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Potentiation of Hemolytic Plaque Formation by Incubation of Immunized Spleen Cells in Phenothiazine Derivatives.* (31848)

GARY A. BECKER† AND ANTHONY V. PISCIOTTA

Department of Medicine, Marquette University School of Medicine, Blood Research Laboratory, Milwaukee County General Hospital, Milwaukee, Wisc.

Numerous studies have shown that chlorpromazine (CPZ) affects membrane function. The literature is confusing because CPZ seems to decrease membrane permeability in certain instances, while it increases passage of substrates through a cell wall in others. Freeman and Spirtes(1) reported that CPZ reduced the rate of hemolysis when erythrocytes were placed in hypotonic solutions. They proposed that CPZ decreased the passive movement of water into the red cell because of an interaction between drug and membrane. Further studies by Spirtes and Guth(2) showed that CPZ inhibited passage of water, sodium and potassium into rat liver mitochondria and thereby prevented swelling of these structures. Furthermore, Guth, Amaro, Sellinger and Elmer(3) presented evidence to show stabilization of rat liver lysosomal membranes by CPZ *in vivo* and *in vitro* so that leakage of acid phosphatase to the surrounding medium was prevented. On the other hand, it has been shown, that CPZ in higher concentrations, *i.e.*, greater than 10^{-4} M, may cause hemolysis of erythrocytes, while less than 10^{-5} M decreased hemolysis caused by hypotonic sodium chloride(4). Fuks, Lanman and Schanker(5) showed that while CPZ may diminish permeability to water it does not affect diffusion of certain organic amine compounds such as DL-norepinephrine, bufotenine or serotonin into erythrocytes or platelets. At variance with these observations was Nathan's report that CPZ enhanced permeability of oxalacetate into *Lactobacillus planatarum*(6) and increased diffusion of L-histidine into *Tetrahymena pyriformis*(7).

In the present study, we tested the *in vitro* effect of CPZ and other phenothiazine derivatives on diffusion of macromolecules through a cell membrane. For this purpose, the hemolytic plaque technique of Jerne(8) was used to demonstrate the effect of these drugs on the diffusion of hemolysin into the surrounding supporting medium.

Materials and methods. White, male, Sprague-Dawley rats weighing 150-200 g were immunized with an intravenous injection of a 10% suspension of sheep erythrocytes in isotonic saline. Four days later, when the number of hemolytic plaques reached a peak value, the animals were sacrificed and spleens removed. The spleens were minced in Eagle's Basal Medium. Three-tenths (0.3) ml of the resulting cell suspension, containing generally about 1.5×10^6 spleen cells were then in-

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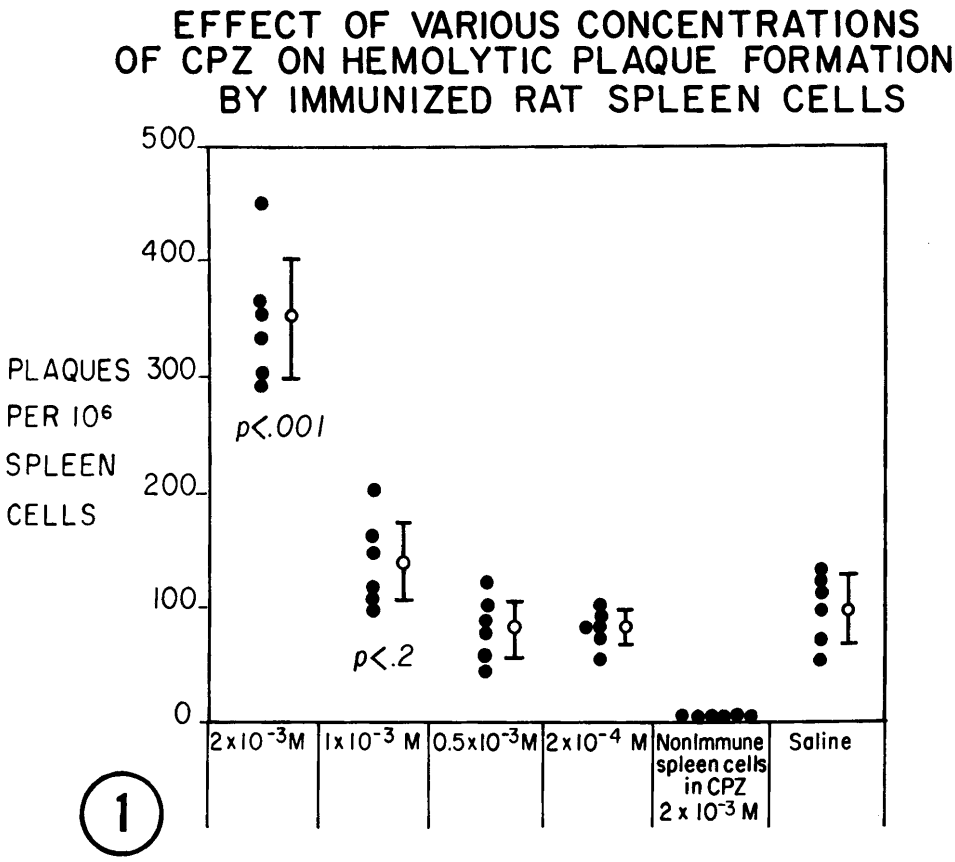

Fig. 1. Effect of chlorpromazine in various concentrations on plaque formation. Each point represents one determination. A mean ± one standard deviation is shown for each concentration.

cubated for one hour at 37°C in equal volume of the test drugs, including CPZ HCL,‡ CPZ sulfoxide,‡ promethazine (PMZ)§ and thioridazine (TDZ).‖ All drugs were diluted with isotonic saline. Cells incubated in saline alone were used as controls. After incubation, cell counts were done using Trypan blue to determine the number of viable cells which remained. The spleen cells were then suspended in agar together with sheep erythrocytes as described by Jerne(8). After the agar hardened, the preparations were incubated at 37°C for one hour. The plates were then covered with complement and incubated an additional one-half hour. The hemolytic plaques that developed were counted under a stereoscopic microscope. All plaque counts were corrected to 10^6 viable spleen cells plated. Two rats were used for each experiment and 3 plates prepared for each drug. All experiments were done twice.

Results. Fig. 1 depicts the effect of different concentrations of CPZ on the development of hemolytic plaques. With a drug concentration of 2×10^{-3} M plaque formation was significantly enhanced as compared to saline treated controls. Ten-fold dilutions of the CPZ dissipated its plaque enhancing effect. Greater concentrations of CPZ, 2×10^{-1} M and 2×10^{-2} M, destroyed all spleen cells

‡ Courtesy of Smith Kline & French Laboratories, Philadelphia, Pa.

§ Courtesy of Wyeth Laboratories, Inc., Philadelphia, Pa.

‖ Courtesy of Sandoz Pharmaceuticals, Hanover, N. J.

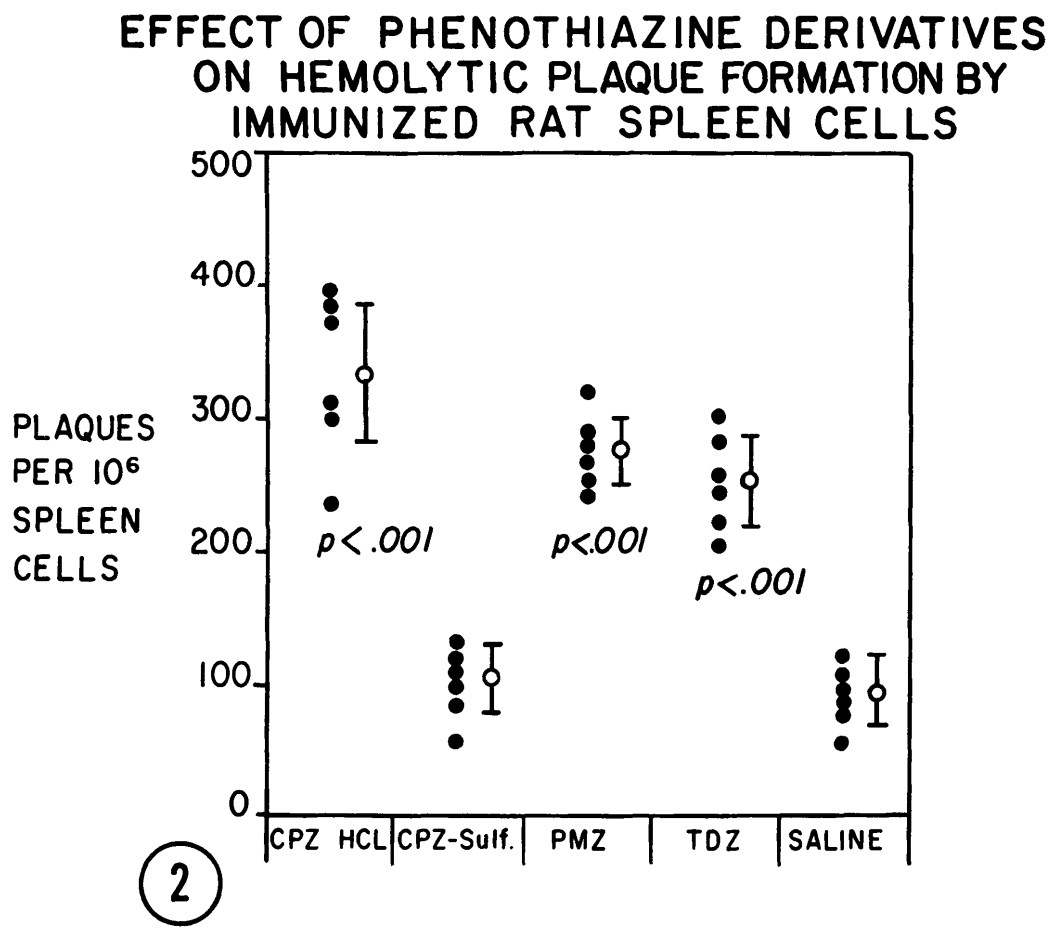

FIG. 2. Each point represents one determination. Mean ± one standard deviation given for each drug.

and no plaques were formed. Because of the known hemolytic properties of CPZ *in vitro*, spleen cells from non-immunized animals were incubated in the drug at 2×10^{-3} M, concentration. No hemolytic plaques were found.

Fig. 2 depicts the results when immunized spleen cells were incubated for an hour prior to plating with various phenothiazine derivatives, all in a concentration of 2×10^{-3} M. More than 2-fold enhancement of plaque formation was observed with CPZ HCL, promethazine and thioridazine treated cells. CPZ sulfoxide, a metabolic oxidation product of CPZ, had no effect on plaque formation compared to controls.

Discussion. When spleen cells from rats immunized with sheep erythrocytes are preincubated with certain phenothiazine derivatives (CPZ, PMZ and TDZ, each 2×10^{-3} M), hemolytic plaque formation is potentiated in an agar plate preparation. The enhancement of plaque formation seems best explained by an increase in cell permeability to preformed hemolysin, induced by drugs. That passage of a macro-molecule such as 19S immunoglobulin is enhanced, suggests a substantial increase in membrane permeability. Whether this action of the phenothiazine derivatives influences the passive movement of antibody across the cell membrane or affects an active transport mechanism is unknown. The data presented indicate that with the agar plaque technique, a greater number of hemolysin containing cells are present than can be accounted for in untreated preparations. These cells do not re-

lease hemolysin unless treated with an agent that allows it to escape.

The *in vitro* effect of CPZ on antibody producing cells is probably different from its action when it is injected into animals. Preliminary studies from this laboratory(9) indicate that CPZ in high doses delays formation of hemolytic plaques, cellular production of RNA and release of antibody after primary immunization. Further, treatment with CPZ results in fewer hemolytic plaques, acridine orange stained cells and lower antibody titers after secondary immunization. This disparity between *in vivo* and *in vitro* effect may be due to the many actions of CPZ after it has entered a viable cell. It is well known that CPZ has a wide variety of biological properties in membraneless systems. For example, it inhibits a number of enzymes including DNA polymerase and thymidylate kinase(10). Since antibody formation depends on many cell functions it is possible that the *in vivo* inhibition of antibody synthesis by CPZ and the *in vitro* enhancement of antibody release by CPZ are unrelated.

Summary. Stimulation of hemolytic plaque formation by rat spleen cells after primary immunization was produced by *in vitro* incubation of these cells with chlorpromazine, promethazine and thioridazine in 2×10^{-3} M concentration. This effect may be the result of increased cell membrane permeability produced by these compounds. The phenothiazine derivatives may be useful agents for increasing the sensitivity of the hemolytic paque procedure by enhancing the release of antibody from immunized cells.

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Electrolyte-Fluid Exchanges and Renal Tissue Composition in Vasopressin Treated Polyuric-Polydipsic Rabbits.* (31849)

M. R. NOCENTI AND L. J. CIZEK

Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City

The male rabbit when deprived of food, but allowed water *ad libitum,* develops a polyuria and polydipsia associated with a significant loss of sodium(1,2). Since the suppression of this polyuric-polydipsic syndrome by estrogens occurs even when sodium loss is enhanced by a spironolactone(3), a change in either vasopressin release or the renal response to this hormone may be involved. In view of the demonstration(4) that the medullary osmotic gradient can be washed out by a water diuresis, the possibility must be considered that this syndrome may be associated with a loss of the medullary osmotic gradient; this could account for an alteration in response to vasopressin. As an attempt to determine the factors promoting the increased fluid exchange and the mechanism of the estrogen inhibition, the response of food deprived rabbits to vasopressin administration and the renal tissue electrolyte

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