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Tuberculous Infection in Animals Prevented from Developing Hypersensitivity.

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Several investigators[1] have shown that guinea pigs rendered hypersensitive and immune with attenuated tubercle bacilli can be desensitized with Koch's Old Tuberculin (O.T.) without loss of their immunity. The present study was begun with the object of investigating the effect of preventing the development of hypersensitivity in experimental tuberculous infection. Since beginning these experiments Willis and Woodruff[2] have reported investigations of like nature, with the conclusion that preventing the development of hypersensitivity made animals unusually susceptible to tuberculosis.

Our first series consisted of 16 adult male guinea pigs. Half were given 1 cc undiluted O.T. subcutaneously daily for 10 days and 2 cc daily thereafter until death. The remaining 8 received nothing and served as controls. On the 4th day all were inoculated in the groin with 0.1 cc of a suspension of a virulent strain of human type tubercle bacilli (5-6 organisms per oil immersion field). Intracutaneous tests were made with 20 mg O.T. at 20 and 37 days and with a suspension of living bacilli 40 days after infection. All treated animals gave negative reactions while the controls gave positive reactions to the tuberculin and characteristic Koch phenomena to the bacilli. At autopsy no differences were found grossly or microscopically in 5 treated animals and their controls dying in the 4th week. Little caseation was found in either group. However, in the 3 remaining treated animals living to the 42nd day, there appeared lesions of an extraordinary nature. In the lungs, many alveoli were packed with polymorphonuclear and mononuclear leucocytes together with enormous numbers of acid-fast bacilli. The

[1] Rothschild, H., Friedenwald, J. S., and Bernstein, C., *Trans. Nat. Tuberc. Assn.*, 1931, p. 149; *Bull. Johns Hopkins Hosp.*, 1934, **54**, 232; Boquet, A., *Compt. Rend. Soc. Biol.*, 1932, **112**, 1168; Cummings, D. E., and Delahant, A. B., *Trans. Nat. Tuberc. Assn.*, 1934, p. 123; Derick, C. L., Branch, E. A. G., and Crane, M. P., *Am. Rev. Tuberc.*, 1934, **32**, 218; Siegl, J., *Beit. z. klin. d. Tuberk.*, 1934, **84**, 311; Birkhaug, K., *Acta Tuberc. Scand.*, 1937, **11**, 25; Higginbotham, M. W., *Am. J. Hyg.*, 1937, **26**, 197.

[2] Willis, H. S., and Woodruff, C. E., *J. Clin. Invest.*, 1937, **16**, 899; *Am. J. Path.*, 1938, **14**, 337.

liver and spleen of these animals revealed relatively few bacilli in comparison. No such lesions were found in the controls. These changes are similar to those described by Willis and Woodruff.[2]

Series II consisted of 61 animals living 36 to 80 days. Eighteen were treated with daily subcutaneous injections of O.T. from the day of infection on, half receiving 1 cc and the rest 2 cc each day. Twenty-two were treated with injections of 10% glycerin in saline (since O.T. contains glycerin), 10 receiving 1 cc and twelve 2 cc each day. Twenty-one received daily injections of 0.9% saline, 11 receiving 1 cc and ten 2 cc. The infecting dose in this group was a suspension containing about 20 organisms per oil immersion field. All were tested intracutaneously with O.T. (10 mg in controls and 100 mg in O.T. treated), and with heat-killed bacilli, at varying intervals, while 2 from each group received live bacilli intracutaneously 56 days after infection. The O.T.-treated animals gave consistently negative reactions both to tuberculin and to bacilli while the 2 control groups reacted strongly to tuberculin and gave necrotic Koch phenomena with the bacilli.

Microscopically there was a distinct tendency for lesions in the O.T.-treated animals to be less caseous than in control groups. However, in both the O.T. and glycerin treated groups, the extent of the lesions in lungs, livers, and spleens was definitely greater than in the saline controls. Areas of pneumonia containing great numbers of acid-fast bacilli, such as were seen in Series I, were observed in 7 of 18 O.T.-treated animals (living 37, 37, 43, 49, 55, 67, and 68 days). However, 6 of 22 glycerin (living 37, 49, 54, 60, 65, and 73 days) and 3 of 21 saline controls (living 39, 52, and 62 days) also showed the same change. Willis and Woodruff[2] found this lesion in only tuberculin treated animals but their controls received nothing, in contrast to the daily injections of glycerin and saline in our animals. Since in the present experiment hypersensitive controls showed the lesion with marked proliferation of bacilli, and since many of the non-hypersensitive O.T.-treated animals failed to show this lesion, it seems that the lack of hypersensitivity does not play a rôle in producing it.

It seems that some change occurs in certain of the animals receiving daily injections which either inhibits the development of their ability to prevent the multiplication and to destroy the bacilli (resistance, immunity) or else interferes with this mechanism when it has developed. We have considered that the administration of glycerin might be a factor, for Long and Vorwald[3] found that tubercle

[3] Long, E. R., and Vorwald, A. J., *Am. Rev. Tubere.*, 1930, **22**, 636.

bacilli grew more profusely in rats receiving glycerin, than in untreated ones. Since 3 of the saline treated pigs showed the enormous numbers of bacilli, glycerin cannot be the only factor. The extraordinary proliferation of bacilli in the lungs in contrast to that in the spleen and liver, and also the fact that the bacilli growing in the lung produce much less necrosis than those introduced intracutaneously, are perplexing phenomena. Further studies of these points and of the antibody titer in the various groups will be reported later.

Summary. When guinea pigs were infected with virulent human tubercle bacilli and prevented from becoming hypersensitive by daily subcutaneous injections of O.T., some developed pulmonary lesions teeming with bacilli. However, some of the control animals, demonstrated to be hypersensitive, showed identical lesions. It is, therefore, concluded that the development of lesions of this type is dependent on factors other than a lack of hypersensitivity.

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Utilization of β-hydroxybutyric Acid by Fed and Fasted Rats.*

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Whenever a substance or some experimental procedure produces a decrease in ketosis, it is of some importance to determine whether this effect is due to a decrease of ketone body production by the liver *(antiketogenesis)*, or to an increase in ketone body utilization by the muscles *(ketolysis)*. Particularly is this significant in considering the influence of glucose administration.[1]

In a series of recent studies, we could obtain no evidence that glucose can accelerate the rate of ketone utilization by the muscles of normal rabbits[2] or by the perfused dog heart.[3] However, the in-

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1 Mirsky, I. A., Heiman, J. D., and Broh-Kahn, R. H., *Am. J. Physiol.*, 1937, **118**, 290.

2 Mirsky, I. A., and Broh-Kahn, R. H., *Am. J. Physiol.*, 1937, **119**, 734.

3 Waters, E. T., Fletcher, J. P., and Mirsky, I. A., *Am. J. Physiol.*, 1938, **122**, 542.