14521

Failure to Demonstrate Alkaline Phosphatase Activity in Inclusion Bodies by the Histochemical Technic.*

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Phosphatase activity of purified elementary bodies of vaccinia was reported by Macfarland and Salaman[1] and confirmed by Hoagland, Ward, Smadel, and Rivers.[2] The first authors concluded from their experiments that the enzymatic activity was due to the virus and not to tissue contaminants, but Hoagland *et al.* found that vaccinia virus may adsorb large quantities of phosphatase from a dilute solution containing the crude enzyme, and considered this strong evidence for the adsorption hypothesis. In conclusion they said, "Until some method can be devised which will distinguish between the enzymes of the host cell and those which may be an integral part of the virus it would seem that the problem of the enzyme constitution of vaccinia virus is incapable of definite solution."

With a full realization of the physical and constitutional differences between the elementary body of vaccinia virus and its inclusion body, histochemical preparations of phosphatase activity of the vaccinia, herpex simplex, fowlpox, and tracheo-laryngitis inclusion bodies were studied.

Gomori[3] and Takamatsu[4] independently introduced an ingenious histochemical technic for the demonstration of phosphatase activity on ordinary histologic slide preparations. Taking advantage of the fact that this enzyme was preserved in alcohol fixation and paraffin sections, they showed that incubation in a solution containing sodium-β-glycerophosphate and calcium chloride buffered at a suitable pH caused the precipitation of calcium phosphate at the site of enzymatic activity. It was then possible to stain for the insoluble calcium phosphate. This technic has the advantage of preserving tissue integrity, revealing clearly the phosphatase activity of the host tissue. It appears that Gomori's technic, although not quantitative, is just as specific for alkaline phosphatase as are the usual biochemical methods of determination.[5,6,7] Gomori has introduced a comparable method for the visualization of acid phosphatase[8] and small amounts of the enzyme can be demonstrated by both the acid[9] and alkaline[10] technic.

It seemed reasonable, therefore, that if vaccina inclusion bodies failed to give a positive histochemical phosphatase reaction when studied *in situ*, the tissue contamination hypothesis might be given added weight. If, however, inclusion bodies stained positively no interpretation could be made since the adsorption phenomenon might occur directly in the tissues. By the use of the same technic Willmer[11] has shown that the chromosomes of dividing cells in tissue cultures give a strongly positive reaction for the presence of phosphatase. It has been shown that alkaline phosphatase prepared from intestinal mucous membrane is capable of hydrolyzing yeast and virus nucleic acid but has no effect on tobacco mosaic virus.[12]

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TABLE I.
Failure to Demonstrate Alkaline Phosphatase in Inclusion Bodies.

Virus	Source inclusion	Histochemical reaction
Vaccinia	rabbit cornea	negative
,,	,, ,,	,,
,,	chorio-allantoic membrane	,,
,,	chorio-allantoic membrane	,,
Fowlpox	chicken epidermis	,,
Herpes simplex	chorio-allantoic membrane	,,
Tracheo-laryngitis	chorio-allantoic membrane	,,

The corneas of 2 rabbits were inoculated with vaccinia virus and the rabbits killed after 24 and 48 hours respectively. Vaccinia virus was inoculated into the chorio-allantoic membrane of 2 chick embryos of 12 days and allowed to incubate for 6 and 7 days. Similar preparations were made with herpes simplex and tracheo-laryngitis virus. A section of epidermis from a chicken dead of naturally occurring fowlpox was studied. Each of these histochemical preparations revealed the presence of their corresponding large inclusion bodies, but in none of the inclusions was there any apparent alkaline phosphatase activity. Likewise, there was little or no phosphatase activity in the chorio-allantoic membrane in the immediate vicinity of the virus. The lesions in skin of the chicken, however, showed slight and scattered enzyme activity.

Summary. We failed to demonstrate alkaline phosphatase in the inclusion bodies of vaccinia, herpes simplex, fowlpox, and tracheo-laryngitis by the use of a slightly modified Gomori technic.[10] While our evidence is not conclusive, it strongly favors the hypothesis of Hoagland, Ward, Smadel, and Rivers that the phosphatase activity of the inclusion body is due to tissue contamination.

14522

Comparative *in vitro* Resistance of Staphylococci to Penicillin and to Sodium Sulfathiazole.*†

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Investigations to be reported elsewhere[1] revealed that sulfonamide-resistant strains of staphylococcus were being recovered with increasing frequency at the University Hospitals, and that the majority of the resistant strains were being obtained from patients who had received sulfonamides prior to isolation of the organisms. In view of this, it appeared highly desirable to compare the *in vitro* resistance of a large number of strains of staphylococcus to the antistaphylococcic action of penicillin and to sodium sulfathiazole. Recent studies carried out with a relatively small number of cultures indicate that some strains of *Staphylococcus aureus* varied only slightly in their resistance to penicillin,[2] while other strains were quite insensitive.[3,4,5,6]

Materials and Methods. Sixty-eight

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