

interface. The  $\text{Na}_3\text{PO}_4 \cdot 12 \text{H}_2\text{O}$  crystals are trigonal and the  $\text{K}_3\text{PO}_4 \cdot 2 \text{H}_2\text{O}$  are needle like. No attempt was made to prepare constant hydrates but the crystals were dried to remove free moisture and analyzed for water of hydration.

The value of tertiary dissociation constant of phosphoric acid determined from the hydrolysis of tertiary sodium and potassium phosphates respectively at  $20^\circ \text{C}$ . has been found to be  $1.02 \times 10^{-12}$ , and at  $38^\circ \text{C}$ .  $1.48 \times 10^{-12}$ .

The tertiary dissociation constant calculated from the data obtained by the electrometric titration of phosphoric acid and from formulas developed by Van Slyke, have been found to be  $0.97 \times 10^{-12}$ , apparently at laboratory temperature.

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### Effect of Immune Sera Upon the Phenomenon of Local Skin Reactivity to *B. Typhosus* Culture Filtrates.

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In a previous communication<sup>1</sup> a phenomenon of local skin reactivity to *B. typhosus* culture filtrates was described. The reactivity was induced by skin injections of the filtrate. 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 skin injections. The mechanism of the phenomenon is not fully understood as yet, since no complete experimental comparison was made between this phenomenon and manifestations of bacterial allergy. There were found, however, certain features which, *considered together*, distinguish this phenomenon from the known phenomena of bacterial hypersusceptibility and the Arthur phenomenon. These features are: the local reactivity; the short incubation period necessary for local preparation of the skin; the short duration of the state of reactivity; the ability to induce the reactivity by a single injection and the necessity to make the second injection of the toxic agent by the intravenous route.

This report concerns the relation of the specific antisera to the skin preparatory factors of the observed phenomenon. There was a double purpose in these studies: first, to add new data leading

<sup>1</sup> Shwartzman, G., *J. Exp. Med.*, 1928, xlviii, 247.

to the elucidation of the mechanism of the phenomenon, and secondly, should there occur neutralization of *B. typhosus* skin preparatory factors by specific sera, to determine whether the phenomenon could be advantageously used for titration of the neutralizing properties of the sera. In order to fulfill this plan the effect of homologous normal and heterologous sera upon the skin preparatory factors of the phenomenon of local skin reactivity to *B. typhosus* culture filtrates was studied.

Most of the sera were prepared by immunizing rabbits and goats with *B. typhosus* culture filtrates. The mixtures of filtrates with specific sera in various proportions were injected into the skin of the abdomen of the rabbits. Numerous experiments were performed with the following results:

In the majority of rabbits these mixtures failed to elicit any local skin reactivity. However, there were some rabbits in which the admixtures of homologous serum did not prevent the phenomenon of local skin reactivity. The neutralized reactions by far outnumbered the non-neutralized ones. On further analysis the reactions could be divided into 3 groups, namely: completely neutralized reactions (C.N.), partially neutralized reactions, (P.N.) and non-neutralized reactions (N.N.) Titrations of various batches of sera established the fact that the potency of a given serum bears a mathematical ratio to all the types of reactions. Thus one has to take into consideration all the data obtained, namely, the titer of serum producing complete neutralization in C.N. and P.N. rabbits as well as the number of rabbits belonging to each type of reaction. For each serum the total of rabbits was not less than 10.\*

The studies on the normal sera brought out the fact that in majority of instances they failed to neutralize the *B. typhosus* skin preparatory factors of the observed phenomenon. These normal animals were able to respond with production of neutralizing substances if treated with the filtrates.

However, there were encountered some normal sera which gave certain evidence of neutralization of these factors in low dilutions. Such sera were found to contain agglutinins or both agglutinins and precipitins for *B. typhosus* in fairly high titer. There were no normal sera obtained as yet which neutralized the reactions and at the same time contained no agglutinins for *B. typhosus*. There were, however, sera which contained agglutinins or both agglutinins and precipitins which failed to give neutralization reactions.

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\* The technique of such determinations with protocols of the results will be described elsewhere.

Several heterologous sera were also investigated. Non related sera were not able to neutralize the factors (Shiga, Scarlet fever, Erysipelas sera). Sera closely allied to *B. typhosus* sera (Para A and B) neutralized these factors in various proportions. The reactions also divided themselves into the same 3 types as described above (C.N., P.N. and N.N.). It is not known as yet whether the neutralization is a group reaction or the skin preparatory factors are identical with those of *B. typhosus*. It will be necessary to titrate a large number of various batches of sera in order to bring out the possible quantitative differences in the titers.

It appears from these studies that a new method is available for quantitative titration of substances in the serum which neutralize the skin preparatory factors of the phenomenon of local skin reactivity to *B. typhosus* culture filtrates. Emphasis should be laid on the fact that it is possible to control the individual susceptibility of rabbits to this phenomenon. This fact permits of considerable accuracy in quantitative titration of the sera. Attempts are under way to determine whether the proposed method can be applied to production of therapeutic sera.

Work is also in progress to determine the effect of specific sera upon the skin reacting factors introduced by the intravenous route.

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### Stability of Luminous Substances of Luminous Animals.

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It is about ten years (1918) since I first collected the luminous ostracod crustacean, *Cypridina*, extracted the luminous materials, luciferin and luciferase, and set some of the extracts aside for later testing as to stability. The animals are collected and dried quickly over  $\text{CaCl}_2$ . When powdered and moistened a bright bluish luminescence appears which is as intense with the 1918 material tested in 1928 as it was in 1918. This powder contains both luciferin and luciferase and light results from mixing the 2 substances in water containing oxygen. Luciferin solution is obtained by making a hot water extract of *Cypridina*. Heating destroys the luciferase, which can be extracted from *Cypridina* with cold water. The luciferin, which dissolves at the same time, is allowed to oxidize in the air, leaving luciferase alone in solution.