TABLE II.
Effect of Various Concentrations of Sodium Thioglycollate in Media for Detecting Surviving Bacteria in Testing Disinfectants.

Disinfectant	Concentration	% of thioglycollate in medium: 0.00 hrs: 24	48	0.01 24	48	0.02 24	48	0.05 24	48	0.10 24	48	0.20 24	48
Phemerol	1:50,000	—	+	—	—	—	—	—	—	—	+	—	+
	1:100,000	+	+	+	+	+	+	+	+	—	+	—	—
	1:250,000	+	+	+	+	+	+	+	+	+	+	+	+
Phenol	1:70	—	—	—	—	—	—	—	—	—	—	—	—
	1:80	—	—	—	—	—	—	—	—	—	—	—	—
	1:90	—	—	—	—	+	+	—	+	—	+	—	+
Mercuric Chloride	1:5000	—	—	—	—	—	—	+	+	+	+	—	—
	1:10,000	—	—	—	—	—	+	+	+	+	+	—	+
	1:50,000	—	—	—	—	—	+	+	+	+	+	—	+
Metaphen	1:500	—	—	—	—	—	+	+	+	—	+	—	+
	1:1000	—	—	—	—	—	+	+	+	+	+	—	+
	1:5000	—	—	—	—	—	+	+	+	+	+	—	+
Merthiolate	1:1000	—	—	—	—	—	—	+	+	+	+	—	+
	1:5000	—	—	—	—	+	+	+	+	+	+	—	+
	1:10,000	—	—	—	+	+	+	+	+	+	+	—	+
Mercurochrome	1:50	—	—	—	—	+	+	+	+	—	+	—	+
	1:250	—	—	—	—	+	+	+	+	—	+	—	+
	1:500	—	—	—	+	+	+	+	+	+	+	—	+

(+) indicates growth; (—) indicates no growth.

TABLE III.
Effect of Addition of Sodium Thioglycollate to Plain Broth at Intervals After Subculturing from Mercurial Solutions.

Disinfectant	Control	Interval after subculturing: Min 1	2	5	10	30	Hr 1	2	5	8	15
Mercuric Chloride, 1:5000	—	+	+	+	+	+	+	+	+	—	—
Metaphen, 1:500	—	+	+	+	+	+	+	+	—	—	—
Merthiolate, 1:1000	—		+				+		+	+	—
Mercuric Oxycyanide, 1:250	—	+	+	+	+	+	+	+	—	—	—
Mercurochrome, 1:50	—	+	+	+	+	+	+	+	—	—	—

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Antigenic Relationship Between *H. Influenzae* Type B and Pneumococcus Type VI.

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Recently, two reports were published pertaining to antigenic relationships between *Hemophilus influenzæ* and pneumococcus. Chapman and Osborne[1] isolated a strain of *H. influenzæ* type a from the spinal fluid and nasal secretion of a child suffering from a fractured skull. This particular strain gave a capsular swelling reaction with anti-

1 Chapman, O. D., and Osborne, W., *J. Bact.*, 1942, **44**, 620.

pneumococcus type 6b serum. A strain of *H. influenzæ* type c, recovered from the sputum of a patient with upper respiratory infection, reacted with antipneumococcus type 21 serum. Neter[2] found that certain anti-*H. influenzæ* horse sera reacted with pneumococcus type 6, causing both precipitation and agglutination. It was reported, furthermore, that anti-*H. influenzæ* types a and b rabbit sera did not contain antibodies against pneumococcus type 6, nor did anti-pneumococcus type 6 horse serum react with *H. influenzæ* type b. In order to determine the nature of the type 6 pneumococcal antibodies in anti-*H. influenzæ* horse sera and the possible antigenic relationship of the two microörganisms involved, precipitation and cross-absorption experiments with the specific soluble substance of pneumococcus type 6 and the specific polysaccharide of *H. influenzæ* type b, were carried out. The results of this investigation are herewith presented.

Material and Methods. Several lots of anti-*H. influenzæ* horse sera were obtained through the kindness of Dr. Leah Seidman, Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts, and Dr. Morris Shaffer, Massachusetts State Antitoxin Laboratory. Dr. H. E. Alexander kindly supplied *H. influenzæ* type b polysaccharide; 2 cc of the material contained 1.96 mg of the substance. Each milligram of this polysaccharide, according to Dr. Alexander,[3] absorbs 6.7 mg of type b *H. influenzæ* antibody nitrogen. Dr. Edwin F. Voight, Lederle Laboratories, furnished 100 mg of pneumococcus type 6 specific soluble substance (No. T6-38).

Precipitation tests were carried out in test tubes (3″x⅜″). The material was measured by means of Kahn pipettes. Precipitation was read macroscopically.

Results. Several lots of anti-*H. influenzæ* horse sera were tested for the presence of antibodies against pneumococcus type 6, as reported previously.[2] Serum No. I 60 markedly reacted with pneumococcus type 6. The titer of type 6 pneumococcal antibodies of several other lots of serum had definitely decreased during storage at icebox temperature for 5 months. The experiments with the specific soluble substance of pneumococcus type 6 and the polysaccharide of *H. influenzæ* were carried out with the serum No. I 60.

A titration of the anti-*H. influenzæ* serum (No. I 60) revealed that, with the supernatant fluid of a pneumococcus type 6 brain heart infusion broth culture as antigen, it caused precipitation in dilutions of 1:1 and 1:2; in dilutions of 1:4 and higher no definite reaction took place. For this reason, all experiments were carried out with undiluted serum.

In the first experiment it was determined whether or not this anti-*H. influenzæ* serum reacts with SSS of pneumococcus type 6. For comparative purposes an anti-pneumococcus type 6 horse serum was included. Various amounts of SSS, ranging from 2 mg to 0.00002 mg (volume 0.1 ml), were mixed with 0.05 ml of the undiluted sera. The results of this experiment are presented in Table I.

It may be seen from Table I that both anti-*H. influenzæ* serum and anti-pneumococcus type 6 serum reacted strongly with SSS of pneumococcus type 6. With both sera a zone-phenomenon was encountered. There cannot be any doubt, therefore, that the reactivity of anti-*H. influenzæ* horse serum with pneumococcus type 6 is due to the presence of antibodies directed against the specific soluble substance of this microörganism.

In order to ascertain the antigenic relationship, if any, between *H. influenzæ* type b and pneumococcus type 6, the following cross-absorption tests were carried out: To 0.6 ml of anti-*H. influenzæ* serum was added 0.025 ml of 1:10 diluted specific soluble substance of pneumococcus type 6 in physiological saline solution (0.05 mg). Precipitation occurred almost immediately. The mixture was incubated at 37°C for 2 hours and then centrifuged. The supernatant fluid, which was perfectly clear, was tested for the presence of antibodies reacting with the specific polysaccharide of *H. influenzæ* type b and also with SSS of pneumococcus type 6. In order to detect small quantities of antibodies the tests were carried out with decreasing amounts of the specific polysaccharide. For comparative purposes precipitation tests were included

[2] Neter, E., *J. Immunol.*, 1943, **46**, 239.

[3] Alexander, H. E., personal communication.

TABLE I.
Reaction Between Anti-*H. influenzæ* (Horse) Serum (No. I 60) and SSS of Pneumococcus Type VI.

Amount of SSS (vol. 0.1 ml) mg	Anti-*H. influenzæ* serum (vol. 0.05 ml)			Anti-pneumococcus type VI serum (vol. 0.05 ml)			Saline solution (vol. 0.05 ml)		
	A	B	C	A	B	C	A	B	C
(1) 2	—	—	—	—	—	—	—	—	—
(2) 0.2	—	—	—	—	—	++	—	—	—
(3) 0.02	++	++++	++++	+++	++++	++++	—	—	—
(4) 0.002	+	++	++	+++	++++	++++	—	—	—
(5) 0.0002	—	—	—	++	++	++	—	—	—
(6) 0.00002	—	—	—	—	—	—	—	—	—
(7) 0	—	—	—	—	—	—	—	—	—

A = After 2 min.
B = After 10 min.
C = After 2 hr.
— = No precipitation.
+ to ++++ = Various degrees of precipitation.

with the original, non-absorbed serum. The results are summarized in Table II.

Table II reveals that absorption of anti-*H. influenzæ* horse serum with SSS of pneumococcus type 6 completely removed the precipitins against pneumococcus type 6, but did not noticeably affect the antibodies directed against *H. influenzæ* type b. Identical results were obtained in repeated experiments employing various amounts of SSS for absorption. Absorption of 0.3 ml of this anti-*H. influenzæ* horse serum with 0.2 mg, 0.02 mg, and 0.002 mg of SSS of pneumococcus type 6 resulted in complete elimination of the pneumococcal antibodies and did not decrease the reactivity of the serum with the polysaccharide of the influenza bacillus. It may be added that absorbed serum also failed to cause precipitation with the supernatant fluid of a pneumococcus type 6 brain heart infusion broth culture as antigen. It may be concluded, therefore, the anti-*H. influenzæ* horse serum contains two distinct antibodies, the one reacting with *H. influenzæ,* the other with pneumococcus type 6.

The question, then, arises as to whether or not the specific polysaccharide of *H. influenzæ* type b is antigenically related to the SSS of pneumococcus type 6. In order to elucidate this problem further, anti-*H. influenzæ* horse serum was absorbed with the specific polysaccharide of the type b influenza bacillus. To 0.5 ml of the serum was added 0.1 ml (0.098 mg) of the specific polysaccharide of *H. influenzæ*. Marked precipitation occurred. After incubation for 30 minutes at 37°C the mixture was centrifuged and the perfectly clear supernatant fluid was then tested for the presence of antibodies against pneumococcus type 6 and *H. influenzæ*. The experiment revealed that absorption with the specific polysaccharide of *H. influenzæ* type b resulted in complete elimination of the pneumococcal antibodies and the precipitins reacting with the specific polysaccharide of the influenza bacillus. The absorbed serum failed to cause precipitation with SSS of pneumococcus type 6 in amounts ranging from 0.2 mg to 0.000002 mg. In contrast, the non-absorbed serum reacted with the specific soluble substance of the pneumococcus in amounts of 0.02 mg to 0.0002 mg. It may be added that the absorbed serum failed to cause precipitation with the supernatant fluid of a pneumococcus type 6 brain heart infusion broth culture as antigen.

The results of these absorption experiments seem to allow of the conclusion that the available specific polysaccharide of *H. influenzæ* type b shares some antigenic component or part thereof with the available specific soluble substance of pneumococcus type 6. Furthermore, these findings throw some light on the possible origin of the type 6 pneumococcal antibodies in anti-*H. influenzæ* horse sera. The fact that the type b polysaccharide of the influenza bacillus absorbed the antibodies to the SSS of pneumococcus type 6 renders it likely that these antibodies were engendered

TABLE II.
Reaction Between Anti-*H. influenzæ* (Horse) Serum, Absorbed with SSS of Pneumococcus Type VI, and Specific Polysaccharide of *H. influenzæ* Type B.

Specific polysaccharide of *H. influenzæ* (vol. 0.1 ml) mg		Absorbed serum (vol. 0.05 ml) A	B	C	Non-absorbed serum (vol. 0.05 ml) A	B	C
(1)	0.098	++++	++++	++++	++++	++++	++++
(2)	0.0098	++	+++	+++	++	+++	+++
(3)	0.00098	—	—	—	—	—	—
(4)	0	—	—	—	—	—	—
SSS of pneumococcus type VI (vol. 0.1 ml) mg		A	B	C	A	B	C
(1)	0.2	—	—	—	—	+	+
(2)	0.02	—	—	—	+	+++	+++
(3)	0.002	—	—	—	—	++	++
(4)	0.0002	—	—	—	—	—	—
(5)	0	—	—	—	—	—	—

A = After 2 min.
B = After 10 min.
C = After 30 min.
— = No precipitation.
+ to ++++ = Various degrees of precipitation.

by *H. influenzæ* and that they are not due to the unintentional immunization with pneumococcus. The demonstration of an antigenic relationship between the specific substances of pneumococcus type 6 and the influenza bacillus type b serves as another example of common antigenic components in otherwise unrelated microörganisms.

Summary. (1) Certain anti-*H. influenzæ* horse serum reacts with SSS of pneumococcus type 6. Quantitatively, the precipitation reaction parallels that obtained with anti-pneumococcus type 6 serum. (2) SSS of pneumococcus type 6 absorbs the homologous antibodies, but does not affect the antibodies against the specific polysaccharide of *H. influenzæ* type b. (3) Absorption of the serum with the polysaccharide of the influenza bacillus removes both homologous antibodies and precipitins against the SSS of pneumococcus type 6. (4) It is concluded that such an anti-*H. influenzæ* horse serum contains two distinct antibodies and that some antigenic relationship exists between the available specific polysaccharide of *H. influenzæ* type b and the available SSS of pneumococcus type 6.

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