Adsorption of Protective Antigen of Hemophilus Pertussis on Human Red Cell Stromata.* (18311)

LOUIS PILLEMER.

From the Institute of Pathology, Western Reserve University, Cleveland, O.

Experiments directed toward the isolation of the protective antigen of *H. pertussis* have shown that this factor can be adsorbed quantitatively, selectively and without loss of potency on human red cell stromata.

H. pertussis (phase I) are grown in a modified Cohen and Wheeler fluid medium(1). The harvested bacteria are disintegrated in a 9 KC Raytheon sonic oscillator at 1° for periods of 15 minutes to 2 hours and the debris removed by centrifugation. The resulting extracts are added to appropriate amounts of red cells, of stromata, or of autoclaved stromata, incubated at 37°, centrifuged at 0° and the supernate discarded. The red cells are lysed with 50 volumes of water and the stromata removed by centrifugation. Details of the methods will be published at a later date.

The stromata protect mice in the intracerebral challenge test(2). Typical experiments are summarized in Table I which show that the protective doses (PD_{50}) of the stromata are equal to those of the bacterial suspensions from which they were prepared, while untreated stromata have no protective properties. Stromata do not adsorb pertussal toxin. They remove only a slight amount of nitrogen from the bacterial extract and cause little or no discernible change in the electrophoretic pattern of the extract. The removal of the protective antigen is therefore a complete and highly selective process.

It has been suggested that the protective potency of culture filtrates and bacterial vaccines of *H. pertussis* are related to their hemagglutinin content(3). Although the hemagglutinin of the supersonic extracts is also adsorbed on stromata, we believe that the hemagglutinin and protective antigen are not identical. No correlation between hemagglutinin titers and protective potency of either bacteria or extracts has been observed in this laboratory. A culture filtrate of 72 hours growth of *H. pertussis* contained as much hemagglutinin as the supersonic extracts. The hemagglutinin was completely adsorbed on stromata but gave no protection in the intracerebral challenge test. Supersonic extracts heated to 56° for 1 hour retain their hemagglutinating and protective power. However, while the hemagglutinin is still adsorbed completely on stromata, such stromata offers little or no protection. Furthermore, certain strains of *H. pertussis* have no hemagglu-

TABLE I. A Comparison of the Protective Doses of Bacterial Vaccines and Stromata-Protective Antigen Complexes Prepared from *H. pertussis* (Phase I).

	Immunizing dose of material in billions of bacteria or their equivalent*			
	2.0	0.5	0.125	Protective
Sample	S/T	S/T	S/T	$dose_{50}$†
Bacterial vaccine No. 138	10/10	9/10	7/10	<.125
Adsorbed stroma No. 138	9/10	8/10	7/10	<.125
Bacterial vaccine No. 134	9/10	7/10	2/10	.310
Adsorbed stroma No. 134	8/10	7/10	2/10	.342
Stroma	0/10	0/10	0/10	—

S = No. of survivals.
T = No. of animals challenged.
National Institutes of Health Reference Vaccine #4 had a PD_{50} of 0.332 when tested with #138 and 0.449 when tested with #134.
* Samples were diluted with 0.9% sodium chloride to contain the immunizing dose in 0.5 ml. Suspensions of adsorbed stroma were adjusted to be equivalent to the bacterial vaccines.
† Calculated by the method of Reed and Muench.

* Aided by grants from Lederle Laboratories Division, American Cyanamid Co.

1. Cohen, S. M., and Wheeler, M. W., *Am. J. Public Health*, 1946, v36, 371.

2. Kendrick, P. L., Eldering, G., Dixon, M. K., and Misner, J., *Am. J. Pub Health*, 1947, v37, 803.

3. Keogh, E. V., and North, E. A., *Australian J. Exp. Biol. Med. Sc.*, 1948, v26, 315.

tinin content and are excellent sources of protective antigen. These observations strongly suggest that the hemagglutinating and protective activities of the extracts reside in distinct chemical structures. Attempts are now being made to elute the protective antigen from stromata. It is hoped that such experiments may help to clarify this problem.

The stromata-antigen complex is a highly potent protective agent as judged by the Kendrick test(2); it is free of pertussal toxin and represents only a very minute portion of the supersonic extracts. The complex may therefore be an improved prophylactic agent against whooping cough. Since group O-Rh negative red cell stromata may be used for the adsorption of the protective antigen, this carrier should offer no clinical disadvantage, but may serve as an important stabilizing adjuvant. The clinical efficacy of the stromata-antigen complex will need to be proven by extensive field trials which are now being planned.

Summary. The protective antigen of *Hemophilus pertussis* has been adsorbed quantitatively, very selectively, and without loss of potency on human red cell stromata. The protective antigen and the hemagglutinin of *Hemophilus pertussis* are not identical. Clinical application of the stromata-protective antigen complex is discussed.

The author wishes to acknowledge the able technical assistance of Mrs. Livia Blum and Mrs. Eleanor Brown. Thanks are due to Dr. J. N. Adam, Jr., and Miss Frances L. Clapp for supplying some of the bacterial suspensions employed in this study and for carrying out the intracerebral challenge tests.

Received October 27, 1950. P.S.E.B.M., 1950, v75.

Porphyrins in the Bone Marrow and Circulating Erythrocytes in Experimental Anemias.* (18312)

RUDI SCHMID, SAMUEL SCHWARTZ, AND C. J. WATSON.
From the Department of Medicine, University of Minnesota Hospital, Minneapolis.

Recent reports(1-3) have described the occurrence of coproporphyrin as well as protoporphyrin in the circulating red blood cells, and have pointed to the correlation of the former compound with erythropoietic activity as revealed by reticulocyte percentage. These studies have induced us to examine the porphyrin content of the bone marrow in certain experimental anemias in rabbits, and to compare it with that of the circulating erythrocytes when examined simultaneously. Very little has been reported in the previous literature bearing on this matter. Vannotti(4) was able to extract considerable amounts of protoporphyrin from the bone marrow of rabbits with experimental hemorrhagic anemia. He also remarked upon the presence of coproporphyrin in the bone marrow of lead poisoned rabbits. He thought that this was derived from protoporphyrin, although there was no supporting evidence for this belief. DeLangen and Grotepass(5) observed small amounts of copro-and larger amounts of protoporphyrin in the bone marrow of a horse poisoned with lead. Stasney and McCord(6) found increased amounts of porphyrin (type not identified) in the bone marrow of cases of iron deficiency anemia and

* Aided by grants from the U. S. Public Health Service, Division of Research Grants and Fellowships (RG-345), and the Atomic Energy Commission, Contract Nos. AT (11-1)-100 and AT (11-1)-108.

1. Schwartz, S., Glickman, M., Hunter, R., and Wallace, J. Plutonium Project Report CH-3720, 1945 (AECD 2109).

2. Schwartz, S., Wikoff, H. M., to be published (*J. Biol. Chem.*)

3. Watson, C. J., a. *Trans. Assoc. Am. Phys.*, 1950, in press. b. *Arch. Int. Med.*, 1950, v86, 797.

4. Vanotti, A., and Siegrist, Th., Z. f. d. gesamte exp. Med., 1940, v108, 336.

5. DeLangen, C. D., and Grotepass, W., *Acta Med. Scand.*, 1938, v94, Fasc. III.