

ods of analysis will be found in a previous publication.¹ Urinary nitrogen excretion increased progressively from 13% of the intake during the control period to 40% 10 weeks after operation. In the paired feeding controls the urinary N excretion increased from 16% of the intake to 25% only, during the same time. The increase in N excretion was accounted for by urea N. Fecal N excretion, expressed in percentage of intake, remained unchanged. The drop in N retention was more pronounced in the thymectomized rats than in the paired feeding controls, though a positive N balance was maintained at all times and the gain in weight was normal in both groups. No difference was found between thymectomized and control animals regarding the excretion of NH₃ nitrogen, uric acid, creatinine and creatine. There was no change in the calcium, magnesium and phosphorus metabolism.

Our studies are being extended to include the metabolism of sulfur and glutathione in thymectomized rats.

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Immunizing Effect of Old Tuberculin on Experimental Tuberculous Infection.

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Several years ago Rothschild, Friedenwald, and Bernstein¹ showed that hypersensitivity could be separated from immunity in experimental tuberculosis in the guinea pig. After rendering the animals both hypersensitive and immune by infection with an attenuated strain of tubercle bacilli, they desensitized them with large doses of Old Tuberculin. Upon reinfection with virulent organisms, the animals' immunity was found to be unimpaired. These observations have been confirmed by other investigators.²

¹ Sandberg, M., and Perla, D., *J. Exp. Med.*, 1934, **60**, 395.

¹ Rothschild, H., Friedenwald, J. S., and Bernstein, C., *Trans. Nat. Tuberc. Assn.*, 1931, p. 149; *Bull. Johns Hopkins Hosp.*, 1934, **54**, 232.

² 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.

In the experiments of Rothschild, *et al.*,¹ extremely large amounts of Old Tuberculin were administered, a total of 142 cc per animal over a period of 126 days. This to our knowledge is more than had ever been given before. Recently Topley and Wilson³ have raised an objection to "the assumption that the only effect produced was the desensitization of the animals. The possible immunizing effect of the tuberculin itself is altogether ignored."

To determine whether the administration of large amounts of Koch's Old Tuberculin might have some immunizing effect when given both before and during the course of experimental tuberculous infection, the present study was undertaken.

Tuberculin was made by growing virulent organisms for 8 weeks on Proskauer and Beck's⁴ synthetic medium. The bacilli were removed by filtration through paper and the filtrate passed through a Berkefeld N candle. After evaporation to one-tenth its original volume, the tuberculin was stored in the ice-chest. Synthetic medium of the same composition was filtered and concentrated in exactly the same manner to make the control concentrated medium. The tubercle bacilli used both for infection and in making the tuberculin were from a virulent strain which was isolated several years ago from the sputum of a human case of active pulmonary tuberculosis. It has been grown continuously on Proskauer and Beck's medium at a pH of 7.4 with no appreciable change in its virulence. A 3-weeks-old culture was used for inoculation.

Thirty adult male guinea pigs, weighing about 500 g, were divided into 3 groups. Ten received 2 cc undiluted Old Tuberculin subcutaneously every other day for 6 weeks (42 cc). Ten more received the same amount of concentrated medium (42 cc) every other day, while the remaining 10 received nothing. At the end of the 6-weeks interval all the animals were tested intracutaneously with 50 mg Old Tuberculin and all gave no reaction. All were then infected in the left groin with a suspension of virulent human tubercle bacilli containing 5-6 organisms per oil immersion field. Treatment with both Old Tuberculin and concentrated medium was continued with daily injections of 2 cc of each for 44 more days when all were killed with ether.

Two days before death all animals were tested intracutaneously with 0.1 cc of a suspension of whole live virulent tubercle bacilli. Those treated with tuberculin gave negative reactions while the 2

³ Topley, W. W. C., and Wilson, G. S., "The Principles of Bacteriology and Immunity," 2nd Ed., 1936.

⁴ Baldwin, E. R., Petroff, S. A., and Gardiner, L. S., "Tuberculosis. Bacteriology, Pathology, and Laboratory Diagnosis," 1927.

control groups gave markedly positive Koch phenomena with necrosis. These skin tests were made on the left side, the entire right side being reserved for the daily injections of tuberculin or concentrated medium. At autopsy the tissues were fixed in Zenker-formol solution. Sections of lungs, lymph nodes, livers and spleens for microscopical study were stained with hematoxylin and eosin. Sections of lung and spleen were stained with carbol fuchsin.

Of the original 30 animals, 18 survived the treatment 23 or more days after infection. One from each group came to autopsy on the 23rd day while all the remaining 15 animals (5 from each group) were killed on the 44th day after infection.

There was very little difference in the extent of the lesions in the 3 groups. As is usually the case in experimental tuberculosis in guinea pigs, the spleens were most extensively involved, then the livers, and lastly the lungs. The difference which was found was one of character. When the lungs and spleens were carefully examined there was found to be a distinct tendency for the tuberculous foci in the Old Tuberculin treated animals to show less caseation than the controls although the former group was not entirely free from caseous lesions.

As far as the number of bacilli in the lungs and spleens of the 3 groups was concerned, all contained comparable numbers with one exception. This was No. 71 of the Old Tuberculin treated group. In the pulmonary alveoli of this animal there were enormous numbers of acid-fast bacilli apparently growing unchecked. There were also numerous polymorphonuclear as well as mononuclear leucocytes but the striking thing was the great proliferation of acid-fast organisms. This type of lesion is exactly similar to that which has recently been described by Willis and Woodruff⁵ and which we have seen in other experiments.⁶ The former investigators have suggested that this lesion develops as a result of lack of hypersensitivity. We have taken up this question in another place⁶ where it is pointed out that this lesion may also develop in hypersensitive animals.

In experimental tuberculosis practically all investigators agree that a definite immunity is conferred by vaccination with living attenuated bacilli or heat-killed virulent organisms. To our knowledge no one has succeeded in producing any demonstrable immunity with fractions of the tubercle bacillus, or with the products of its break-down in cultures, *e. g.*, Old Tuberculin. However, because

⁵ Willis, H. S., and Woodruff, E. C., *J. Clin. Invest.*, 1937, **16**, 899; *Am. J. Path.*, 1938, **14**, 337.

⁶ Follis, R. H., Jr., in press.

only recently has it been realized that animals will tolerate large daily doses of Old Tuberculin, it seemed possible that some degree of immunity might be conferred by treatment with large amounts of this material previous to and during infection.

In 1910 Krause⁷ had given guinea pigs as much as 25 cc of water extract tuberculin before infection, with no apparent beneficial effects. A little later Klopstock⁸ administered 24 cc of Old Tuberculin over a period of 149 days before infection, and found no significant decrease in tuberculosis between treated guinea pigs and their controls. In the present experiment a total of 130 cc of undiluted Old Tuberculin were administered, 42 cc before infection and 88 cc over the 6-weeks period following inoculation with virulent bacilli.

From the results of our experiment, therefore, it seems quite unlikely that Old Tuberculin contains any substance which plays a rôle in augmenting the guinea pig's power to prevent the spread and hinder the growth of the tubercle bacillus or to destroy this organism. And it is thus apparent that in the experiments of Rothschild, *et al.*,¹ as well as those of other observers, the administration of large doses of Old Tuberculin for the purpose of desensitization, did not play a rôle in bringing about the resistance to infection which animals deprived of their hypersensitivity exhibit. Therefore, we do not feel that the objection raised by Topley and Wilson² is a valid one. The only effect of continued large doses of Old Tuberculin was on the character of the lesions; those in the treated animals showed less caseation than those in the controls. In the light of the general knowledge concerning the action of hypersensitivity in rendering tissues susceptible to injury by bacterial products, the fact that desensitization decreased the amount of necrosis in these experiments and those of others, is not surprising.

Summary. Old Tuberculin when administered subcutaneously in large amount both before and during experimental tuberculous infection in guinea pigs, confers no demonstrable immunity. The view that the immunity that persists after desensitization by tuberculin is a result of the treatment is, therefore, not a valid one.

⁷ Krause, A. K., *Studies from the Saranac Laboratory for the Study of Tuberculosis*, 1911, p. 1.

⁸ Klopstock, F., *Z. f. exp. Path. u. Ther.*, 1913, 13, 56.