

7 days after the feeding, typical minute vesicles appeared on the normal fowl wherever the proboscis of the insect had been inserted.

In one experiment transmission was obtained under conditions approximating those existing in nature. Two healthy and one infected chickens were placed in a box covered with fine-mesh copper screen. Four freshly hatched *Culex* mosquitoes were released in this box. After 7 days 2 typical discrete lesions appeared on the left wattle of one of the healthy fowls. In a control group without mosquitoes none of the chickens showed evidences of infection after a period of 17 days.

It seems, therefore, that where the lesions are superficial, as in fowl-pox, interrupted feedings by mosquitoes and possibly also by blood-sucking flies may play an important, if not the most important rôle in the dissemination of the disease. Our experiments thus far indicate that transmission of this kind is a mechanical transference of the virus; consequently it is not essential that the infectious agent be able to establish itself in the insect. In diseases where the virus is present in the circulation in high concentration, as in fowl plague, mechanical transference of the virus by interrupted feedings of insects may also be an important factor in the dissemination of such infections.

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Determination of Tertiary Dissociation Constant of Phosphoric Acid.

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Quantitative studies of the mechanism of calcification require a knowledge of the physico-chemical constants of the blood equilibria involved in the process. The tertiary dissociation constant of phosphoric acid, K_3 , thus far available seems incompatible with the apparent dissociation constant pK_3 from the dissociation curve for the phosphates which is of an order of 1×10^{-12} .

The value of K_3 was determined experimentally from a study of aqueous systems of the tertiary sodium and potassium phosphates at equilibrium, from the standpoint of hydrolysis. The value thus obtained was correlated with calculations made from the data obtained by the electrometric titration of phosphoric acid.

The tertiary phosphates hydrolyze to a great extent, the secondary and primary salts inappreciably. In the mechanism of the first stage hydrolysis of the tertiary salts, the essential equilibrium, may be expressed ionically:



Applying the law of mass action,

$$\frac{[\text{HPO}_4^{2-}] [\text{OH}^-]}{[\text{PO}_4^{3-}]} = K_h \dots \dots \dots (1)$$

Where the brackets include the ion activities, and K_h is the activity hydrolysis constant. The degree of hydrolysis, h , is by definition,

$$h = \frac{[\text{HPO}_4^{2-}]}{\gamma_B C} = \frac{[\text{OH}^-]}{\gamma_B C} \dots \dots \dots (2)$$

and $1-h = \frac{[\text{PO}_4^{3-}]}{\gamma_T C}$

Where γ_B , γ_S , γ_T are the activity coefficients of hydroxide, secondary and tertiary phosphates of sodium or potassium respectively. Substituting the values of $[\text{OH}^-]$, $[\text{HPO}_4^{2-}]$ and $[\text{PO}_4^{3-}]$ from these relations in Equation 1, we have

$$\frac{h^2 \cdot \gamma_B \cdot \gamma_S \cdot C}{(1-h) \gamma_T} = K_h$$

Also, the condition maintains that $[\text{H}^+] \cdot [\text{OH}^-] = K_w$. Dividing this by Equation 1,

$$\frac{[\text{H}^+] [\text{PO}_4^{3-}]}{[\text{HPO}_4^{2-}]} = \frac{K_w}{K_h} = K_a$$

where K_a is the tertiary ion activity or true constant.

Purely prepared tertiary phosphates were made up to definite concentrations with conductivity water and kept in pyrex flasks. The hydron concentrations of these solutions were measured potentiometrically in a thermostat maintained at 20° and 38° C. ± 0.02 respectively. The gas chain consisted of Pt—H₂—solution χ saturated KCl—HgCl—Hg. The hydron concentration measurements were standardized daily against a 0.1 N HCl prepared from constant boiling acid. The pH is 1.085 at 20° and 1.09 at 38°, extrapolated from the activity coefficients determined by Noyes and Ellis. The calculated EMF values of the entire system against the acid standard are 0.3119 at 20° and 0.3022 volts at 38°. The average divergence was 0.003 pH. The observed voltages were corrected to one atmosphere of hydrogen.

The tertiary phosphates were recrystallized twice from distilled water free from CO₂ and again by adding an equal volume of 95% alcohol and cooling in ice water. Crystallization proceeds at the

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°C . has been found to be 1.02×10^{-12} , and at 38°C . 1.48×10^{-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 0.97×10^{-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 communication¹ 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

¹ Shwartzman, G., *J. Exp. Med.*, 1928, xlviii, 247.