4551

Improved Method of Preparing Bacillus Typhosus Toxic Substances Necessary for Local Skin Reactivity.

GREGORY SHWARTZMAN.

From the Laboratories of the Mount Sinai Hospital, New York City.

In previous communications[1, 2, 3, 4] a phenomenon of local skin reactivity to *Bacillus typhosus* culture filtrates was described. The reactivity was induced by the injection of the filtrates into the skin of a rabbit. If 24 hours later an intravenous injection of the same filtrate was given to the rabbit, there appeared an extremely severe hemorrhagic necrosis at the site of the previous injection. The factors determining the local skin reactivity were termed "skin preparatory factors" and those involved in the production of local hemorrhagic necrosis following the intravenous injection were called "reacting factors." There were found a number of features which considered together distinguished this phenomenon sharply from the known phenomena of bacterial hyper-susceptibility and from the Arthus phenomenon. The same phenomenon was later reproduced with a variety of pathogenic microorganisms.[5] The skin preparatory factors of *Bacillus typhosus* were inactivated only by autoclaving, were not affected by a pH range of 4.0 to 9.0 and were specifically neutralized by immune sera. The *Bacillus typhosus* skin preparatory and reacting factors were obtained in filtrates of 6 day old cultures in tryptic digest broth.[2] These filtrates undoubtedly contain a considerable amount of extraneous material (products of tryptic digest and bacterial cell autolysis).

For the purpose of other work, it was desirable to obtain filtrates containing a minimal amount of these extraneous substances. After trying out a number of procedures, this object was attained by the use of washings of bacterial growth on solid media. The following is the method employed:

Kolle flasks containing plain veal infusion agar of pH 7.4 were seeded each with 3-4 cc. of 20 hour old plain broth culture of *Bacillus typhosus* (strain T_L) diluted 1-4 with 0.9% NaCl solution. The dilution was made immediately before use. After 20-22 hours of incubation the growth of each flask was washed off with 14-16 cc.

[1] Shwartzman, PROC. SOC. EXP. BIOL. AND MED., 1928, xxv, 560.

[2] Shwartzman, *J. Exp. Med.*, 1928, xlviii, 247.

[3] Shwartzman, PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 131.

[4] Shwartzman, *J. Exp. Med.*, 1929, xlix, 593.

[5] Shwartzman, PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 207.

of 0.9% NaCl solution containing 0.4% phenol. The washings were then pooled, centrifuged within the following 1-2 hours and the clear supernatant fluid filtered through Berkefeld "V" candles shortly after centrifuging. The filtrates prepared in this manner will be referred to as T_L "agar washings" filtrates.

The following is a summary of results obtained with these toxic substances:

Forty rabbits each received 6 simultaneous injections in dilutions of 1:2, 1:8, 1:16, 1:24, 1:36, and 1:48. The readings of the primary effect of these injections were made 23-24 hours later. The dilution 1:2 produced well-marked erythema (4+) in 1 rabbits, moderate erythema (2+) in 3 rabbits, slight reddening (1+) in 7 rabbits, and doubtful reddening or no erythema in the remaining 29 rabbits. With the dilution 1:8, only 1 rabbit showed a moderate erythema (2+), and the remaining rabbits very slight erythema or no reactions whatsoever. None of the higher dilutions had any visible primary effect upon the skin. Twenty-four hours after the intradermal injections, the rabbits were divided into 5 groups of six, and 2 groups of 5. Each group received a single intravenous injection of the filtrate. The first group received 1.8 cc., the second group 1.4 cc., the third group 1.0 cc., the fourth group 0.5 cc., and the sixth group received 0.3 cc., respectively, per kilo of body weight. There was an irregular general toxic effect of the filtrate. From 1 to 4 rabbits of each group were killed in 1-5 hours by the intravenous injection. The skin reactions of the surviving 26 rabbits were read 5 hours after the intravenous injection. Three rabbits showed no reactions (resistant). One of these had received intravenously 0.8 cc. and two 0.3 cc. of the filtrate per kilo of body weight. The remaining 23 rabbits showed very severe and typical reactions. Although, as observed before,[2] fluctuations were noticed in the skin preparatory effect of diluted filtrate, nevertheless there seemed to be a distinct reciprocal relationship between the amounts of filtrate necessary to be injected intradermally and intravenously in order to elicit the phenomenon. Thus, even the highest dilution (1:48) was able to prepare the skin of some rabbits for severe hemorrhagic necrosis providing from 0.8-1.8 cc. per kilo was injected intravenously. As the intravenous doses decreased, well-pronounced reactions were elicited only in areas prepared with dilutions lower than 1:48, so that in the last group which received intravenously 0.3 cc. per kilo, only areas prepared with dilution 1:2, 1:8 and 1:16 showed severe reactions.

Essentially similar results were obtained with 2 other batches of identically prepared T_L "agar washings" filtrates.

Summary and Conclusions: It is possible to obtain highly potent skin preparatory and reacting factors in filtrates of washings of 20-22 hour old *Bacillus typhosus* cultures on plain agar. Under these conditions there is avoided much of the extraneous material present in tryptic digest broth previously employed, as well as much of the bacterial autolysis occurring in the older method. This would indicate that the skin preparatory and reacting factors are bacterial soluble substances, the production of which is independent of massive cell destruction.

The filtrates prepared in this manner produce negligible primary effect upon the skin (erythema, swelling), notwithstanding their high skin preparatory potency. This is in agreement with the view previously expressed[1, 2, 5] that the skin preparatory potency of a given filtrate bears no relation to its primary effect on the skin.

It is of considerable interest to note that there exists a distinct reciprocal quantitative relationship between the skin and intravenous doses. This observation merits further study.

4552

Nicotine Tolerance in the White Rat.

C. H. THIENES.

From the Department of Pharmacology, University of Oregon Medical School, Portland, Oregon.

The minimal effective dose (M.E.D.) of nicotine for white rats of both sexes, from 29 to 33 days old, was 0.3 mg. per kilo body weight. This dose was sufficient to cause slight but definite weakness of the hind legs. After determining the M.E.D., one-half of each of 3 litters were injected twice daily with 2 to 3 times the M.E.D., producing symptoms ranging from instability to convulsions and prostration. At intervals of 3 and 9 weeks following the first injections, after a rest day of no injections, all rats were tested for the M.E.D. Whereas the controls, 10 in number (injected twice daily with salt solution), responded each time to 0.3-0.4 mg. nicotine per kilo body weight, the test rats, 13 in number, showed a definite decrease in susceptibility, in that at the end of 3 weeks, the M.E.D. averaged 0.65 mg. nicotine, and at the end of 9 weeks, 0.85 mg. per kilo body weight. This was considered to be evidence of an acquired nicotine tolerance.