siderations, we have undertaken the production of Rh antisera by inoculating experimental animals with Rh+ human erythrocytes.

Guinea pigs were inoculated with varying doses of red blood cells over a period of 3 months. The cells used were from an Rh+, group O, type MN individual. The presence of the Rh antigen in these cells was assured by the fact that they agglutinated in several absorbed guinea pig anti-rhesus sera and in 6 human anti-Rh sera. Immune serum obtained in this manner when absorbed, according to our previously reported technic[2] with group O, type MN, Rh— cells, distinguished sharply between known Rh+ and Rh— human erythrocytes of all blood groups.

In view of these findings it would appear that the possibilities for producing Rh antisera, useful in testing the red blood cells of human beings belonging to any blood group, have been greatly extended.

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Effect of Propylene Glycol on Bacterial Spores.

EDWARD BIGG. (Introduced by H. L. Alt.)

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Recent publications[1,2] have demonstrated the bactericidal and viricidal activity of propylene glycol. Using air-suspended bacteria as well as broth cultures *in vitro*, the following organisms have been found susceptible to this substance: Hemolytic and non-hemolytic staphylococci, *B. coli*, hemolytic streptococci, *Streptococcus viridans*, pneumococci (Types I, II, and rough), *Hemophilus influenzæ*, *B. pertussis* and PR8 strain of influenza virus.

Bactericidal action is demonstrable in dilutions as high as one part of propylene glycol in 50 million parts of air when air-suspended organisms are tested, while *in vitro* experiments demonstrate that organisms grow in broth containing as much as 15% glycol but are killed when the concentration of glycol in the media reaches 85%.

In view of the widespread interest in the use of glycol vapor as a means of controlling cross infections, this study was carried out in an attempt to throw further light on its possible mode of bacterial killing. Using *B. subtilis* as the test organism, observations were made to determine the effect of propylene glycol on bacterial spores.

I. *Effect of Propylene Glycol Vapor on Air-Suspended Bacteria.* These tests were done in small glass chambers of 2 cubic feet capacity; the air was agitated by means of small fans and standardized broth cultures were atomized into the space. Measured dilutions of propylene glycol vapor were introduced. Samples of 2 liters of air were taken for culture and analysis.

A. *Vegetative Forms.* Eighteen-hour cultures of *B. subtilis* were sprayed into the chambers and results similar to those reported occurred; *i.e.*, there was immediate killing in dilutions as high as 1 g of glycol in 25 million cc of air.

B. *Spores.* A 72-hour culture of *B. subtilis* showing many sporulated forms was heated for 20 minutes at 80°C. After this period of heating a loop of the culture was inoculated in broth. Abundant growth occurred, showing that the spores were viable. An 18-hour culture showing no spore forms was killed by this amount of heat. When the suspension of spores was atomized into "glycolized air" no bactericidal effect was observed even when the introduction was made into a saturated

1 Robertson, O. H., Bigg, E., Puck, T. T., and Miller, B. F., *J. Exp. Med.*, 1942, **75**, 593.

2 Robertson, O. H., Loosli, C. G., Puck, T. T., Bigg, E., and Miller, B. F., *Science*, 1941, **94**, 612.

atmosphere (1 g of glycol in 200,000 cc of air).

II. *Effect of Propylene Glycol in Vitro.* A. *Vegetative Forms.* Inoculation of glycol into broth cultures resulting in glycol concentrations ranging from one to 100% produced the expected effects. Growth occurred in dilutions up to 20% and killing was obtained in solutions of 80% or more.

B. *Spores.* No effect on the viability of spores resulted from the inoculation of glycol into these cultures even though spores were exposed to the glycol for as long as 72 hours.

Discussion. Although no actual proof has been demonstrated as to how the glycols exert their bactericidal action, it would appear that it is brought about by the chief physical property of glycol, namely, hygroscopicity. Due to the moisture contained in each droplet of suspended bacteria and/or the film of moisture about each bacterial cell, molecules of glycol are attracted. This develops a high glycol concentration in direct contact with the cell membrane. As shown in test-tube experiments these high concentrations produce bacterial death. The lethal effects may take place by actions which possibly include dehydration of the cell, interference with respiration, denaturation of protein, inhibition of enzyme activity, and others. Since spores are particularly resistant to drying, the possibility is suggested that removal from, or entrance into the cell body, of water, would be difficult, whereas in vegetative forms this action could occur more readily, mediated by a high glycol concentration on the cell surface.

Conclusion. Propylene glycol in its liquid or vapor state demonstrates no killing effect on spores of *B. subtilis*. This observation tends to support the hypothesis that the bactericidal action of the glycols is due to the hydroscopic properties of these substances.

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Effect of Hormones on Contraction of Striated Muscle and on Choline Esterase Activity.

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While sex hormones and some of the hormones secreted by the hypophysis do not contract striated muscle in low concentrations, they may modify the biochemical equilibrium of the muscle. The purpose of this study is to investigate whether or not the presence of the above substances modifies the contraction of the muscle produced by known chemical agents.

Experimental. Effect of the Hormones on the Acetylcholine Sensitivity of Striated Muscle. The *Rectus abdominis* muscle of the frog was excised and suspended in a muscle chamber containing 20 cc of Ringer's solution at pH 7 and room temperature. The muscle was immersed alternately in a 0.1 mg per 100 cc acetylcholine solution for 30 seconds and in Ringer's solution for 10 minutes until the height of contraction remained constant for 3 cycles. This contraction served as control. The height of contraction was registered with an isotonic lever on a kymograph. The muscle was then immersed in a solution or suspension of one of the hormones in Ringer's solution for 5 minutes before each immersion in acetylcholine. The hormones were used in increasing concentrations. Changes in the height of contraction of the muscle due to the presence of the hormones were measured and expressed as percent of control. Experiments were performed in each case using instead of hormones the solvents alone. The solvents alone (sesame oil, peanut oil, and water with acacia) did not modify the effect of acetylcholine on the muscle. Other solvents (alcohol, ether, acetone, bile salts) were not used