ference in the amount of enzyme in the liver, but preliminary experiments with blood and with the spleen indicated that almost twice as much inorganic phosphate was liberated in experiments with Akh animals as with Rfi animals.

Finally it is worthy of note that the leukocytosis produced in the Akh strain is primarily a segmented-cell type of response. A typical hemogram four hours after the injection of thymonucleic acid was: lymphocytes, 13; monocytes, 1; segs, 63; stabs, 14; juveniles, 5; eosinophiles, 2; and basket cells, 2.

It should be emphasized that the experiments deal with the response of the bone marrow, while the type of leukemia in the Akh animal is lymphoid. We have not been able as yet to establish any connection between the type of response and the subsequent development of leukemia, in a given animal, but experiments along this line are under way.

Summary. By comparison of the response of the bone marrow to the injection of nucleic acid and its split products a difference has been demonstrated between two strains of mice, Akh and Rfi, the former of which carries lymphoid leukemia and the latter myeloid leukemia.

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Promin in Treatment of Experimental Tuberculosis.

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Promin* is the sodium salt of p,p¹-diaminodiphenylsoulfone-N,N¹—(dextrose sulfonate).

Greey, MacLaren, and Lucas[1] reported that promin is effective in streptococcus infections in mice. Feldman, Hinshaw, and Moses[2] reported that it exerts an impressively beneficial effect on the course of experimental tuberculosis in guinea pigs, even when[3, 4] treatment

* Supplied through the courtesy of Parke, Davis and Co., Detroit.

1 Greey, P. H., MacLaren, D. B., and Lucas, C. C., *Can. Med. Assn. J.*, 1939, **40**, 319.

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

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

4 Hinshaw, H. C., and Feldman, W. H., *J. A. M. A.*, 1941, **117**, 1066.

was delayed until 6 weeks after infection. These authors administered the drug orally. Woodruff[5] reported that promin was not beneficial when administered subcutaneously to tuberculous guinea pigs. Toomey and Roach[6] obtained favorable results in acute streptococcus infections and have summarized the toxic reactions observed in 150 patients who were given large doses daily by the intravenous route. Coggeshall, Maier, and Best[7] have described the beneficial effects of promin on certain types of naturally acquired malaria in man, using large doses given intravenously for a short period.

We think it is noteworthy that every report on the promin treatment of humans indicates that the toxic reactions are serious only when the drug is administered orally. We also have had this experience while treating patients at the Montefiore Hospital. There are theoretical grounds for believing that promin is hydrolyzed in the stomach, splitting off the glucose groups and releasing the toxic parent diaminodiphenylsulfone. This substance has given favorable results in avian tuberculosis of rabbits,[8, 9] and we are beginning a study of its effect on mammalian tuberculosis in guinea pigs. Because of these considerations, we decided to use the subcutaneous route in our experiments.

Experimental. 1. Four experiments with 2 strains of human tubercle bacilli (H37 and a recently isolated strain) showed that when promin is added to Sauton's medium in concentration of 40 mg %, it inhibits the growth of tubercle bacilli for about 30 days, after which time the organisms began to grow. This phenomenon is being investigated. There was no change in morphology, staining reactions, or perhaps virulence, in organisms that had remained under these conditions for 30 days. Guinea pig inoculation with these cultures produced invasive tuberculosis.

2. Nineteen tuberculin-negative guinea pigs, average weight 400 g, were infected subcutaneously in the groin with a suspension of organisms extracted from fresh human sputum by the NaOH concentration method, and containing from 3 to 5 acid-fast bacilli per oil immersion field. Eight of these animals served as controls. The other 11 were each given three daily doses of 140 mg promin in 40% aqueous solution subcutaneously, starting on the day of infection. When an animal in either group died, an animal from the other group was sacrificed for purposes of comparison.

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[5] In Proc. Am. Soc. Bact. and Path., *Am. J. Path.*, 1941, **17**, 578.

[6] Toomey, J. A., and Roach, F. E., *J. Ped.*, 1941, **18**, 1.

[7] Coggeshall, L. T., Maier, J., and Best, C. A., *J. A. M. A.*, 1941, **117**, 1077.

[8] Rist, N., *C. R. Soc. Biol.*, 1939, **130**, 976.

[9] Rist, N., Bloch, F., and Hamon, V., *Ann. Inst. Past.*, 1940, **64**, 203.

Results. For the first 3 weeks, the deaths occurring were due to intercurrent infection, and there was little difference noted in the amount of tuberculosis in the 2 groups. After this time, however, there was marked spread of the disease in the control animals, while this spread was absent or minimal in the treated ones. When the experiment was terminated 7 weeks after infection, 5 animals in each group survived. Upon autopsy the controls showed maximal tuberculosis of all organs, while the treated animals showed little or no progress from the picture seen at the 3-week stage.

These results encouraged us to repeat the experiment with a larger series of animals.

3. Thirty-seven male tuberculin-negative guinea pigs, average weight 500 g, were infected subcutaneously in the groin with .001 mg of a culture of the organism used in Experiment 2. Starting on the day of infection, 20 of these were each given 160 mg of promin in 40% aqueous solution subcutaneously 3 times daily. The remaining 17 animals served as controls. With 2 exceptions, no animals were killed until the end of the experiment. The entire group of animals was tuberculin tested 2 weeks after infection. The blood levels of promin were estimated in the treated animals at various times in the day. All animals were weighed once a week, and upon autopsy, the amount of disease was noted on a scale from 0 to 4, the lungs, liver, and spleen were weighed, and representative portions of the lungs, liver, spleen, and lymph gland were obtained for histological studies.

Results. Tuberculin Tests. All 37 animals reacted positively to 0.1 mg O.T. intracutaneously.

Promin Level of Blood. This ranged between 5 and 44 mg %, the average being 18 mg %. The lowest values were found between the evening (6 p.m.) and the morning (8:30 a.m.) doses; the highest value was found an hour after any dose.

Weight. The treated animals lost about 25% more than the controls. We believe a large part of this difference may be due to the greater amount of handling to which the treated animals were subjected, as well as to the toxic effects of the drug, which will be briefly described below.

Survival Time and Autopsy Findings. Our observations on these topics have been pictured in Chart 1. Reference to this chart shows that one control and 3 treated animals died of intercurrent disease shortly after infection. The remaining 16 controls show the typical survival times of a group of animals infected with a standard dose of tubercle bacilli and kept under identical conditions. Two animals

died of tuberculosis relatively early, 12 died between 80 and 165 days after infection, and 2 lived for longer periods, namely 185 and 250 days after infection, when they died of tuberculosis. Only 3 of these 16 controls showed less than maximal tuberculous involvement. When we examine the findings in the treated animals, we see that this normal pattern is greatly disarranged. Deaths are only one-third as frequent as in the controls. Seven of the treated animals were living and apparently in good health on the 271st day after infection, when they were sacrificed to terminate the experiment. Only 2 of the treated animals showed maximal tuberculous involvement. Six of them showed minimal tuberculosis and died from some other cause, while 9 showed moderate or severe involvement. The averages of the autopsy scores of the 2 groups were 3.5 for the controls, and 2.0 for the promin-treated animals. Furthermore, after the hundredth day after infection, every treated guinea pig showed gross evidence of fibrosis. This occurred in about half of the controls, but was not as marked or as consistent.

Histologic. Examination of sections of the organs confirmed the gross autopsy findings; the treated animals had less tuberculosis and it was more fibrotic and chronic in type than that seen in the controls. Sections stained for acid-fast organisms showed the tissues of the treated animals to contain about ⅔ of the number of acid-fast bacilli found in the controls. However, all of the treated animals had tuberculosis.

Toxic Reactions. The injections were apparently quite painful to the animals, who cried and scratched the site of injection for 2 or 3 minutes. The injection caused ulcers in about 50% of the animals.

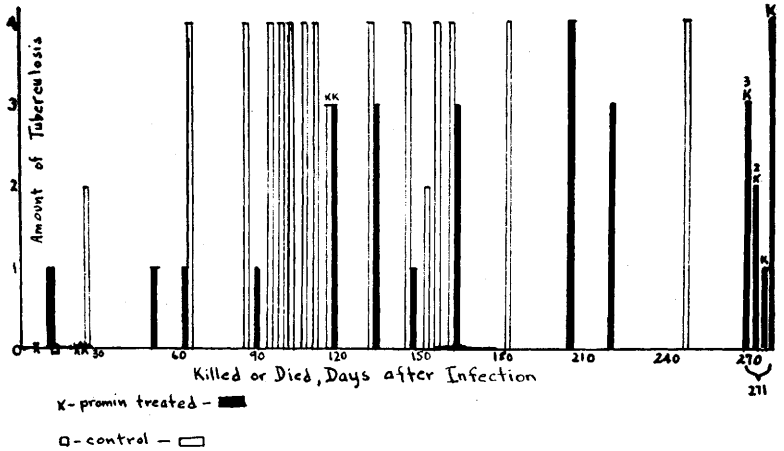

FIG. 1.

A few of these penetrated the muscle and attained a diameter of 1.5 cm. However, all of them healed in 2 or 3 weeks without secondary infection. Seven of the treated animals showed varying grades of fatty degeneration of the liver, while this condition was found in only one control guinea pig. However, because of the small numbers involved, we do not wish to draw conclusions from this latter observation.

Discussion. From the evidence presented above, we think it reasonable to conclude that promin exerts a retarding effect on the course of experimental tuberculosis in guinea pigs. Under the conditions of our experiments, this beneficial effect is manifested by a retardation of the spread and multiplication of tubercle bacilli in the animals. While these results are far superior to those which we have obtained with any other therapeutic agent in experimental tuberculosis, they fall far short of the wonderful effects reported by Feldman and his coworkers. These differences may be explained in part by variations in experimental procedure in such external and internal factors as route of administration of the drug, organism used, and breed of guinea pig. However, an adequate chemotherapeutic agent for humans should be able to cope with bacteria of wide differences in virulence, and should be of help in individuals of various constitutional makeups and from a wide variety of environments. Nevertheless the results reported, in our opinion, indicate that promin should have an exhaustive and prolonged study in the treatment of human tuberculosis.

Summary and Conclusions. 1. Promin exerts a bacteriostatic effect on tubercle bacilli *in vitro*. 2. Promin exerts a retarding effect on guinea pig tuberculosis, but does not cure the disease. 3. The effects of promin on human tuberculosis are being investigated.

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Complementing Action of Eserine and Acid in Neurohumoral Activation.

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Since eserine permits a greater accumulation of acetylcholine at junctional points of activated ganglion cells, muscle fibers and other