respect the inhibitory influence of saliva differs from that of the normal serum inhibitor.

Summary. Saliva of human adults possesses the capacity to inhibit agglutination of erythrocytes by influenza virus. Under the conditions of study the effectiveness varies among individuals and at different times. Its action is not significantly affected by heating at 56° C for 30 minutes; nor is its behavior influenced by heating of the virus. Nevertheless, the action of the inhibitor appears to be upon the virus rather than upon the erythrocytes. The nature of the inhibitor has not been determined but it is suggested that saliva represents a physiologic source of materials such as have been shown to interfere with hemagglutination in *in vitro* systems.

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Alteration of *Pasteurella pestis* Bacteriophage Following Successive Transfer on *Pasteurella pseudotuberculosis* and on Shigellae.

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A previous report[1] has described the activity of a strain of *Pasteurella pestis* bacteriophage which was able to lyse 19 of 27 strains of *Pasteurella pseudotuberculosis* and 6 of 37 strains of shigellae. By repeated transfer on *P. pseudotuberculosis* (Spokane strain) this phage became adapted so that it lysed all 27 strains of this species in high dilution. After transfer on shigellae, the phage increased its potency for sensitive strains of this genus. The present report describes the effect of successive transfer of the same *P. pestis* phage on *P. pseudotuberculosis* and on certain species of shigellae in various combinations.

The methods used in this study have been recorded.[1] All tests were incubated at 37°C. The transfers of phage were made by adding 0.5 ml of the previous passage to 10 ml of a broth culture of bacteria about two hours old and incubating until clear. After about 5 transfers no secondary resistant growth appeared and filtration of the lysate was unnecessary. The "purified" *P. pestis* phage previously described was used as a starting point in this study. The bacterial cultures were the same as those studied in the first report. Thirteen additional strains of *P. pseudotuberculosis* were investigated, of which all but one were lysed by the original phage.

In the first experiment, the *P. pestis* phage was transferred 22 times on *P. pseudotuberculosis* (Spokane strain) and then five times on each of the following in succession: *S. dysenteriae* (No. 44), *S. paradysenteriae* (type 103 No. 12), *S. sonnei* (No. 6), and *S. ambigua* (No. 33). It was found that any significant change occurred within 5 transfers on a given culture and that further transfers up to 25 made no difference. After each series of transfers, the adapted phages were tested on agar plates against each of the organisms listed and against *P. pestis*. Representative results are shown in Table I.

The results after transfer on 2 or 3 of the species of shigellae were essentially the same as those after transfer on all 4 species. The right hand column in Table I represents the effect of successive transfer on all 4 shigellae. It is noteworthy that after passage first on *P. pseudotuberculosis* and then on *S. dysenteriae*, the phage no longer lysed *P. pseudotuberculosis*.

A second series of experiments was planned to show the effect of successive transfer on

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[1] Lazarus, A. S., and Gunnison, J. B., *J. Bact.*, 1947, **53**, 705.

TABLE I.
Highest Dilution of Original and Adapted Phages Producing Lysis of Plate Cultures of Various Organisms.

Organism tested	Original *P. pestis* phage	Original *P. pestis* phage adapted to: PTB	PTB then *S. dysenteriae*	PBT then *S. dysenteriae, paradysenteriae, sonnei,* and *ambigua*
P. pestis	10^{-7}	10^{-7}	10^{-7}	10^{-6}
PTB	—	10^{-7}	—	—
S. dysenteriae	10^{-3}	10^{0}	10^{-6}	10^{-5}
S. paradysenteriae	10^{-2}	10^{0}	10^{-4}	10^{-4}
S. sonnei	10^{-1}	10^{0}	10^{-2}	10^{-5}
S. ambigua	10^{-4}	10^{-4}	10^{-6}	10^{-5}

— = no lysis. PTB = *P. pseudotuberculosis* (Spokane).

TABLE II.
Lysis After Transfer of *P. pestis* Phage on Pasteurellae and Shigellae.

Phage transferred on	Tested on *P. pestis*	Agar plate cultures of: 9 PTB strains not lysed by original phage	31 PTB strains lysed by original phage	Shigellae*
P. pestis	++	—	++	+
PTB (Spokane)	++	++	++	+
Shigellae*	++	—	++	++
PTB—then *S. sonnei, paradysenteriae,* or *ambigua*	++	++	++	++
PTB then *S. dysenteriae*	++	—	++	++
PTB then *S. dysenteriae* then PTB again	++	++	++	++

++ = lysed by phage in dilution of 10^{-3} or higher.
+ = lysed by phage in dilution lower than 10^{-3}.
— = not lysed.
PTB = *P. pseudotuberculosis.*
* Includes *S. dysenteriae, S. paradysenteriae, S. sonnei,* and *S. ambigua.*

different organisms in altering the characteristics of the original phage. The plan and the results are summarized in Table II.

It was again noted that phage first adapted to *P. pseudotuberculosis* (Spokane) and then transferred on *S. dysenteriae* lost its ability to lyse *P. pseudotuberculosis* (Spokane). This adapted phage was then tested against all 40 strains of *P. pseudotuberculosis.* It failed to lyse any of the nine strains which were resistant to the original phage, but lysed all 31 strains sensitive to the original phage. Hence this phage adapted to the Spokane strain and then to *S. dysenteriae* behaved just like the parent *P. pestis* phage in its action on *P. pseudotuberculosis* cultures.

Transfer of phage adapted to the Spokane strain on shigellae other than *S. dysenteriae* did not change its ability to lyse all the *P. pseudotuberculosis* strains. Therefore the loss of activity against certain *P. pseudotuberculosis* cultures was produced only by *S. dysenteriae.* The potency of this phage for all strains of *P. pseudotuberculosis* was completely restored by again transferring it on the Spokane culture after passage on *S. dysenteriae.* Furthermore, the original phage adapted first to shigellae, including *S. dysenteriae,* and then to *P. pseudotuberculosis* (Spokane) lysed both the shigellae and all strains of *P. pseudotuberculosis.* Therefore, it seemed that transfer of phage adapted to *P. pseudotuberculosis* on *S. dysenteriae* produced a selective blocking action resulting in loss of ability to lyse a group of cultures previously sensitive.

Tests were made to determine whether this blocking action might be due to metabolic

or disintegration products of *S. dysenteriae* which, of course, would be present in phage adapted to this organism. A lysate of *S. dysenteriae* was prepared from a broth culture by adding phage adapted first to *P. pseudotuberculosis* and then to *S. dysenteriae*. This lysate was mixed with equal parts of phage adapted to *P. pseudotuberculosis* and allowed to stand at 37°C for two hours. The mixture was then added to broth and agar cultures of *P. pseudotuberculosis* using the same technic as usual. There was complete lysis in all tests. Hence, the presence of a lysate of *S. dysenteriae* did not inhibit lysis of *P. pseudotuberculosis* by phage adapted to the latter.

The plaques produced by the original *P. pestis* phage on agar cultures of *P. pestis* were uniform in size and measured about 3 mm in diameter after 24 hours. On plates of *P. pseudotuberculosis* and of shigellae, the plaques formed by the original phage varied in diameter from 0.5 to 3 mm. Large and small plaques were isolated repeatedly in attempts to separate two strains or mutants of phage, but without success. Isolated plaques gave rise to both large and small plaques even after many subcultures. Successive transfer on four species of shigellae followed by passage on *P. pseudotuberculosis* did not alter the size of plaques. The plaque size for a given culture was no different with the various adapted phages than with the original phage.

No explanation can be given at present for the alteration of phage adapted to *P. pseudotuberculosis* by subsequent transfer on *S. dysenteriae* so that it longer lyses certain strains of pasteurellae. It seemed possible that the original phage might contain two components and that the one acting on these strains could not multiply in the presence of *S. dysenteriae*. However, this is unlikely because the activity toward *P. pseudotuberculosis* was readily restored by readaptation to the Spokane strain after many transfers on *S. dysenteriae*.

The variation in plaque size shown by the original phage suggests that mutant types might be present which might vary in their host range, although attempts to separate such mutants were unsuccessful. Delbruck[2] has reported the occurrence of biochemical mutants of phage with differing requirements for cofactors for adsorption of the phage on the host bacteria. One of his mutants required tryptophan or its analogues and its adsorption was inhibited by indole. These substances are not concerned here for the medium used is rich in tryptophan and neither *P. pseudotuberculosis* nor *S. dysenteriae* produce indole. Lysis was not inhibited by the addition of lysates of *S. dysenteriae* to mixtures of *P. pseudotuberculosis* and its adapted phage.

Likewise there is no apparent explanation for the difference in behavior of those strains of *P. pseudotuberculosis* resembling *P. pestis* in their sensitivity to the phages and of those not resembling *P. pestis*. No correlation could be demonstrated between the sensitivity of the strains to the various phages and their microscopic morphology or their antigenic structure. The only detectable difference was that the majority of the strains behaving like *P. pestis* produced rough colonies while those of the other group were usually smooth.

Summary. Bacteriophage, originally recovered as a lytic agent for *P. pestis*, also lysed 31 of 40 strains of *P. pseudotuberculosis*. After adaptation to one of these (Spokane strain), it also lysed the remaining nine strains. When the adapted phage was transferred on *S. dysenteriae*, it reverted to its original activity; *i.e.*, it again failed to lyse the nine strains of *P. pseudotuberculosis* which resisted the original phage. Activity for all strains was restored by transferring it on the Spokane strain. Lysates of *S. dysenteriae*, mixed with the adapted phage, caused no reversion to the original selective activity for only 31 strains of *P. pseudotuberculosis*. Transfers of the adapted phage on *S. paradysenteriae, S. sonnei, S. ambigua*, or on all of these species in succession, did not alter its ability to lyse all strains of *P. pseudotuberculosis*. The size of plaques did not change during transfers on either the shigellae or the pasteurellae used.

[2] Delbruck, M., *J. Bact.*, 1948, **56**, 1.