

estimate the deflection on the scale closer than 0.1 mm, this constitutes the limit of resolution which corresponds to about 0.1 cc. The sensitivity could, of course, be easily increased by choosing a longer pointer or by reflecting a beam of light from a mirror mounted at the balance pivot.

We have made comparative measurements of blood flow through the hand using this method and the standard plethysmograph. The results were found to check well.

The author wishes to thank Dr. Zazeela and Mr. Marrus for their active coöperation in the physiological tests of the method.

10809

On the Toxicity of "Q" Substance from *Eberthella typhosa*.

E. W. DENNIS.

From the Department of Bacteriology, School of Medicine, American University of Beirut, Beirut, Lebanon.

Bruce White^{1, 2} described an acid-alcohol-soluble "protein", extracted from *Eberthella typhosa* and other enteric bacilli, the presence of which was necessary for agglutination of specifically sensitized R bacilli. This substance, which he called "Q" substance, was antigenic but not species-specific. Anti-"Q" sera agglutinated p-forms of *Salmonella* bacilli; R-forms were agglutinated more slowly and to a lesser titer. S-forms contained "Q" but were inagglutinable by "Q" antisera. "Q" antisera gave heavy precipitates with solutions of "Q" substance. "Q" substance also gave a precipitate with normal serum but the specificity of the reaction with immune serum could be demonstrated by suitable dilution of the antigen. The "Q" substance could be differentiated from the P substances extracted by Furth and Landsteiner³ by means of hot 75% alcohol. White reported "Q" substance as being non-toxic for rabbits, but the dosage was not stated.

E. typhosa, strain Ty 2, was grown on sodium-chloride infusion-agar, pH 7.4-7.6, for 24 hours at 37°C. The growth was washed from the agar with 3-5 cc of saline per Roux bottle. The bacilli were killed by the addition of one volume of pure acetone at 37°C for one hour. The killed flocculated bacilli were collected by centri-

1 White, P. Bruce, *J. Path. and Bact.*, 1932, **35**, 77.

2 White, P. Bruce, *J. Path. and Bact.*, 1934, **39**, 529.

3 Furth, J., and Landsteiner, K., *J. Exp. Med.*, 1928, **47**, 171.

fugation and extracted 3 times (18-24 hours) with 4 volumes of acetone at room temperature. The defatted bacilli were collected and dried *in vacuo* over calcium chloride; 28.5 g of bacilli (from 170 Roux bottles) were extracted for one hour at 45-46°C in 2 liters of .2N HCl alcohol (100 cc .2N HCl 1900 cc absolute alcohol). Extraction was carried out in a flask fitted with a reflux condenser. After extraction the bacteria were filtered off on a double layer of hard filter paper and washed with warm HCl alcohol. The washings were added to the extract, which was then chilled and made slightly alkaline (pH 7.8) with NaOH. An orange-colored precipitate appeared which was collected after ½ hour at 5°C. The precipitate was washed with cold absolute alcohol and with ether, and dried (weight, 1.462 g). Nine hundred and seven mg of crude "Q" was extracted with 40 cc of distilled water at pH 8, and centrifuged. The insoluble material was discarded. The clear, brown extract was chilled and .1N HCl carefully added to pH 5.5 when a heavy flocculent precipitate appeared. The precipitate was collected by centrifugation, dissolved in 10 cc dilute alkali and reprecipitated at pH 5.5. The recovery was complete. The final precipitate was washed in the neutral absolute alcohol and ether and dried. The final product weighed 147 mg. Qualitative tests on the crude "Q" substance showed the presence of a large amount of pentose, but the final product gave a negative Molisch test for carbohydrate. The Biuret test was strongly positive and Millon's test was positive after standing at room temperature. The substance was readily soluble in dilute acid, dilute alkali, and warm acid alcohol, but dissolved with difficulty in neutral water. This fraction, labeled Q₁W, showed on analysis: total N, 12.2%; total P, faint trace; reducing sugar after acid hydrolysis, faint trace; Sakaguchi's test for arginine grouping, positive.

Toxicity. "Q" substance dissolved in saline at pH 7.8-8 was injected intravenously into rabbits and intraabdominally into mice. When injected into rabbits, 5 mg per kilo induced lethargy, rise in temperature, rapid loss in weight and diarrhea; 10 mg per kilo killed a 2 kg rabbit in 36 hours; 2 doses of 10 mg each, 24 hours apart, killed a 1.8 kg rabbit in 48 hours. Five milligrams of "Q" substance was lethal for 22 g mice. At necropsy the rabbits did not show any gross pathological changes which would account for death. The generalized hyperemia and hemorrhage which characterizes the action of the polysaccharide "endotoxin" was absent; microscopically the only findings were small foci of necrosis, without polynuclear infiltration, in the liver, in the Malpighian corpuscles in the spleen, in the lymph nodes and in the Peyer's patches.

Skin-toxicity. An attempt was made to produce the Shwartzman phenomenon,⁴ using "Q" substance as a preparing agent. "Q" substance, in doses up to 1 mg, injected intracutaneously, produced within 18 hours, sharply circumscribed ulcers of the skin at the sites of injection (Fig. 1). There was little or no reddening prior to, and no hemorrhage subsequent to intravenous injection of 7.5 mg of endotoxin F/17A⁵ which produced severe hemorrhagic necrosis at the site of injection of .001 mg of the same fraction. It was concluded that "Q" substance in saline solution is not capable of preparing the skin for the Shwartzman reaction.

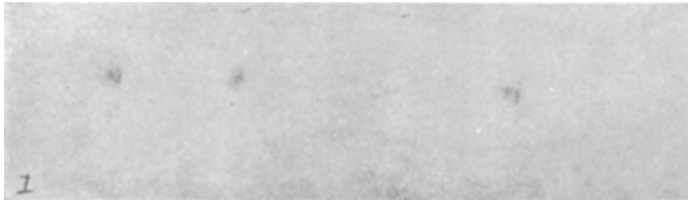


FIG. 1.

Skin-necrotizing action of typhoid "Q" substance in dose of 0.25, 0.1, 0.5, and 0.025 mg (read from left to right).

An experiment was then carried out to determine if the necrotizing action of "Q" substance could be neutralized by typhoid antiserum. The serum used was Felix's concentrated antityphoid immune-globulin solution, prepared at the Lister Institute. Aseptic precautions were taken throughout. The serum was diluted 1:25, 1:50; 1:100; 1:150; and 1:200. One cc of each dilution was added to each of a series of tubes containing 1 cc of 1:500 solution of "Q" substance, thoroughly mixed and the serum-antigen mixture incubated for 1 hour at 37°C. Precipitation, proportional to the concentration of serum, occurred in all tubes as well as in tubes containing undiluted horse- and rabbit-sera plus "Q" substance. The tube containing the highest concentration of immune globulin was centrifuged and the supernate and washed precipitate injected separately (Fig. 2a and e). The serum-"Q" substance mixtures were injected in .25 cc amounts into the left side of a rabbit. The right side of the rabbit was used for a parallel neutralization-test using Felix's serum against .01 mg doses of Shwartzman active polysaccharide F/20AE. Twenty-four hours after the endermal injections the sites of injection of the serum-"Q" mixtures were markedly inflamed but there were no ulcers. An eliciting dose of 7.5 mg of F/17A was injected intravenously.

⁴ Shwartzman, G., *J. Exp. Med.*, 1928, **48**, 247.

⁵ Dennis, E. W., to be published.

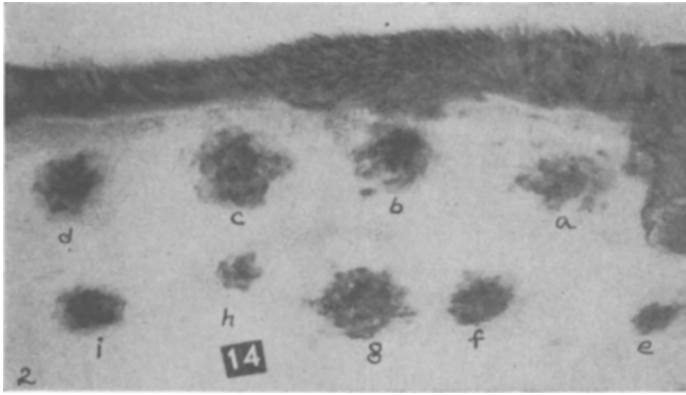


FIG. 2.

Shwartzman activity of serum-"Q" substance mixtures. Constant dose of 0.25 mg of "Q." Serum-dilutions: (a) 1:50; (b) 1:100; (c) 1:200; (d) 1:300; (f) 1:400; (e) Washed precipitate from (a); (g) Normal horse serum plus "Q"; (h) Normal rabbit serum plus "Q"; (i) Shwartzman active F/17A (.001 mg) plus normal rabbit serum, control.

The results are shown in Figs. 1 and 2. There was an intense Shwartzman reaction at all sites of injection, the intensity being proportional to the concentration of serum. There was no difference between the activity of normal horse serum-"Q" mixture and immune globulin-"Q" mixture. Removal of the precipitate reduced the intensity of the reaction (Fig. 2a); the washed precipitate was highly active (Fig. 2e).

Shwartzman⁶ has reported the skin-preparatory activity of the specific serum-precipitates. However, the components and the serum-antigen mixtures were inactive; hence, the two phenomena are not analogous. Typhoid "Q" substance also precipitates dilute egg-albumen and normal human serum; skin-tests with the egg-albumen have been inconclusive but the precipitate with human serum is toxic. "Q" substance, in its property of precipitating complex proteins from solution, resembles the histones, but in its high solubility in alcohol it is different.

A specific "Q" antiserum, prepared by intravenous injection of 45 mg of "Q" substance into a rabbit over a period of 8 weeks, agglutinated both "H" and "O" suspensions of Ty 2 to a titer of 1:200 and gave a precipitate with 1:50,000 dilution of "Q" but did not prevent the necrotizing action of "Q".

Conclusions. "Q" substance prepared from typhoid bacilli is lethal to rabbits and mice when given in adequate doses. We attribute its toxicity to its necrotizing action, which appears to be due to the action of "Q" in forming a toxic compound with serum-protein.

⁶ Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 193.