sponding purine monophosphates gave very little inhibition, producing K_i values of approximately 125 mM. In the case of the pyrimidine nucleotides, both the cytidine and uridine triphosphates were not as effective as the purine triphosphates. The K_i values obtained with the pyrimidine triphosphates were approximately seven times greater than for the purine triphosphates. Inhibition by ATP was found to be pH dependent in the range studied, (pH 7.0–10.2) with the greatest amount of inhibition observed at pH 8.6. Michaelis-Menton kinetics were observed with all of the nucleotides studied. However, when considering inorganic pyrophosphate, sigmodial type kinetics were observed. Inorganic phosphate was without effect on the diesterase; 5′-AMP and cyclic AMP did not reverse ATP inhibition; $MgCl_2$ alleviated the inhibition produced by ATP.

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An Evaluation of the Effects of Polyvinylpyrrolidone on Blood Typing with Anti-Rh Serum: Importance of Albumin Concentration (33857)

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Polyvinylpyrrolidone (PVP) as an agent for increasing the sensitivity of agglutination tests evoked considerable interest more than a decade ago (1–5), though current reports have been limited largely to those dealing with automated agglutination techniques (*e.g.*, 6, 7). In view of recent interest in the use of PVP in manual blood typing, particularly as an additive to anti-Rh sera, we undertook an evaluation of the effects of this polymer in such typing systems.

Experimental Methods. Preliminary testing was done to determine whether, as suggested by various early reviewers (8–10), the danger of nonspecific results would be increased if, in an effort to increase the sensitivity of a test system, one simply added PVP to a commercial typing system. The PVP used was of two types, designated K-30 and K-90, which according to the manufacturer[2] had average molecular weights of 40,000 and 360,000, respectively. Two blood typing procedures were carried out under various conditions with red cells from 56 different donors and commercial Rh antisera of 4 different specificities, *viz.*, anti-Rh_0 (anti-D), anti-rh′ (anti-C), anti-rh″ (anti-E), and anti-hr″ (anti-e). Both were tube agglutination methods, one of which utilized a measured suspension of cells and the other of which used only an

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[2] General Aniline and Film Corp., New York, New York.

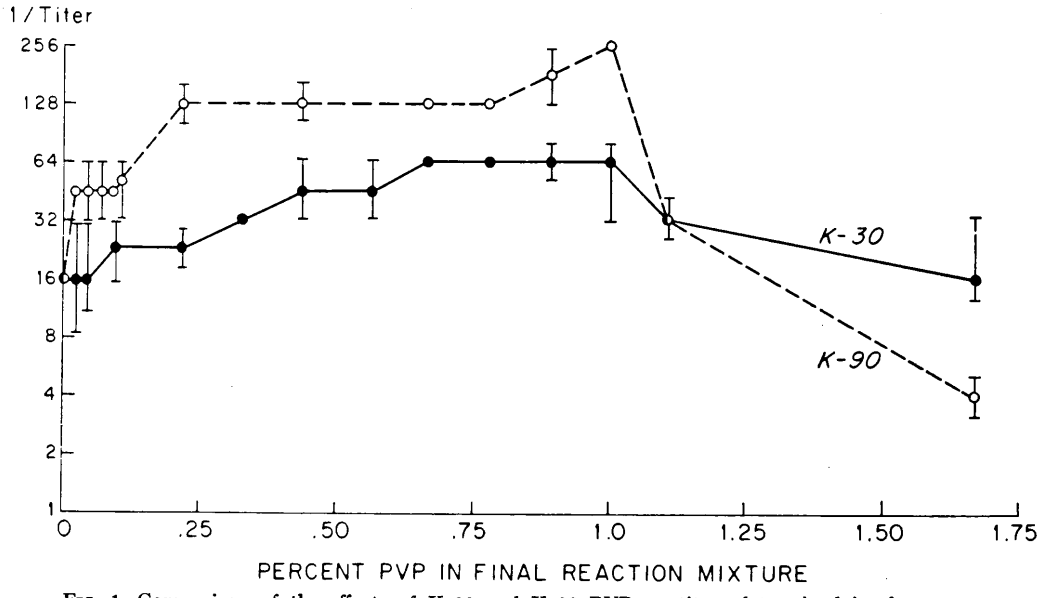

FIG. 1. Comparison of the effects of K-30 and K-90 PVP on titers determined in the presence of 18.3% bovine albumin. Each level of PVP was tested 1–4 times; when tested two or more times, averages are plotted and vertical lines indicate maximum range of titers observed. Vertical lines showing a range of less than one tube indicate that titers obtained in replicate tests were identical. The 0% PVP titer was that of the control in which PVP was replaced with 20% albumin.

approximate cell concentration prepared by transferring cells with an applicator stick. In a total of 500 tests in the presence of PVP at concentrations ranging from 0.5 to 4.5%, 49 false positive and 19 false negative results were observed.

For subsequent studies we chose a system which would permit closer control of experimental conditions, in the hope of pinpointing factors which affect the enhancement of agglutination by PVP. A 30% solution[3] of bovine albumin was diluted with isotonic saline. The resulting solutions of bovine albumin of 5, 10, or 20% concentration were used to reconstitute an incomplete anti-Rh_0 (anti-D) serum, which was a dried reference preparation (Division of Biologics Standards Reference Anti-Rh Typing Serum, Lot No. 4). Group O, Rh_0 (D) positive human red cells were washed three times with isotonic saline and prepared as 2% suspensions in bovine albumin (5, 10, or 15% concentration).

The PVP was dissolved in pH 7 Evans buffer as described by Stroje *et al.* (11). The solutions were filtered through three layers of Whatman No. 12 filter paper, autoclaved, and readjusted (if necessary) to pH 7 by dropwise addition of 2.5 *N* NaOH. The resulting solutions were mixed with 30% bovine albumin and isotonic saline so as to yield the desired concentrations of both PVP and albumin (see below). Twofold serial dilutions of the antiserum were prepared in 5, 10, or 20% bovine albumin, and 0.1-ml aliquots were placed in 10× 75-mm glass test tubes. To each tube was added 0.1 ml of PVP in the corresponding concentration of albumin. A 0.1-ml aliquot of the cell suspension, also in the corresponding albumin concentration,[4] was then added to each tube. Thus a given titration was carried out at a constant level of PVP and a constant level of bovine albumin.

After incubation for 15 min at room temperature (25°), the tubes were centrifuged for 2 min at 1000 rpm (approximately

[3] Bovine Albumin 30%, Lot No. A30511, Armour Pharmaceutical Co., Kankakee, Illinois.

[4] The sole exception to this procedure was the use of cells in 15% albumin when the antiserum and PVP were in 20% albumin. This yielded a final albumin concentration of 18.3% rather than 20%.

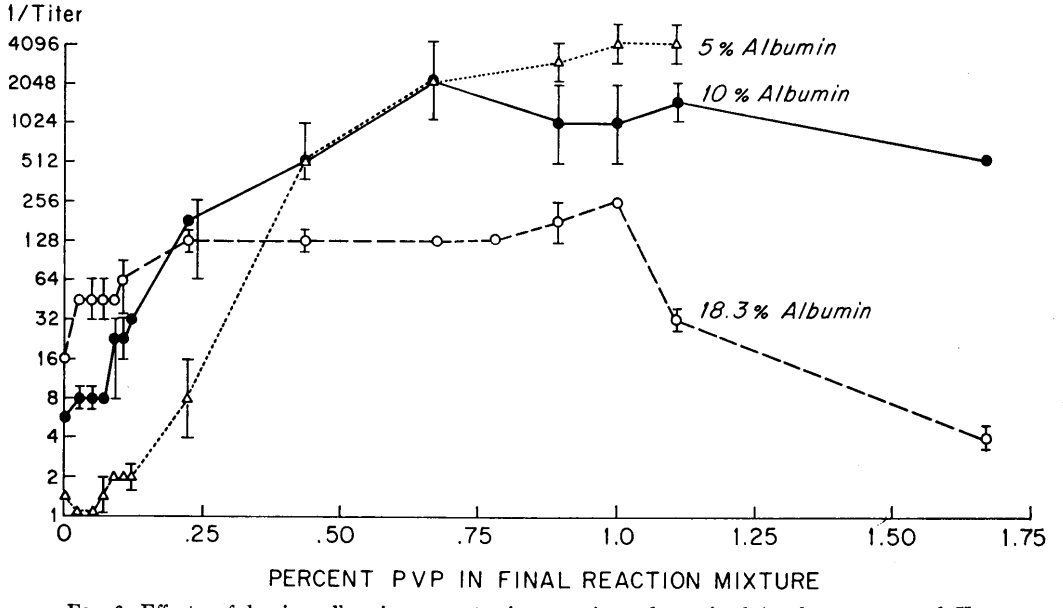

FIG. 2. Effects of bovine albumin concentration on titers determined in the presence of K-90 PVP. Each level of PVP was tested 1–4 times; when tested two or more times, averages are plotted and vertical lines indicate maximum range of titers observed. Vertical lines showing a range of less than one tube indicate that titers obtained in replicate tests were identical. The 0% PVP titers were obtained from the controls in which PVP was replaced with 5, 10, or 20% albumin, respectively.

150*g*), read macroscopically, and reread 15 min later (12). The titer was recorded as the highest dilution at which there was 2+ agglutination in the second reading. PVP-free controls were prepared by omitting the PVP solution or by replacing it with 0.1 ml of isotonic saline, Evans buffer, or the corresponding concentration (5, 10, or 20%) of bovine albumin.

When K-30 and K-90 PVP were tested in the presence of bovine albumin (final concentration, 18.3%), titers were appreciably increased over that of the PVP-free control (Fig. 1). The K-90 material was somewhat more effective in this respect, in agreement with the report of Jandl and Castle (13); however, both the high and low molecular weight PVP could bring about decreases in titer at high concentration.

Since K-90 PVP was the more effective, its action was tested in the presence of various levels of bovine albumin. As expected, titers of the PVP-free controls varied directly with the level of albumin (Fig. 2, 0% PVP). On the other hand, the values of the *maximum* titers obtained varied *inversely* with the albumin concentration (Fig. 2, 1.0–1.1% PVP).

The fact that the highest titers were obtained in the presence of 5% bovine albumin (the lowest level tested) suggested that PVP might bring about even greater enhancement of agglutination if the albumin were omitted together. However, when testing was performed in the absence of albumin, titers were not increased above that observed in the 18.3% albumin, PVP-free control. Tests carried out with Rh_0 (D) negative cells revealed no evidence of nonspecific agglutination by the reconstituted reference serum in the presence of PVP.

Discussion and Conclusions. The present work, in agreement with the results of others (14, 15), indicated that the titer can vary as a function of PVP concentration (Fig. 1). In addition, however, the present experiments showed the importance of the relationship between bovine albumin and PVP concentrations in systems utilizing both of these polymers. For example, the order of titers ob-

tained with various levels of bovine albumin could be reversed (*i.e.*, from 18.3%> 10%> 5% to 5%> 10%> 18.3%) simply by changing the concentration of K-90 PVP from 0 to 1% (Fig. 2).

There is no specific requirement for the level of protein in commercial typing sera (12), and there is appreciable variation in the albumin content of such antisera. It is therefore apparent that if an investigator intends to use a system involving PVP and commercial anti-Rh serum, different levels of PVP may be necessary to achieve optimal results with different antisera. Furthermore, the possibility of false results in Rh typing in the presence of PVP requires that the use of this polymer to enhance agglutination sensitivity be weighed carefully against the potential hazard of nonspecificity and, in any case, be made only under the most stringently controlled conditions.

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