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New Heat-Stable Agglutinogens in the Suipestifer Group.

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Observations on absorption of phage by heat-killed bacilli particularly those on the typhosus-paratyphosus B group and some strains of suipestifer suggested that further investigations along this line might yield information revealing new relationships in this highly complex group of organisms.[1, 2, 3] We here report the existence of differences in the suipestifer group first detected by characteristic phage absorption effects and confirmed by subsequent immunization and experiments on agglutinin absorption.

The observations were made with a phage derived from chicken stool filtrates and propagated with a particular American strain of suipestifer, No. 26* in our collection. This phage lysed to a high titer numerous smooth cultures of the biochemically different types, namely American, Kunzendorf and one out of 4 strains of the Hirschfeld type, the other 3 being comparatively resistant. As already reported,[1, 3] the strain Glässer-voldagsen was completely resistant even to the action of undiluted phage. We have since found one culture, No. 92 (American*) in our collection which was poorly lysed but differed from other suipestifer strains in failing to absorb actively from the suipestifer 26 phage, particularly from some passages. Somewhat similar effects were observed with strains 80 and Glässer-voldagsen.

Curiously enough, the same cultures, 80 and 92 were 2 of several strains, mostly of the Kunzendorf type, which on repeated occasions absorbed small quantities of the phage for paratyphosus B in con-

[1] Levine, Philip, Frisch, A. W., and Cohen, E. V., *J. Immunol.*, 1934, **26**, 321.

[2] Levine, Philip, and Frisch, A. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 339.

[3] Levine, Philip, and Frisch, A. W., *J. Inf. Dis.*, 1935, in press.

* For strain No. 26, we are indebted to Dr. Landsteiner, who received it originally from the British National Type Culture Collection. From the same source we obtained the Hirschfeld cultures. Suipestifer No. 80 is in the American Type Culture Collection and is recorded in their catalogue as No. 800; it was isolated by Shaw[4] from the blood stream of a patient with clinical influenza. In our hands this strain produced H_2S. Dr. Ten Broeck[5] supplied us with culture No. 92 which he isolated from a pig.

[4] Shaw, F. W., *J. Lab. and Clin. Med.*, 1926, **12**, 141.

[5] Ten Broeck, C., written communication.

TABLE I.

Absorbed with heat-killed organism No.	Test-organism—suipestifer 26 Dilutions of Anti-suipestifer 26 phage						Test-organism—paratyphosus B 16 Dilutions of Anti-paratyphosus B 16 phage				
	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
26 A	+±	++++	++++	++++	++++	+++	cl	cl	cl	cl	+
84 A	+±	++++	++++	++++	++++	+++	cl	cl	cl	cl	+
100 H	++++	++++	+++	++++	++++	+++	cl	cl	cl	±	++
63 GV	tr	+±	+++	++++	++++	+++	cl	cl	cl	±	++++
80 K	cl	++	++++	++++	++++	+++	cl	+±	+++	++++	+++
92 A	cl	cl	cl	++	++++	++++	cl	+	+±	+++±	++++
16	cl	cl	cl	++	+++	++++	++++	++++	+++	++++	+++
Unabsorbed	cl	cl	cl	tr	+±	++++	cl	cl	cl	cl	tr

Culture No. 16 is paratyphosus B; all other cultures are in the suipestifer group.
A = American; H = Hirschfeld; GV = Glässer-voldagsen; K = Kunzendorf.
The turbidity reading recorded is that made 6 hours following the addition of the test organisms; cl indicates complete clearing; ±, +, +±, ++, etc, indicate increasing degrees of turbidity.

trast to the other suipestifer strains which did not at all absorb. These effects are indicated in Table I.

In view of these results, it seemed desirable to determine whether serological characteristics could be found to correspond more or less with the qualitative differences shown in phage absorption.

Accordingly rabbits were immunized intravenously with increasing quantities of boiled bacillary suspensions of several varieties. Specimens of sera obtained after 3 and 6 injections were tested both by direct titration and by absorption. The absorptions were made with sera diluted 1:100; the bacilli employed for the absorption were previously heated for one hour at 80°C., while the suspensions used in the tests for the quality of the absorbed sera were previously treated with absolute alcohol to prevent the action of flagellar agglutinins, if present. In Table II is recorded a typical experiment with sera vigorously absorbed with an excess of bacterial bodies.

TABLE II.

Rabbit Serum No.	Absorbed with Strain No.	Tested with alcohol-treated suipestifer cultures, No. 26A	84A	100H	63GV	80K	92A
413 (anti-100)	84	0	0	±	+±	+±	+±
	92	+++	+++	+++	++	0	0
426 (anti-92)	84	0	0	+	++	++	++±
	92	0	0	0	0	0	0

The results indicate that suipestifer Nos. 80 and 92 form one sub-group, whereas the great majority of the American type (represented in Table II by Nos. 26 and 84) and Kunzendorf strains (not recorded in the table) form a second sub-group. Apparently Gläs„ser-voldagsen and perhaps also suipestifer 100 (Hirschfeld) have still other properties. It is interesting to note that sera for strain 100 contain several types of qualitatively different antibodies, whereas most other cultures of the American or Kunzendorf types fail to produce an antibody specific for 80 and 92. Such an antibody was found to be present in each of 3 rabbits injected with suipestifer No. 92 and also 100. Direct titration of these sera did not readily reveal the relationships described in the agglutinin absorption tests.

Experiments are under way to determine whether or not the strains 80 and 92, in contrast to others are capable of specifically absorbing small quantities of the paratyphosus B antibody.

In view of the relationship of newport to the suipestifer group, it became necessary to re-examine this question. Our preliminary ex-

periments showed that all actively agglutinating sera except those produced by strain 92, agglutinated newport also, and, in harmony with this result, all suipestifer cultures except 80 and 92 removed this agglutinin.

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Polyvalency Demonstrated by Antiphages.

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A general procedure was described for the study of the specificity of absorption of bacteriophage by heat-killed bacilli.[1, 2, 3] Polyvalent phages derived from chicken stool filtrates and propagated either against a strain of paratyphosus A or enteritidis were shown to consist of at least 2 prominent qualitatively different fractions, the one selective for suipestifer strains and another for the typhosus-paratyphosus B group. This effect was readily demonstrated by testing the quality of the residual phage with several serologically different sensitive organisms. The question naturally presented itself as to whether or not antiphages may be employed to indicate the presence in a phage of the several fractions.

Accordingly 4 series of rabbits were injected intravenously with the 2 polyvalent phages for paratyphosus A and for enteritidis and also with the presumably monovalent paratyphosus B and suipestifer phages. After 4 injections of 2 cc. each, potent antiphages, practically lacking in agglutinins were readily obtained in all instances except in the case of the suipestifer phage.

Experiments demonstrating the production of 2 antibodies corresponding to the fractions in the polyvalent phages are given in Table I. The results show that the antisera for the paratyphosus A and enteritidis phages contain potent antibodies which neutralize the action of the typhosus-paratyphosus B fraction of the polyvalent enteritidis phage (Table I A, aertrycke of the paratyphosus B group, as test organism); both antisera also possess antibodies for the suipestifer fraction but to varying degrees of activity. Although

1 Levine, Ph., Frisch, A. W., and Cohen, E. V., *J. Immunol.*, 1934, **26**, 321.
2 Levine, Ph., Frisch, A. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 339.
3 Levine, Ph., Frisch, A. W., *J. Inf. Dis.*, 1935. In press.