nificant. It is concluded that under the conditions of these experiments the presence of agglutinating and phagocytosis-promoting antibodies in the plasma fails to increase or prolong chemotaxis.

Thus no evidence has been obtained from these experiments favoring the view that chemotaxis is dependent upon or is increased by the presence of agglutinating and phagocytosis-promoting antibodies. It seems rather that chemotaxis is brought about primarily by substances produced by the organisms,[3] and that such substances are given off by all kinds of bacteria.[4] If this is true, chemotaxis is not a specific immune reaction as are, in part, phagocytosis and agglutination, but a non-specific response. It appears that antibodies play their part only after the cell has been attracted to the bacteria; then, by facilitating the spread of cell on particle,[5] antibodies aid in bringing about phagocytosis.

Summary. Sensitization of a strain of hemolytic streptococcus with rabbit antiserum did not increase the chemotactic attraction of these bacteria for rabbit polymorphonuclear leukocytes *in vitro.* Also the chemotactic response to hemolytic streptococci was no greater when leukocytes and plasma were obtained from immunized rabbits than when they were obtained from normal animals. Under these conditions, chemotaxis, unlike phagocytosis and agglutination, is not increased by specific antibodies, but appears to be rather a non-specific response of leukocytes toward microörganisms.

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Studies on *H. pertussis.** I. Liberation by Sonic Vibration of a Soluble Component That Absorbs Phase I Agglutinins.

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(Introduced by D. W. Bronk.)

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The antibacterial action of protective antisera has been shown to involve combination with antigens present on the bacterial surface.

[3] Dixon, H. M., and McCutcheon, M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 378.

[4] McCutcheon, M., and Dixon, H. M., *Arch. Path.*, 1936, **21**, 749.

[5] Mudd, S., McCutcheon, M., and Lucke, B., *Physiol. Rev.*, 1934, **14**, 210.

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The preparation from non-flagellated organisms of a soluble antigen detectable by such surface-reactions as agglutination or phagocytosis is therefore of cardinal importance in relation to antibacterial immunity. With this as a guiding principle we have tested the commercial preparation of Krueger's Ball-mill "Endoantigen" of *H. pertussis*[1] but have found it, as purchased, not to contain detectable quantities of agglutinin-absorbing components. In view of this and the apparent lack of value of Endoantigen in the prophylaxis of whooping cough[2, 3] we have considered it worthwhile to apply the sonic method of extraction[4] to suspensions of *H. pertussis* in Phase I with the hope of obtaining a soluble agglutinogen. Other available preparations of soluble antigens of *H. pertussis* have not been so extensively studied as Endoantigen and this report includes results obtained with others currently used in clinical practice.

Phase I organisms freshly regenerated from Cryochem-dried form[5] were grown in Roux flasks on Bordet-Gengou medium containing 20% of fresh defibrinated horse-blood. Not more than 10 culture-generations were used following the opening of a container of dried organisms and the final generation was never more than a month removed from the dry form. The organisms were harvested by scraping the medium after 72 hours of incubation at 37°C and were suspended in saline. Less blood was removed with the organisms by scraping than by washing. Such suspensions in concentration of approximately 1000 billion organisms per milliliter were subjected to sonic disintegration for one hour in apparatus similar to that previously described.[6] The suspensions were then centrifuged at high speed and the opalescent supernate was diluted with sterile distilled water to 10 times its volume. The diluted extract was passed through a Seitz bacterial filter by suction and the clear filtrate was then concentrated. This was done by drying from the frozen state in the Cryochem apparatus[7] and restoring with sterile distilled water to the volume of the original supernatant solution. Small amounts of material not readily soluble were removed by centrifugation.

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1 Kreuger, A. P., *J. Infect. Dis.*, 1933, **53**, 237.

2 Roundtable Conference on Vaccine Prophylaxis of Whooping Cough, *J. Ped.*, 1938, **13**, 277-300.

3 Singer-Brooks, Charlotte H., *J. Pediat.*, 1939, **14**, 25.

4 Chambers, L. A., and Flosdorf, E. W., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 631.

5 Flosdorf, E. W., and Kimball, A. C., to be published.

6 Chambers, L. A., and Flosdorf, E. W., *loc. cit.*

7 Flosdorf, Earl W., and Mudd, S., *J. Immunol.*, 1938, **34**, 469.

Antiserum against Phase I organisms of a titer of 1:25,000 was then absorbed with this reconstituted filtrate; .05 ml of undiluted serum was incubated for 30 minutes at 37° with 0.45 ml of the filtrate and then for 4 hours at 4 to 8°C. The precipitate was removed by centrifugation and 0.05 ml of the supernate was incubated 30 minutes at 37° with another 0.45 ml portion of the sonic filtrate and overnight in the refrigerator. The precipitate was removed and 0.3 ml of the supernate was incubated 30 minutes with 0.9 ml of the filtrate and overnight in the refrigerator. At this point there remained only a slight trace of precipitate which was removed. The supernate accordingly represents a serum-dilution of 1:400.

The absorbed serum was tested by agglutination in serial dilutions from 1:400 to 1:25,600 in steps of 1:2; 0.5 ml of each dilution was incubated 30 minutes at 37° and overnight in the refrigerator with 0.5 ml of a two billion per ml suspension of living Phase I organisms. In each case normal serum was exposed to the same absorbing antigen under identical conditions in order to detect possible non-specific agglutination. Also the immune serum was treated identically using saline in place of the absorbing antigen. The organisms used for production of the immune rabbit serum were grown on medium containing horse blood. Therefore, in some cases the test-antigen was grown on medium containing sheep blood in order to evaluate the remote possibility that horse-blood constituents in the sonic filtrate could cause absorption.

The serum absorbed with sonic filtrate showed no agglutinating power even at 1:400 (1:800 final dilution) when tested with the homologous Phase I organisms as antigen. (Table I). However, 6 absorptions using undiluted serum absorbed with equal volumes of sonic filtrate, rather than the lower ratio of serum to absorbent as described above, resulted in only partial absorption of agglutinins, which indicates that the antibody-content of such high-titer serum cannot be removed to completion unless the serum is diluted. The nitrogen-content of the sonic filtrate as used in the absorption was only 0.7 to 1.6 mg per ml. This low concentration of the sonic filtrate suggests why the technic of diluting the serum as described was necessary. The control experiments described above in every case validate the specificity of absorption by the sonic filtrate.

Using even the higher ratio of antigen-volume to serum-volume, saline washings of the whole living organisms, Kreuger's endo-antigen, a commercial preparation known at Topagen† and another

† Samples of "Topagen" with and without a mercurial preservative were kindly furnished by Dr. John Reichel and Mr. C. Roos of Sharp and Dohme.

TABLE I.
Typical Agglutinin-titrations.*

Absorbing antigen	Mg nitrogen per ml	Precipitation during absorption			Final antiserum-dilutions						
		First	Second	Third	1/800	1/1600	1/3200	1/6400	1/12,800	1/25,600	1/51,200
Unabsorbed	—	—	—	—	4	4	3	2	2	1	1
Sonic filtrate*	0.70	2	1	tr	0	0	0	0	0	0	0
Saline washings of whole living organisms	—	tr	tr	0	4	4	4	3	tr	tr	tr
Endo-antigen	0.11	tr	tr	tr	4	4	3	2	1	1	1
Topagen	0.54	1	tr	tr	4	4	3	3	2	2	tr
"Detoxified" antigen	3.14	tr	tr	tr	4	4	4	3	2	2	1

*Similar results have been obtained in each of the 12 experiments in which absorption was carried out with sonic filtrate using the lower ratio of serum to absorbent as described. Five different batches of sonic filtrate were used in the course of these experiments. In the case of the other absorbents, the experiments were carried out at least 3 times and except in the case of "detoxified" antigen at least 2 different preparations or containers were used.

known as Detoxified Pertussis antigen‡ effected no detectable removal of agglutinins (Table I). This finding with respect to the last 2 preparations cannot be interpreted *necessarily* in terms of their possible effectiveness. The mechanism of action postulated for the reported effectiveness of Topagen[8] is not immunization against the antigens at the surface of the organism. Likewise the last preparation is being offered as a possible control of whooping cough through a toxin of *H. pertussis.*

The results of precipitin-testing have not been included. This method of assay, like complement fixation, does not distinguish components of the surface of the cell from other cellular or metabolic and medium-components.

In view of the fact that intense sonic treatment produces chemical changes in certain systems and not in others[9-12] the sonic filtrates were exposed to further sonic treatment for an hour. No detectable loss in agglutinin-absorbing capacity was observed.

The agglutinin-absorbing material present in the sonic filtrate may be either a whole antigen or a hapten. Further work is in progress to determine this by experiments with animals and to isolate and identify the component chemically. The authors wish to express their appreciation to Dr. Stuart Mudd for his helpful suggestions during the course of this work.

Summary. Filtered extracts of *H. pertussis* obtained by sonic disintegration of Phase I organisms have been found to absorb to completion the agglutinins from homologous rabbit antiserum of high titer. The soluble preparations now available and known as Endoantigen, Topagen and Detoxified Pertussis antigen were found to be devoid of such activity within the limits of sensitivity of the method used.

‡ Manufactured by Lederle Laboratories, Pearl River, New York. (Purchased on the market.)

[8] Gold, H., *J. Ped.*, 1937, **10**, 641.

[9] Flosdorf, E. W., and Chambers, L. A., *J. Am. Chem. Soc.*, 1933, **55**, 3051, 1934, **56**, 2795.

[10] Flosdorf, E. W., Chambers, L. A., and Malisoff, *J. Am. Chem. Soc.*, 1936, **58**, 1069.

[11] Chambers, L. A., and Flosdorf, E. W., *J. Biol. Chem.*, 1936, **114**, 75.

[12] Flosdorf, E. W., and Chambers, L. A., *J. Immunol.*, 1935, **28**, 297.