communication indicating a sudden potency loss of thromboplastic suspensions due to bacterial contamination can now be attributed to the elaboration of the enzyme thromboplastinase by a specific micro-organism. So far as we know, thromboplastinase is the only enzyme capable of destroying tissue thromboplastins of diverse sources, prepared in several different ways. The mode of action of this enzyme is apparently highly specific, involving a split of organic phosphorus seemingly correlated with the loss of thromboplastic potency. A conclusion from such evidence that tissue thromboplastin of different organs or of different species are the same is no doubt premature. It would appear, however, that they do contain a common structural unit, necessary for thromboplastic activity which is susceptible to thromboplastinase cleavage. Analysis of 3 different thromboplastic preparations indicates that this common moiety contains phosphorus. There seems, moreover, to be a quantitative relationship between thromboplastin destroyed and phosphorus liberated as a result of thromboplastinase action. This suggests the possibility of calibrating different thromboplastic preparations in terms of this functional phosphorus rather than in terms of non-specific nitrogen content of the tissue preparation. Further work reported elsewhere(14) indicates that the site of thromboplastinase action is on that portion of thromboplastic lipid which is associated with the inositol phosphatide fraction.

Summary. Thromboplastinase, a new enzyme of bacterial origin has been reported and its preparation and mode of action described. To our knowledge, this is the only enzyme which is apparently specific for thromboplastin. The split of a phosphorus moiety, apparently necessary for thromboplastic activity has been correlated with enzyme action. The theoretical and practical implications of these observations are discussed.

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Reaction of Certain Phytoagglutinins with Flexner-Jobling Carcinoma Cells of the Rat.* (20516)

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The complexity of the surface of all mammalian tissue cells is suggested by our knowledge of the various antigens that make up the surface of human erythrocytes. It is possible that the surfaces of cells vary not only with their histological classification, but also with their state of health. It is not illogical to postulate that the surface of epithelial cells of a carcinoma could be differentiated from the cell surfaces of related normal cells if proper

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means for making such measurements were available.

Several years ago the senior author and his colleagues(1) made a limited comparative study of the cell surfaces of guinea pig leukocytes and erythrocytes as measured by several different criteria, namely the reaction of these cells with 1) specific antiserums, 2) influenza virus, 3) pneumococcus polysaccharides, and 4) a phytoagglutinin. The results suggested that these or other biological substances might be selectively adsorbed to various mammalian cells. There is evidence that tumor antiserum and some viruses react with the surfaces of certain tumor cells. At least such substances will interfere with growth of such cells *in vivo*.

The work here reported comprises one portion of an investigation concerned with the determination of cell surface differences which may exist between cells obtained from normal and malignant tissues of the rat.

Phytoagglutinins have long been known to agglutinate various types of cells(2). More recently Boyd(3,4) has investigated numerous types of plant extracts for their hemagglutinating ability, looking especially for those plant substances which were able to specifically agglutinate cells of the different human blood groups. In the present study 5 different phytoagglutinins were used by the authors in a comparison of their action on rat leukocytes, erythrocytes, and tumor cells.

Materials and methods. The Flexner-Jobling carcinoma was used as a source of tumor cells. The tumor was maintained in white stock rats by the periodical subcutaneous inoculation of 0.5 ml of a 5% suspension of tumor cells. 12 to 15 day old subcutaneous tumors were aseptically excised, homogenized in a solution described by Krebs(5), filtered through 20XX mesh nylon, washed, and made up to the desired suspension on the basis of volume of packed cells.

White blood cells were obtained by the inoculation of 100 ml of Locke's solution containing 0.1% glucose into stock rats, and harvesting the peritoneal exudate approximately 12 hours later. The cells were spun down, washed, and resuspended to the desired concentration on the basis of volume of packed cells. Rat and chicken red blood cells were collected in citrate solution, washed, packed, and also resuspended to the desired concentration in saline (0.85% NaCl).

Four different phytoagglutinins were obtained from Dr. W. C. Boyd, of Boston, Massachusetts. These 4 lyophilized samples were reconstituted in saline solution so as to contain 10 mg per ml. A fifth phytoagglutinin was made from white navy beans after the method of Li and Osgood(6). The action of these phytoagglutinins against human red cells is as follows:

Phytoagglutinin No.	Source	Type of human erythrocyte agglutination
1.	*Phaseolus limensis* Macfadyen var. "Westside Lima"	A
2.	*Phaseolus vulgaris* Linnaeus var. "Golden Cluster Wax"	A, B, O
3.	*Phaseolus limensis* Macfadyen var. "Finast Lima"	A
4.	*Phaseolus vulgaris* Linnaeus var. "Rustproof Golden Wax"	A, B, O
5.	*Phaseolus vulgaris* Linnaeus var. "White Navy Bean"	A, B, O

Results. The agglutinating titers of the phytoagglutinins for the cells under consideration was determined using doubling serial dilutions of the stock solutions of the plant extracts made in saline. To each dilution an equal volume of 1% cells was added. The agglutination in each case was read after standing at room temperature for 2 hours. Chicken red blood cells were also tested for their agglutinability with these phytoagglutinins. The results of these tests are given in Table I.

From this table it is to be noted that phytoagglutinin numbers 2, 4, and 5 actively agglutinate rat erythrocytes, while only no. 5 agglutinates white blood cells, and no agglutination was observed with tumor cells. It was of further interest to determine whether or not the phytoagglutinins were removed from solution by the red cells that had been agglutinated, and also by the tumor cells which were not clumped by this material.

To determine this, the supernatant fluids of the tubes representing the end points in the agglutination reactions were assayed for the presence of remaining agglutinin. The supernatant from each series tested was di-

TABLE I. Agglutination Titers Obtained with Phytoagglutinins.

Phyto No.	Rat RBC	Rat WBC	Chicken RBC	Tumor cells
1	0	0	0	0
2	1:40	0	1:640	0
3	0	0	0	0
4	1:160	0	1:640	0
5	1:200	1:200	1:640	0

* 0 = no agglutination.

TABLE II. Tumor Growth Observed from Tumor Cells Treated with Unabsorbed Phytoagglutinins.

Phyto No.	10 days	15 days	20 days	25 days
1	4/10*	8/10	8/10	8/10
2	9/10	10/10	10/10	10/10
3	3/10	7/10	9/10	9/10
4	10/10	10/10	10/10	10/10
5	0/10	0/10	0/10	0/10
Control	10/10	10/10	10/10	10/10

* No. of tumor takes/No. of sites inoculated.

TABLE III. Tumor Growth Observed from Tumor Cells Treated with Adsorbed Phytoagglutinin No. 5.

Phyto No. 5	10 days	15 days	20 days	25 days
CRBC adsorbed	5/10*	6/10	6/10	6/10
Rat RBC adsorbed	5/10	10/10	9/10	9/10
Unadsorbed	1/10	1/10	1/10	1/10
Control	2/10	6/10	9/10	9/10

* No. of tumor takes/No. of sites inoculated.

vided into two portions, one to be titrated against rat erythrocytes and the other against chicken red blood cells. No agglutination of rat erythrocytes was obtained with the supernatant fluid following agglutination of rat erythrocytes by phytoagglutinins nos. 2, 4, or 5. However, these supernates did agglutinate chicken erythrocytes in a dilution of 1:640.

Using the supernates obtained from the first 2 dilutions in the tests with phytoagglutinins nos. 2, 4, and 5 on tumor cells, new agglutination tests with rat and chicken erythrocytes were also done. The rat erythrocytes were not agglutinated, indicating adsorption of phytoagglutinins 2, 4, and 5 by the rat carcinoma cells. Chicken erythrocytes were again agglutinated in a dilution of 1:640.

The results indicate that while agglutination occurs with rat erythrocytes and not with tumor cells, there is still evidence for the adsorption of the red blood cell agglutinin by both the red blood cells and tumor cells. On the other hand, the substance responsible for chicken red blood cell agglutination is not adsorbed by either rat erythrocytes or tumor cells.

Since the tumor cells obtained from the rat adsorbed phytoagglutinins 2, 4, and 5, it was desirable to ascertain whether this adsorption had any effect on the viability of the tumor cells. A known suspension of tumor cells was mixed with an equal volume of the stock solution of phytoagglutinin and placed on a shaker at room temperature for 2 hours, subsequent to which rats were inoculated subcutaneously with each mixture. Results as indicated by tumor growth are given in Table II. Phytoagglutinin no. 5 appears to have a marked inhibitory effect on tumor growth.

Phytoagglutinin no. 5 was adsorbed with chicken and rat erythrocytes in order to determine the inhibitory action of the adsorbed phytoagglutinin against tumor growth. A known tumor cell suspension was mixed with rat red blood cell adsorbed, chicken red blood cell adsorbed, and unadsorbed no. 5 phytoagglutinin, incubated at room temperature for 2 hours, and inoculated subcutaneously into rats. Results are given in Table III.

It appears that the rat red blood cell agglutinin is associated with the tumor inhibiting fraction, since after removal of the rat red blood cell agglutinin by adsorption, a greater incidence of tumor takes is noted.

Since the *in vitro* protective action of no. 5 agglutinin gave such significant results against tumor growth in rats, it was also of interest to determine the *in vivo* effect. Immediately after the inoculation of subcutaneous and intraperitoneal tumors, a defined amount of phytoagglutinin was injected intraperitoneally every day for a period of 10 days. In another group of rats, the same type of treatment was begun 5 days after tumor inoculation. In neither group did the treatment with phytoagglutinin *in vivo* have an inhibitory effect on tumor growth.

Summary. A phytoagglutinin obtained from the white navy bean, when added to a suspension of Flexner-Jobling rat carcinoma cells, destroyed the ability of these cells to produce tumors when inoculated into rats. The phytoagglutinin had no apparent effect *in vivo*,

when administered daily to rats beginning at the time of the tumor inoculation.

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Development of Antibodies in Children Convalescent from Whooping Cough.* (20517)

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The nature of the immunity which develops as a result of infection with *H. pertussis* has never been clearly defined. Many investigators believe that resistance to this disease is determined by the level of circulating antibacterial agglutinins. Evidence that this type of antibody frequently fails to appear as a result of the disease is, however, present in the literature. Other antibody responses which have been studied are complement fixation, opsonocytophagic reaction, and mouse protection. Recently Keogh and North(1) studied antihemagglutination and reported that in experimental murine pertussis the protective power of serum, given to mice intraperitoneally, was a function of the antihemagglutinin content of the serum—a conclusion not supported by the work to be reported here.

The present study is concerned with a comparison of 3 types of serum antibody in patients convalescent from pertussis, namely: 1) antibacterial agglutinin, 2) antihemagglutinin, and 3) mouse protective antibody (the capacity of serum to protect mice against intranasal infection with *H. pertussis*). It was the purpose of this study to ascertain which of these antibodies appeared most consistently with clinical recovery; hence which was the most significant measure of acquired immunity.

Methods and materials. Sera were obtained on patients ill with whooping cough from whom *H. pertussis* was isolated on nasopharyngeal culture.† The patients varied in age from 3 months to 9 years; their illnesses were characterized as moderately severe or severe. Blood was drawn immediately on admission and at 2- to 3-week intervals during convalescence. The sera were stored in the dry ice chest; all the specimens collected on each individual patient were tested together.

A. Antibacterial agglutinins. H. pertussis strain No. 18323 (obtained from Dr. Pearl Kendrick, Michigan Department of Health) and strain No. 484 (obtained from Dr. Anne Kimball, Minnesota Department of Health) were employed to prepare the standard antigen. The 24-hour growth from Bordet-Gengou Media was suspended in saline to a concentration of 20 billion organisms/ml, 0.3% neutral formaldehyde was added, and the bacterial suspension stored in the refrigerator. The antigen was used in a final concentration of 5 billion organisms/ml and was added to serial 2-fold dilutions in saline of the unknown serum. The tubes were shaken, and after 1-hour incubation at 37°C, the test was

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