be explained. If complete hydrolysis of fats precede absorption it should be expected that fatty acids and glycerides should be absorbed equally well, or that free acid should have an advantage. Since it has been known since the time of Frank's work that ingested fatty acids appear in the lymph as glycerides one is tempted to conclude that it is this synthesis that delays their absorption but that triglycerides need undergo no change and are absorbed as such.

However, there is an alternative interpretation. Thus the important physical state of comparatively large quantities of free fatty acid in the lumen of the intestine may be pronouncedly different from that of fat plus a small amount of fatty acid slowly released by hydrolysis and constantly removed by absorption. The resynthesis of fat from fatty acid is an uncontested fact. The requisite glycerol for this resynthesis is on hand from a recently hydrolyzed fat, but must first be synthesized in or carried to the mucosa for synthesis from free fatty acid. This could conceivably cause a delay in absorption.

Summary. From spectrophotometric examination of the tissue lipids of rats at 10, 16, 24 and 48 hours after being fed free conjugated linoleic acid or its glyceride, it is concluded that glycerides are absorbed more rapidly, and possibly more completely, than free fatty acids but that the pathways of absorption are the same.

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Competitive Elution of Pertussis Hemagglutinin. (18010)

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Keogh, North and Warburton(1) have observed that erythrocytes of various mammalian and avian species are agglutinated by saline suspensions of *Hemophilus pertussis* and related members of this genus. Hemagglutination was also induced by filtrates or cell-free supernatants of liquid-medium cultures of these organisms. Other investigators (2,3) have subsequently demonstrated similar hemagglutination with other bacterial species. Conditions for the production of the hemagglutinating substance in casein-hydrolysate broth, and the relation of the hemagglutinating substance to the antigen or antigens which induce protective antibodies against *H. pertussis* infection in mice, have been described by Fisher(4) and by Keogh and North(5) respectively.

In the course of a study of the behavior and properties of the pertussis hemagglutinin we attempted to elute the hemagglutinin from erythrocytes by various methods. Hemagglutination was obtained with the supernatants of 7- to 9-day-old cultures of virulent strains‡ of *H. pertussis* grown in Cohen and Wheeler's medium(6). The potency of such preparations was determined by adding

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4. Fisher, S., *Australian J. Exp. Biol. and M. Sc.,* 1948, v26, 299.

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

‡ Strains No. 16945, 18323, 18530 and 18560, kindly furnished by Dr. Pearl L. Kendrick, Michigan Department of Health.

6. Cohen, S. M. and Wheeler, M. W., *Am. J. Pub. Health,* 1946, v36, 371.

0.5 ml aliquots of 0.5% suspensions of human red cells, to 0.5 ml volumes of serial 2-fold dilutions of *H. pertussis* supernatant, incubating at 37°C for 2 hours, and reading the agglutination pattern after the manner of Salk's technic for titration of influenza hemagglutinins(7).

Attempts to elute the hemagglutinating factor by varying the temperature of the mixture were unsuccessful. Agglutinated red cells were incubated at 37°C, 20-25°C or 4-6°C for as long as 3 days, and were then resuspended by agitation and allowed to settle. Agglutination was invariably re-established within 2 hours, with a pattern and a cohesiveness comparable to that originally observed.

In view of Lowell and Buckingham's report (8) that influenza virus could be eluted from erythrocytes by washing the cells in a 5% solution of glucose containing no added mineral salts, we attempted to elute pertussis-agglutinated erythrocytes in a similar manner. Following one washing of agglutinated cells with salt-free 5% glucose, the cells were resuspended in 0.9% NaCl solution; agglutination again readily reappeared.

It was observed (as Fisher(9) has independently noted) that the supernatant fluid from frozen and thawed red cells, resuspended in an equal volume of distilled water, was capable of blocking the agglutination of fresh red cells by *H. pertussis*. An experiment was therefore designed to determine the capacity of such a red-cell extract to elute the active substance from pertussis-agglutinated cells. Agglutinated cells were mixed with 5 volumes of a 1:5 dilution of red-cell extract in saline, shaken to resuspend the cells, and incubated at 37°C until the cells had settled. No hemagglutination appeared. Controls, consisting of agglutinated cells mixed with saline solution, showed normal reagglutination. The red-cell extract treated cells were then centrifuged, the supernatant discarded and the cells washed twice with normal saline solution. On adding fresh *H. pertussis* supernatant to the cells, hemagglutination reappeared after storage overnight at 4-6°C. To another aliquot of these cells, normal saline solution was added instead of *H. pertussis* supernatant; no hemagglutination appeared after storage in the cold.

Discussion. The failure of erythrocytes agglutinated by *H. pertussis* factor to resuspend, either after exposure to temperatures of 37°C or higher, or in the presence of lowered salt concentrations, is one of several features which distinguish this type of hemagglutination from that induced by the influenza-mumps-Newcastle disease group of viruses. Another distinction is found in the capacity of cell-free supernatants of *H. pertussis* suspensions to induce hemagglutination, in contrast with the influenza-mumps-NDV group. In both respects the reactions of the *H. pertussis* factor with susceptible erythrocytes resemble those exhibited by viruses of the pox group(10,11). Fisher(9) has also noted that *Vibrio cholerae* mucinase, which inactivates the cell receptor for influenza virus, does not affect pertussis hemagglutination.

However, hemagglutination by virus particles is not invariably followed by elution. It has been fairly conclusively demonstrated that, for influenza virus in particular, the power to elute runs closely parallel to the power to infect and to multiply; thus, influenza-virus particles, gently inactivated by heat, will adsorb to red cells but will not elute under the usual circumstances(12). On the other hand, influenza-virus particles, rendered non-infectious by brief ultraviolet irradiation, do not lose their capacity to elute unless the capacity to hemagglutinate has also been destroyed(13). The mechanism of attachment of the virus particle to the red cell appears therefore to be distinguishable from, although closely related to, the pre-

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12. Hirst, G. K., *J. Exp. Med.*, 1948, v87, 315.

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sumably enzymatic property of the virus that leads in time to destruction of the receptor, and to elution. Thus the dynamics of hemagglutination by the *H. pertussis* factor, and by mildly inactivated influenza-type virus particles, may differ less markedly than would at first appear to be the case.

The observation that the bond between *H. pertussis* factor and red cells can apparently be dissociated in the competitive presence of red-cell extract, and that the cells thus released can again be agglutinated by addition of fresh *H. pertussis* factor, indicates that attachment of this factor to the red cell does not result in appreciable destruction of the receptor. The dissociation and reassociation reported here bears a closer resemblance to a familiar type of interaction which has been observed between many cellular antigens and their antibodies. Morgenroth, for example(14), noted that if sheep cells were saturated with hemolysin in the absence of complement, and then mixed and incubated with fresh cells for a certain minimum period, the addition of complement would then result in hemolysis of all the cells in the mixture. The influence of time, of amount of antibody added, and of the interaction of 2 red-cell species with an antibody active against both, have been studied in detail by Philosophow(15); and Mayer *et al.* (16) have recently confirmed and extended these observations. Another recent observation, parallel in principle, is that when Rh-positive red cells (presumably saturated with the corresponding maternally produced antibody) from an erythroblastotic infant are mixed with homologous Rh-positive adult blood, the cells of both infant and adult may hemolyze (17). Thus hemagglutination of the type induced by *H. pertussis* factor bears a limited resemblance not only to conventional virus hemagglutination, but also to conventional antigen-antibody interaction.

Summary. The hemagglutinating factor of *Hemophilus pertussis* culture supernatants can apparently be eluted from human erythrocytes by suspending such erythrocytes in a distilled-water extract of frozen and thawed red cells. Following the removal of the *H. pertussis* factor from agglutinated cells, these cells can be re-agglutinated by addition of fresh *H. pertussis* culture supernatant. The factor cannot be eluted by incubation of agglutinated cells at various temperatures, or by suspending the cells in salt-free isotonic glucose solution.

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Effect of 3-Hydroxy-2-phenylcinchoninic Acid on Renal Secretion of Phenyl Red and Penicillin.* (18011)

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Certain derivatives of cinchoninic acid have been found to be effective antidiuretics when administered to dogs with induced water diuresis(1,2), to cause an increased rate of excretion of uric acid when administered to man(3,4,5), to stimulate the anterior pituitary causing a decrease in the ascorbic acid content of the adrenal glands of rats(6), and to suppress the secretion of phenol red by the kidney due to blocking the tubular secretory mechanism(7,8). The present communication is concerned with this last pharmacological action, the blocking of renal tubular

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