | 27.3 | 6.1 | 0–8 | 4 † | 2.6 |
| 97 | C3H | " | 11 | 19–34 | 25.3* | 3.7 | 15–24 | 18.5‡ | 2.7 |
| | | " +PVP | 6 | 14–26 | 21.3 | 4.7 | 1–8 | 4 ‡ | 2.5 |

*, †, ‡ Difference between paired values statistically significant.

Chlorazol Black E was used (C-118). The cellular distribution of this dye was similar to that of EFR. Evidence for a displacement of dye from its previous sites of storage was furnished by the appearance of dye in serum and urine. Histologic examination of animals injected subsequently with PVP disclosed a marked decrease in reticulo-endothelial dye storage. The diminished dye content of tissues was due to a change in the type of storage rather than to a decrease in the number of storing cells: instead of the dark red coarse granules in controls, PVP-treated mice presented predominantly either fine granules of dye, or "dye-vacuoles," similar to those noted after *simultaneous* administration of dye and PVP.

In experiments C-88 and C-97, the extent and type of dye storage was estimated by means of the Chalkley tissue analysis(16). Hematoxylin-eosin stained tissue sections of uniform thickness (6 $\mu$) were examined under oil immersion. The reference pointer was moved at random and placed on nuclei of liver cells; a total of 200 nuclei were aimed at in each section. Hits of the 4 pointers on dye material were recorded in 2 categories: (a) coarse granules, (b) other forms of storage (fine granules or dye-vacuoles). Table III shows that administration of PVP brought about a highly significant depression of coarse dye storage. Incidentally, a difference in dye storage, found on statistical analysis to be significant, was also found between control animals of strains C57 Black and C3H, with the former exceeding the latter in storing ability. This corroborates earlier observations on greater storage of carmine in C57 Black than in C3H mice(13,14).

C. Table IV lists data on mice injected with *dye (CBE) after PVP administration.* The reticulo-endothelial storage of CBE, found in untreated mice of all 3 strains, was uniformly prevented by preceding PVP administration (18 injections). Evidence of PVP storage in the form of "foam cells" was prominent in liver and spleen, being most pronounced in mice of strain C3H, and least marked in C57 Black animals.

*Discussion.* These experiments indicate that

TABLE IV. Administration of Dye after PVP (1 ml of 1% CBE).

| Strain | Group | No. and sex of animal | Color of—Urine | Color of—Serum | Dye storage |
|---|---|---|---|---|---|
| C57 Black | C* | 5 ♂ | Y† | Y | 2+ |
| | PVP | 10 ♂ | Light P‡ | Smoky G§ | — |
| C3H | C | 5 ♂ | Y | Y | + |
| | PVP | 10 ♂ | Light P | Smoky G | — |
| dba | C | 5 ♂ | Y | Y | + |
| | PVP | 10 ♂ | Light P | Yellow to G | — |

* C = control. † Y = yellow. ‡ P = purple. § G = gray.

TABLE V. Comparative Dosage of PVP.

| Species | Approx. wt, kg | 3.5% PVP, ml | PVP, g/kg | No. of inj. |
|---|---|---|---|---|
| Man | 70 | 1000 | .5 | ? |
| Rabbit* | 3 | 30 | .35 | 16/8 wk |
| Mouse | .025 | .5 | .7 | 12-24/2-4 wk |

* Nelson and Lusky (8).

PVP, or a derivative of it, is stored in reticulo-endothelial tissues of the mouse, leading to formation of vacuolated and distended cells. The extent of storage appears to vary with the strain, inasmuch as C57 Black mice exhibited less PVP storage, or exhibited it only after doses of PVP larger than those required for C3H or CBA mice. In previous work (13,14) C57 Black mice showed a superior storing ability for dyes as compared with C3H mice, and in studies of Davidsohn and Stern (17-20) they exceeded most other strains in production of hemoantibodies.

In attempts to evaluate the biologic activity of PVP, a comparison of dosage in animal experimentation and in clinical use should be included. According to Table V, the g/kg amount of PVP for single administrations is similar for the 3 examples listed. However, the total *amount of PVP* given will obviously depend on the *frequency* of administration.

The results of simultaneous administration of dye and PVP can be interpreted as indicating renal excretion of a portion of the dye, *before* it had a chance to be deposited in reticulo-endothelial tissues, while the remainder of the dye was stored together with PVP. The observations in mice injected with PVP *after* administration of dye appear to indicate, in accord with similar findings by Schubert (12), that dye already ingested by macrophages can be dislodged by subsequently administered PVP. Thus eluted dye reappears in the circulation, and is at least partially excreted in urine. It is possible that some of the circulating dye may be again taken up by reticulo-endothelial cells. This may explain the presence of "dye-vacuoles," representing a mixture of dye and PVP, which was found in this type of experiment as well as after simultaneous administration of dye and PVP. Finally, a "blocking" effect on the storing ability of reticulo-endothelial tissue was observed in mice injected with PVP *prior* to administration of dye. The latter finding is paralleled by observations of Barfuss and Eichler (4) who reported an impaired reticulo-endothelial activity in rabbits injected with PVP, as expressed in delayed disappearance of Congo Red from the circulation.

These experimental findings suggest the following tentative generalizations regarding biologic properties of PVP: 1) it enhances renal excretion of dyes; 2) it can displace and elute at least partially certain dyes from their site of storage; 3) preceding storage of PVP may prevent dyes from being deposited in reticulo-endothelial tissues. If it is permissible to consider the dyes used in our experiments as models of macromolecular substances in general, a number of possible implications can be considered. Schubert (12) proposed to utilize PVP for promoting rapid elimination of toxins (diphtheria, botulinum), and quoted several reports in which this procedure was used with favorable results in animals as well as clinically. The "eluting" effect of PVP on colloids previously stored in reticulo-endothelial tissue is in need of additional investigation. It is possible that the displacement by PVP of stored material depends on chemical and/or physical properties of the latter. Experiments with hemosiderin, thorothrast, India ink, and a number of additional dyes are under way to test this assumption. The inhibition of dye storage by PVP raises the question whether this signifies an impaired functional activity of reticulo-endothelial tissue. Preliminary results of immunization experiments suggest that mice of some strains, after treatment with PVP produced lower

levels of hemoantibodies than controls; these findings, however, are not yet conclusive.

All experiments described in this report were terminated shortly after the last PVP administration. For this reason no statement can be made as to the duration of the storage of PVP. It still remains to be established whether morphologic and functional changes attributable to the administration of PVP are wholly or partially reversible, or permanent.

*Summary.* 1. Storage of PVP, or of a derivative of PVP, was observed in mice after 12-24 injections of a 3.5% solution. The extent of storage appeared to depend on the mouse strain. 2. Simultaneous administration of PVP and sulfonated azo dyes led to renal excretion of dye, and to a decrease in reticulo-endothelial dye storage. 3. PVP administered after dye was given resulted in elution of dye from reticulo-endothelial tissues. 4. PVP administered prior to dye injection prevented storage of dye. 5. On the basis of these findings, an attempt was made to evaluate biologic properties of PVP.

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## Studies on Platelets. IV. A Thrombocytopenic Factor in Normal Human Blood, Plasma, or Serum.* (19466)

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In abnormal conditions, individuals may develop sensitivity to normal plasma and respond to its administration with a series of symptoms which have been grouped under the definition of "plasma transfusion reaction" (1). This has been found to occur consistently in paroxysmal nocturnal hemoglobinuria and, occasionally, in some types of blood dyscrasia and extensive carcinomatosis. Most of these patients exhibit some degree of thrombocytopenia.

Marked, but transitory thrombocytopenia appeared, in our earlier observations(2), to be one of the characteristic features of plasma transfusion reaction. Further study, directed to investigate the specificity of this finding revealed that temporary but significant reduction in blood platelets usually followed the transfusion of compatible blood, plasma or serum of normal donors into normal recipients

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