occur is attested by the observation that the pigeon-fancier, whose father died from ornithosis, had specific antibodies against the virus in his serum. Aerogenic contamination of the respiratory mucosa by the public in a great many civic places can be readily visualized. A patient may not have been cognizant of this exposure. Thus, the fortuitous isolation of a saprophytic ornithotic virus from nose- or throat-washings, in the course of an attack of influenza or a cold, might often happen.

In the future, it appears highly desirable (a) that the sera of patients with atypical pneumonias, influenza and other respiratory infections, in the absence of specific bacterial agents, be tested for specific antibodies against the psittacotic or ornithotic virus, (b) that in the course of epidemiological investigations, the prolonged or even fleeting exposure to pigeons or barnyard birds like fowls and ducks be kept in mind, and (c) that the term psittacosis be limited to the human infections definitely proven to be of association with psittacine birds, while those due to contact with other birds be described as ornithosis of fulmar, pigeon, or chicken origin.

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Toxicity of Washings from *Hemophilus pertussis* for Mice.*

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While studying the toxic substance that can be obtained by growing *Hemophilus pertussis* in a liquid medium, as described by Wood,[1] we have observed that a substance with similar toxic properties can be obtained by simply washing the organisms of freshly isolated strains grown on a solid medium. After 48 hours of growth, the organisms are removed from the surface of Bordet's medium with a cotton swab and suspended in 0.85% NaCl solution. When density of the suspension has been adjusted to approximately 40 billion organisms per ml, the suspension is thoroughly mixed for 5 minutes with a pipet. The organisms are then sedimented in an angle centrifuge at a temperature of 4°C. After several periods of centrifuga-

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[1] Wood, M. L., *J. Immunol.*, 1940, **39**, 25.

TABLE I.
Toxicity of Saline Washings from *Hemophilus pertussis* for Mice.

Test No.	Strain	No. of organisms washed in billions per ml	Dilution of washings* None	1-2	1-4	1-8	1-16
1	Jen.	10	0/2†	0/2	0/2		
2	"	20	4/4	0/4	0/4	0/4	
3	"	30				1/2	0/2
4	"	40	4/4	4/4	4/4		
5‡	"	40	3/3	3/3	1/2		
6§	"	80	1/2	0/2	0/2		
7	"	100	2/2	2/2	2/2		
8	Kin.	80		3/4	2/4	0/4	0/4
9	Ash.	80		4/4	2/4	0/4	0/4

*Dose = 0.2 ml given intravenously.
†The numerator indicates the number of mice that died in a group containing the number given in the denominator.
‡Same preparation as test 4, after 10 days storage at 4°C.
§Second washings from same organisms as test 4.

tion of an hour each, the supernatant fluid is clear but still contains a few organisms, as tested by culture. After from 6 to 8 such periods of centrifugation, however, a bacteria-free supernatant fluid can be obtained. Supernatant fluids so prepared have been lethal for 3-week-old Swiss mice when injected intravenously (Table I).

Our results show that the supernatant washings and the toxic substance obtained by the method of Wood[1] are lethal in comparable dilutions of 0.2 ml doses. The mice injected with sublethal doses

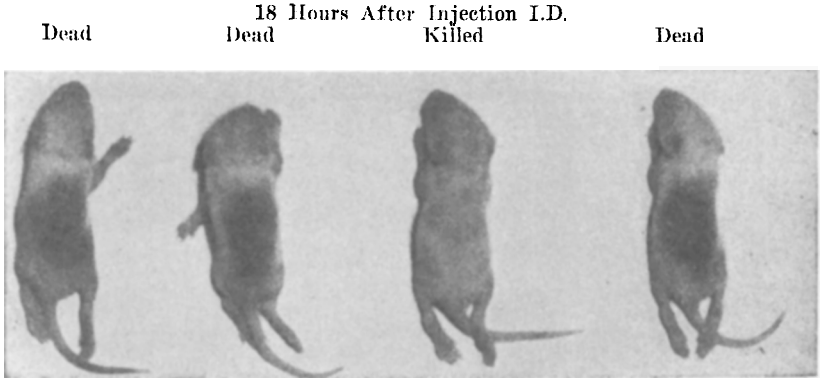

FIG. 1.
Effects of refined antitoxic and antibacterial sera upon the skin-reacting factor in washings from *H. pertussis*. 0.05 ml of a 1-10 dilution of serum was mixed with 0.05 ml of undiluted washings and the mixture incubated for 30 minutes at 37°C before endermal injection.

showed an atrophy of the spleen and other pathological changes resembling those described by Wood. When 0.05 ml was injected endermally into 10-day-old mice, it produced a marked hemorrhagic necrosis at the site of injection and frequently killed the animal (Fig. 1). Both the lethal and the skin-reacting factors were destroyed by heating in a water bath at 54°C for 10 minutes. After 10 days of storage at 4°C, the lethal factor remained essentially unaltered in bacteria-free washings. A considerable amount of the toxic component was removed by passage through a Seitz filter. Neutralization experiments gave identical results with the toxic substance prepared by Wood's method and with our toxic washings.

It was observed that the lethal and skin-reacting factors were neutralized by a refined antitoxic serum, but not by either of 2 antibacterial-globulin solutions, one of which was prepared by immunizing rabbits with killed organisms, the other, with living organisms (Table II and Fig. 1). All of these findings closely resemble those obtained by Wood with her toxic substance and support her suggestion that the toxic factor in broth cultures may have been derived from the surface of the growing organism.[1] That surface-washings are not the only source of heat-labile toxin is well

TABLE II.
Neutralization of Toxic Washings by Refined Antitoxic and Antibacterial Sera.

Dilution of serum	Sera tested*			Titration of lethal factor in washings		
	A	B	C	Strain	Dilution	
1-10	0/4	3/4		Jen.	1-8	1/2
1-100	0/4	4/4			1-16	0/2
1-5		4/4		Jen.	none	2/3
1-10		4/4			1-2	3/3
1-100	0/4	4/4			1-4	1/2
1-500	1/4					
1-10		4/4	4/4	Kin.	1-2	3/4
1-100	0/4		4/4		1-4	2/4
1-500	0/4		4/4		1-8	0/4
1-10			4/4	Ash.	1-2	4/4
1-100			4/4		1-4	2/4
1-500			4/4		1-8	0/4

Serum A—*Pertussis* antitoxin (rabbit) obtained through the courtesy of Dr. A. L. Joyner of Lederle Laboratories, Pearl River, N.Y.

Serum B—Immune-globulin solution produced by immunizing rabbits with killed organisms, obtained through the courtesy of Dr. W. E. Bunney of E. R. Squibb and Co., New Brunswick, N.J.

Serum C—Immune-globulin solution produced by immunizing rabbits with living organisms (supplied by Dr. Bunney).

*0.2 ml of diluted serum was mixed with 0.2 ml of undiluted washings and the mixture incubated for 30 minutes at 37°C before intravenous injection.

known, because it has been obtained from extracts of disrupted, washed organisms.[2, 3] The toxic substance in washings from *H. pertussis* seems to be analogous to that obtained by Zinsser and by Parker[4] from a variety of other organisms and called by them the "X-substance."

In *H. pertussis* washings other components, such as a heat-stable factor, an agglutinogen, and derivatives from the culture medium, are no doubt present in varying amounts. That a specific soluble substance could be removed by washing the organisms with saline or water has been demonstrated by Miller.[5]

The chemical composition, the antigenic properties and the effect of the toxic washings upon blood leucocytes are interesting points now being studied.

Summary. A heat-labile substance, toxic for young mice has been obtained by washing suspensions of freshly isolated strains of *Hemophilus pertussis* with 0.85% NaCl solution. Both the lethal and the skin-reacting factors of the toxic substance can be neutralized by antitoxin but not by antibacterial rabbit sera.

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Non-Specificity of Sulfonamides.

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(Introduced by N. Kopeloff.)

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The notion that individual members of the sulfonamide series are specific for certain bacterial species is widespread, although contrary to the most widely accepted hypothesis of the mode of sulfonamide action. The following experiments serve to clarify this point:

Staphylococci were grown on Knight's medium modified by Fildes, *et al.*,[1] and *Escherichia coli* on the asparagine-glucose-mineral

[2] Evans, E. G., and Maitland, H. B., *J. Path. and Bact.*, 1937, **45**, 715.

[3] Flosdorf, E. W., and Kimble, A. C., *J. Immunol.*, 1940, **39**, 475.

[4] Zinsser, H., Enders, J., and Fothergill, L., *Immunity Principles and Application in Medicine and Public Health*, The Macmillan Co., New York, 1939, 78-101.

[5] Miller, J. J., PROC. SOC. EXP. BIOL. AND MED., 1937-38, **37**, 45.

[1] Fildes, P., Richardson, G. M., Knight, B. C. J. G., and Gladstone, G. P., *Brit. J. Exp. Path.*, 1936, **17**, 481.