to a study of the factors affecting the stability of such suspensions. Other workers[9] have found that the stability is unaffected by a relatively wide pH range. Certain enzymes are active only within certain oxidation-reduction ranges.[10] Among viruses, that of tomato spotted wilt is highly susceptible to oxidation by air and may be protected by reducing agents such as cysteine and sodium thioglycollate.[11] Mueller found that the virus of Rous's sarcoma may be protected against oxidation by the addition of cysteine.[12]

Zinsser and Tang[13] have found that cysteine stabilizes herpes virus.

The results in Table III and Fig. 1 show the effect of certain reducing agents under vaseline and of glycerine in several concentrations on the stability of virus. Unless otherwise indicated the virus was ultracentrifuge purified; the pH was held at 7.0-7.6 with M/10 phosphate; the concentration of reducing agent was M/10; and the temperature was 5-10°C. Methylene white was prepared by reducing methylene blue with metallic zinc and hydrochloric acid followed by rapid neutralization and centrifugation of the precipitate. The supernatant was used. Cyanide was used in two instances to destroy some cellular enzymes which might be present and causing the inactivation. In several instances after the experiments were finished there was still some active reducing agent as detected with methylene blue.

In the experiments shown in Table III the temperature accidentally rose to something above 10°C. As a result of this accident, however, the stabilizing effect of cysteine was forcibly brought to our attention for there was only a perceptible drop of virus activity in the tube containing cysteine but a marked drop in all others including the control.

Summary. The virus of Eastern equine encephalomyelitis may be concentrated and partially purified by ultracentrifugation. M/10 cysteine retards spontaneous inactivation of the ultracentrifuge purified virus.

9 Finkelstein, H., Marx, W., Bridgers, W. H., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 103; Finkelstein, H., Marx, W., Beard, D., and Beard, J. W., *J. Inf. Dis.*, 1940, **66**, 117.

10 Hellerman, L., *Cold Spring Harbor Symp. Quant. Biol.*, 1939, **7**, 165.

11 Best, R., *Aust. J. Biol. and Med. Sci.*, 1939, **17**, 1.

12 Mueller, J. H., *J. Exp. Med.*, 1928, **48**, 343.

13 Zinsser, H., and Tang, F. F., *J. Immunol.*, 1929, **17**, 343.

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Action of Promin and Diamino-Diphenyl Sulfone upon Tubercle Bacilli; Antipromin Action of p-Aminobenzoic Acid.

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A series of test tube experiments was undertaken to determine the action of promin* and its parent substance, diamino-diphenyl sulfone, upon the tubercle bacillus, and to learn whether or not *p*-aminobenzoic acid would inhibit the effect of these compounds as it has been shown to inhibit the effect of sulfanilamide upon other organisms.

All these tests were made upon both virulent and avirulent mutants of the human strain H_{37} used by Feldman, Hinshaw and Moses,[1-4]

* Promin used in this study was kindly supplied through the courtesy of Dr. E. A. Sharp, Parke, Davis and Company, Detroit, Mich.

1 Feldman, W. H., Hinshaw, H. C., and Moses, H. E., *Proc. Staff Meet., Mayo Clin.*, 1940, **15**, 695.

2 Feldman, W. H., Hinshaw, H. C., and Moses, H. E., *Ibid.*, 1941, **16**, 187.

3 Feldman, W. H., Hinshaw, H. C., and Moses, H. E., *Am. Rev. Tuberc.*, 1941, **45**, 303.

4 Feldman, W. H., Mann, F. C., and Hinshaw, H. C., *Ibid.*, 1942, **46**, 187.

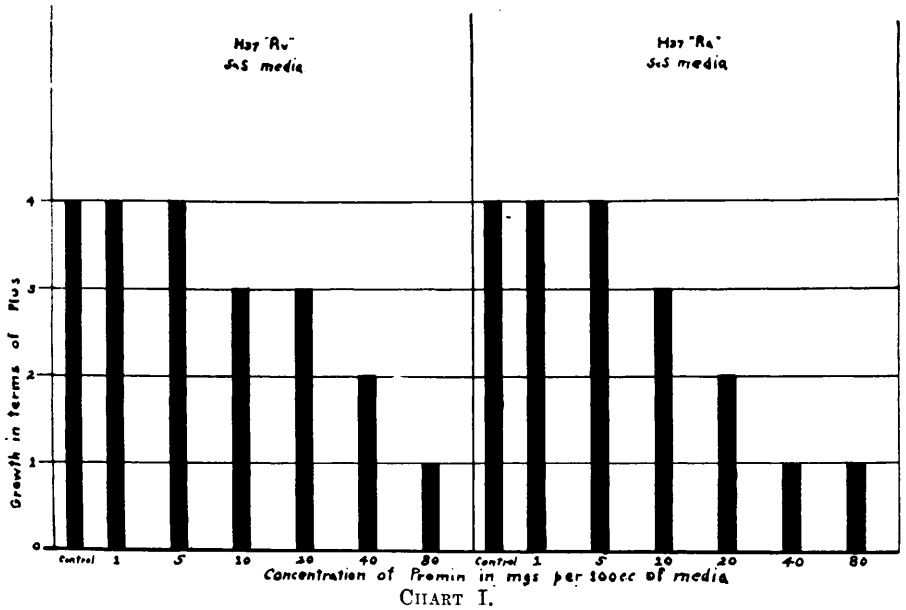

CHART I.

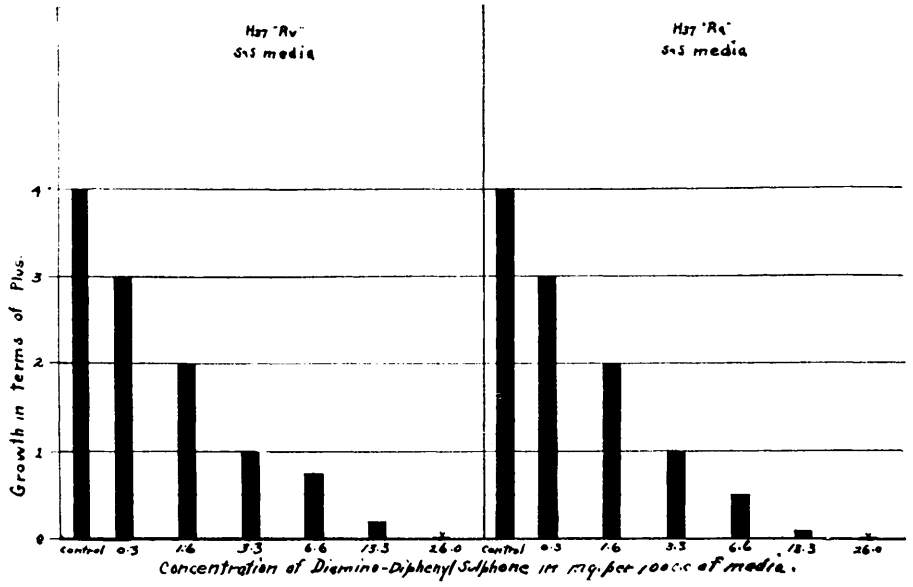

CHART II.

in their work on promin, and by writers in previous studies on sulfonamide drugs. The cultures were grown on Steenken and Smith's solid medium, or on Proskauer and Beck's fluid medium.

Promin in Steenken and Smith's Medium. Promin was added to tubes of this medium in concentrations from 1 to 80 mg% and the mixtures were then seeded with the 2 variants of the standard bacillus. Chart I demonstrates comparatively slight inhibitory action until concentration of 40 mg% is attained. The effect is more marked upon the avirulent (R_a) mutant.

Diamino-Diphenyl Sulfone† *in Steenken*

† Diamino-diphenyl sulfone was kindly supplied by Abbott Laboratories, North Chicago, Ill.

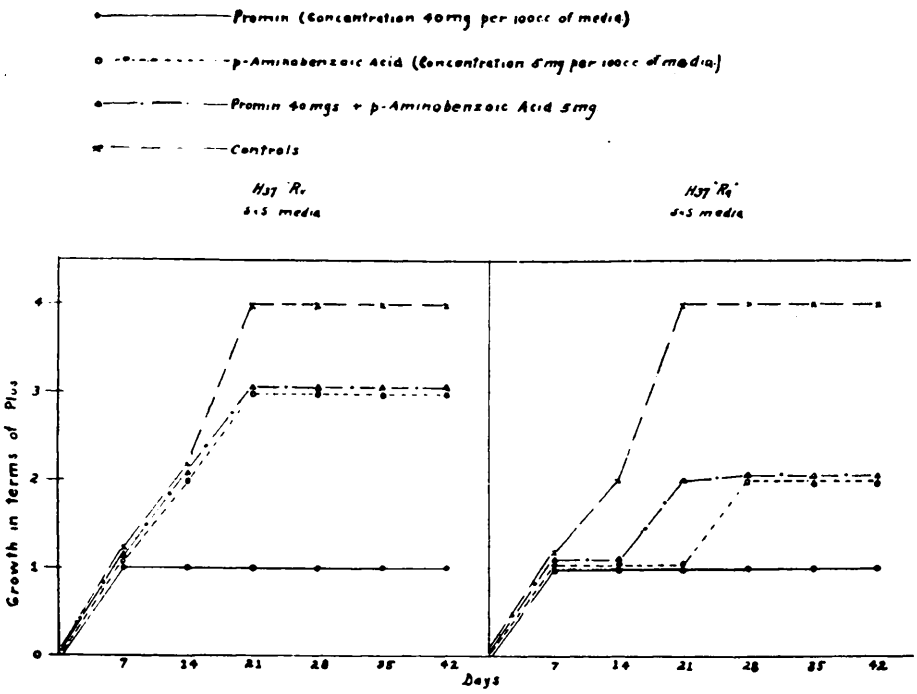

CHART III.

and Smith's Medium. The quantities of this drug used were based on the amount of the parent substance, diamino-diphenyl sulfone, which constitutes about one-third of the molecular weight of promin. Therefore the weights used in this test were in the ratio of one part of the diamino-diphenyl sulfone as compared to 3 parts of promin used in the previous test. These quantities varied from 0.3 to 26 mg% as shown in Chart II. These drug concentrations had much more effect upon the growth of the tubercle bacilli than the larger quantities by weight of promin. The additions of 1.6 mg% reduced growth to the same degree produced by 40 mg% of promin.

P-Aminobenzoic Acid and Promin in Proskauer and Beck's Fluid Medium. These tests involved observations upon the action of this substance alone and in combination with 40 mg% of promin, together with appropriate controls. The results are plotted in Chart III on the basis of the amount of growth observed at intervals during 42 days after planting the cultures. It will be noted that the untreated control medium produces maximum growth after 21 days and that promin alone limits bacterial growth to a low level which does not increase appreciably after the first week.

The addition of *p*-aminobenzoic acid in a concentration of 5 mg% exerts a moderate degree of inhibition of growth, and this effect is not appreciably increased by the simultaneous presence of 40 mg% of promin. The result was checked further by determining the weight of organisms produced in 4 weeks on Proskauer and Beck's fluid medium to which the test drugs had been added in the concentrations just mentioned.

Chart IV shows that the results were comparable to those based upon an estimate of the extent of the growth. Promin alone kept the growth down to very low levels. In the presence of *p*-aminobenzoic acid, promin caused only slightly less inhibition of growth than *p*-aminobenzoic acid alone.

In both the last 2 groups of tests the effects were somewhat more marked upon the avirulent dissociant R_a of the H_{37} strain.

Discussion. Our experiments indicate that the concentration of promin required to produce inhibition of growth of tubercle bacilli (H_{37} R_v) *in vitro,* is far greater than the concentration required to retard the progres-

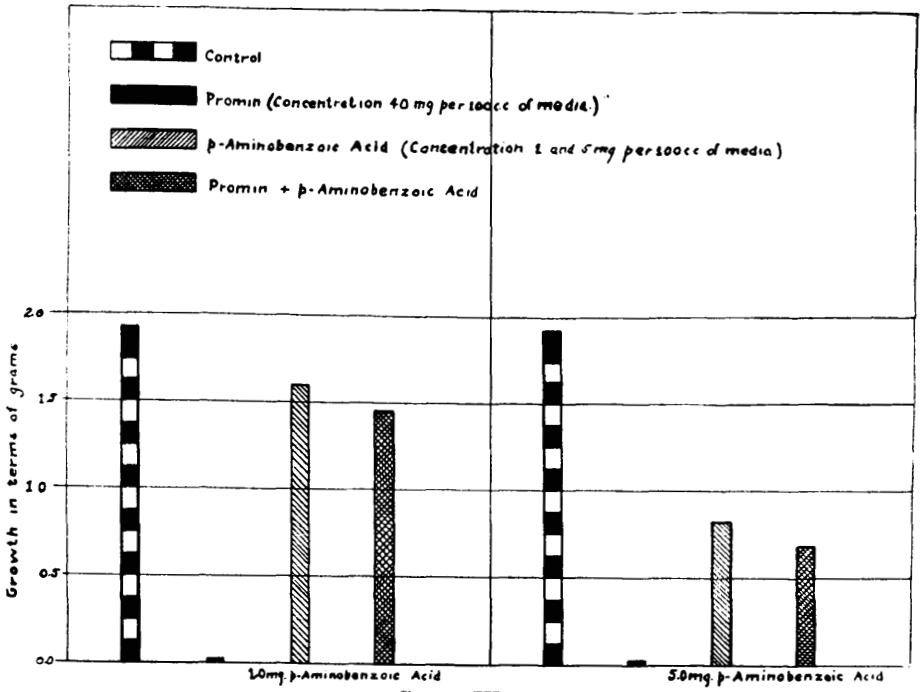

CHART IV.

sion of the disease in the animal body as reported by Feldman, Hinshaw and Moses.

Apparently, the action of promin in the animal body (guinea pig) is quite similar to the action of other sulfonamides against other microörganisms, in that they produce a bacteriostatic and not a killing effect upon the microörganisms.

Diamino-diphenyl sulfone produces a much greater inhibition of growth upon the H_{37} (R_v and R_a) microörganisms when added to media on the calculated basis of the available amount of the parent substance diamino-diphenyl sulfone present in promin, which is about one-third of the molecular weight of promin.

On the basis of this finding it is possible that the parent substance diamino-diphenyl sulfone may be equally, or more, effective than promin against tuberculous disease in guinea pigs.

From these studies it can be seen that *p*-aminobenzoic acid exerts a marked antipromin effect upon the growth of the tubercle bacilli. This action is similar to the antisulfonamide action of *p*-aminobenzoic acid upon the growth of other bacteria as described by Woods.[5]

It might also be suggested that *p*-aminobenzoic acid be added to the cultures when culturing for tubercle bacilli from wounds treated with sulfonamides. Since the drugs are usually applied as powder or a thick paste, the drug in such form is in a concentration that would retard or inhibit the growth of the tubercle bacillus.

[5] Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.