11. Shaffer, J. G., and Sienkiewicz, H. S., *Science*, 1952, v116, 306.

12. Shaffer, J. G., *Ann. N. Y. Acad. Sci.*, 1953, v56, Art. 5, 1033.

13. Baernstein, H. D., Rees, C. W., Bartgis, I. L., *Jour. Parasitology*, 1957, v43, 143.

Received August 26, 1957. P.S.E.B.M., 1957, v96.

Detection of Incomplete Enterobacterial Antibodies in Cord Blood by Means of Antiglobulin Hemagglutination Test.* (23516)

ERWIN NETER

Statler Research Laboratories and Department of Pediatrics, Children's Hospital and Departments of Pediatrics and Bacteriology, University of Buffalo School of Medicine, Buffalo, N. Y.

The passive transfer of antibodies through the placenta from the mother to the fetus depends upon two main factors, namely, the structure and function of the placenta and the characteristics of the antibody. In some animal species, notably rodents, apes, and man, certain antibodies readily pass the placental barrier. In other species, such as the pig and ruminants, placental transmission does not take place, but antibodies are transferred to the offspring through colostrum. The role of the antibody molecule in the transmission through the placenta is illustrated by the fact that, for as yet poorly understood reasons, incomplete Rh antibodies pass the human placenta far more readily than complete antibodies of identical serologic specificity. Recently, it was shown by means of the indirect bacterial hemagglutination test, which is considerably more sensitive than the conventional bacterial agglutination method, that antibodies against the somatic (O) antigens of enteropathogenic *Escherichia coli* are not quantitatively transferred from mother to newborn infant. In a series of 26 cord blood specimens antibodies could be demonstrated in only 3, although the maternal serum of all contained such antibodies(1). Similarly, Stulberg and associates(2) found 77% of 45 cord serum specimens lacking in such antibodies. Further, when antibodies were present in cord blood, their titer was substantially lower than that of the corresponding maternal antibodies. The possibility was considered that incomplete antibodies to enterobacteriaceae may pass the human placenta, provided that the mother has such antibodies. Weil and Felsen(3) demonstrated incomplete *Shigella flexneri* agglutinins in approximately half of 30 human sera by combining the conventional bacterial agglutination method with the antiglobulin technic. To determine whether the hypothesis just mentioned is correct, maternal and cord serum specimens were examined by means of a hemagglutination-antiglobulin test for complete and incomplete enterobacterial antibodies.

Material and methods. Fifty paired maternal and cord blood specimens from healthy individuals were obtained through the cooperation of the Obstetrical Department of the Children's Hospital. The serum specimens were stored in the deep freeze until used. Data on blood groups and Rh antibodies were available. The hemagglutination test was carried out as described previously(4,5). As O antigens supernates of the following agar-grown cultures were employed: *E. coli*, serogroups 111 and 55, *Shigella flexneri* (2 freshly isolated and serologically different types), *S. sonnei*, *Salmonella paratyphi* B (group B), *S. montevideo* (group C_1), *S. muenchen-oregon* (group C_2), and *S. enteritidis* (group D). These supernates (refrigerated centrifuge) were prepared from heated (1 hour 100°C) suspensions and were employed in a dilution of 1:10. Erythrocytes obtained from blood donors (blood group O, Rh negative) were washed 3 times, treated with the antigens in a waterbath at 37°C for 30 minutes, and again washed three times. Serial two-fold dilutions of the sera (0.2 ml) were mixed with the modified red blood cell suspensions (0.2 ml). Non-

* Study aided by research grant from Natl. Inst. of Allergy, U. S. Public Health Service.

TABLE I. Enterobacterial Antibodies in 50 Cord Sera.

Method of antibody demonstration	% of positive sera								
	Escherichia coli		*Shigella*			*Salmonella* group			
			flexneri		*sonnei*				
	111	55	a	b		B	C_1	C_2	D
Bacterial agglutination	0	0	0	0	0	0	0	0	0
Hemagglutination	18	6	14	78	6	12	52	20	10
Antiglobulin agglutination	30	28	24	14	22	30	18	46	30
Idem & hemagglutination	48	34	38	92	28	42	70	66	40

modified red blood cells were employed as controls to eliminate erroneous results due to blood group antibodies. After incubation for 30 minutes at 37°C in a waterbath the mixtures were centrifuged at 1000 rpm for 1 minute. The resulting hemagglutination was noted. After three washings antiglobulin (Coombs) serum (0.05 ml), procured on the open market, was then added to all tubes showing no hemagglutination. The tubes were centrifuged and the resulting hemagglutination, indicative of incomplete antibodies, was recorded.

Bacterial agglutination tests were carried out with the identical serum dilutions (0.5 ml) used in hemagglutination tests. Heated suspensions (0.5 ml) of the same cultures were used. The mixtures were incubated in a waterbath at 50°C for 18 hours, and the resulting bacterial agglutination was read after centrifugation for 5 minutes at 1500 rpm (6). As diluent phosphate hemagglutination buffer (Difco) was used in all tests.

Results. Fifty cord serum specimens from healthy infants were examined for O antibodies to 9 representatives of enterobacteriaceae by means of the conventional agglutination, enterobacterial hemagglutination, and antiglobulin hemagglutination methods. Sera were considered positive if antibodies were demonstrated in a dilution of 1:5 or higher. The antiglobulin hemagglutination test was regarded as positive only if the hemagglutination test itself was negative. The results are summarized in Table I.

It is seen from this Table that (1) none of the sera contained agglutinins, (2) from 6 to 78% yielded positive results in the hemagglutination test, and (3) an additional 14 to 46% were positive for incomplete antibodies in the antiglobulin hemagglutination test. By combining the hemagglutination and antiglobulin hemagglutination tests a total of 28 to 92% of the serum specimens yielded positive results. It is interesting to note that the percentage of positive sera differs markedly with different antigens, either because of actual differences in antibody content or because of differences in sensitivity of the antigens used. Particularly striking is the high percentage of sera containing antibodies to one of the *S. flexneri* strains.

Regarding the presence of incomplete enterobacterial antibodies in maternal and cord serum specimens the following observations were made. The antiglobulin serum raised the antibody titer of maternal sera only 2-fold in 17 instances and caused no increase in the remaining 33. In contrast, the same antiglobulin serum caused four-fold and greater increases in 22% and 8-fold and greater increases in 10% of 50 cord sera. In several serum specimens the titer was increased from 32 to 256 times. If one accepts a four-fold or greater enhancement in the antibody titer by the antiglobulin serum as evidence for incomplete enterobacterial antibodies, then, it may be concluded that such antibodies are present in certain cord sera and cannot be demonstrated by the same technic in the corresponding maternal sera. No difference was noted between cord sera with and without Rh antibodies.

A comparison of the maternal and cord serum titers obtained with both hemagglutination and antiglobulin hemagglutination tests revealed that with one single exception the maternal titers were higher than those of the cord sera. In the majority of instances the ratio was between 4:1 and 16:1.

In order to determine the specificity of the antiglobulin hemagglutination method, absorption experiments were carried out. Cord serum containing both *S. sonnei* and *S. flexneri* antibodies, demonstrable only by means of

antiglobulin serum, was absorbed with *S. sonnei* or *S. flexneri* antigens. Then, the absorbed and non-absorbed serum was titrated with both antigens in the antiglobulin hemagglutination test. It was found that absorption with *S. sonnei* or *S. flexneri* antigens had removed the homologous but not the heterologous antibodies. Identical results were obtained in experiments with other cord serum specimens. It is evident, therefore, that the antiglobulin hemagglutination method detects antibodies which become attached specifically to erythrocytes modified by the homologous enterobacterial antigen.

Discussion. The present study revealed that O antibodies to various enterobacteriaceae can be demonstrated in some human cord sera by means of the hemagglutination test and thus extends previous observations regarding hemagglutinins against enteropathogenic serotypes of *E. coli*. Since the conventional agglutination procedure yielded negative results, this observation confirms the greater sensitivity of the hemagglutination method. It is evident also that the sera differed markedly in their capacity to agglutinate red blood cells modified with different antigens. In the present series the high percentage of cord serum specimens with antibodies against one of the *S. flexneri* strains is particularly noteworthy. It remains to be determined whether these data reflect exclusively differences in antibody content or whether crude antigens prepared by the same method, as in the the present study, differ in sensitivity. In addition, the locality of the population may influence the antibody titers.

Incomplete enterobacterial antibodies are considered here to be present in cord sera if anti-human globulin serum enhances the serum titer obtained in the hemagglutination test at least 4-fold or produces a positive result when the latter test is negative. According to these criteria 22% of 50 cord sera contained incomplete enterobacterial antibodies against one or more antigens, but the corresponding maternal sera did not. These findings suggest the following hypothesis. Adult sera may contain both complete and incomplete enterobacterial antibodies, but the titer of the latter does not materially exceed that of the former; during pregnancy the incomplete antibodies pass the placental barrier more readily than do the complete antibodies. Additional studies are needed to determine whether the antiglobulin bacterial agglutination method of Weil and Felsen(3) measures the same antibodies as the antiglobulin hemagglutination test used in the present study. One must keep in mind that minor modifications of a serologic method may substantially enhance its sensitivity; for example, centrifugation renders the enterobacterial hemagglutination test considerably more sensitive. Therefore, the difference in titer between an agglutination procedure and the corresponding antiglobulin technic is not necessarily indicative of incomplete antibodies. Further, the possibility exists that the enterobacterial hemagglutination test does not solely measure bacterial agglutinins in a more sensitive manner; it actually may demonstrate more than one kind of antibody with identical serologic specificity. This possibility is suggested by the observation that no constant ratio exists between the titers determined by the two procedures. A similar suggestion was made by Ceppellini and De Gregorio and is based on comparative studies on *S. typhosa* antibody titrations by means of hemagglutination, hemolysis, hemagglutination in proteic media, and indirect Coombs test(7). Utilization of various serum fractions conceivably may aid in the elucidation of this moot question.

Summary. Examination of 50 paired maternal and cord sera for O antibodies against various enterobacteriaceae revealed the following results. 1. Agglutinins could not be demonstrated in the cord sera. 2. The hemagglutination test revealed the presence of antibodies in 6 to 78% of the cord sera depending upon the antigen used. 3. The antiglobulin hemagglutination test detected incomplete antibodies in an additional 14 to 46% of the cord sera. 4. The antiglobulin hemagglutination test enhanced the hemagglutinin titers of cord sera to a greater extent than those of maternal sera. 5. With one exception the maternal antibody titers were higher than those of the corresponding cord sera. 6. The specificity of the antiglobulin hemagglutination test was demonstrated by means of ab-

sorption experiments. 7. The placental transfer of enterobacterial antibodies from mother to fetus is discussed with particular reference to incomplete antibodies.

1. Neter, E., Westphal, O., Lüderitz, O., Gino, R. M., and Gorzynski, E. A., *Pediatrics,* 1955, v16, 801.

2. Stulberg, C. S., Zuelzer, W. W., and Page, R. H., *J. Immunol.,* 1956, v76, 281.

3. Weil, A. J., and Felsen, J., *ibid.,* 1955, v74, 488.

4. Neter, E., Gorzynski, E. A., Gino, R. M., Westphal, O., and Lüderitz, O., *Can. J. Microbiol.,* 1956, v2, 232.

5. Neter, E., Westphal, O., Lüderitz, O., and Gorzynski, E. A., *Ann. N. Y. Acad. Sci.,* 1956, v66, 141.

6. Braun, O. H., Seeliger, H., and Wagner, N., *Zschr. Kinderheilk.,* 1954, v75, 50.

7. Ceppellini, R., and De Gregorio, M., *Boll ist. sieroterap. milan.,* 1953, v32, 429.

Received August 29, 1957. P.S.E.B.M., 1957, v96.

Toxicological and Pathological Studies with 3,3-Dimethyl-1-Phenyltriazene: with Special Reference to Hepatic Damage.* (23517)

JEAN SCHOLLER, FREDERICK S. PHILIPS, AND STEPHEN S. STERNBERG
(With the assistance of Barbara B. Adams and Barbara Wheelock)
Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, Sloan-Kettering Graduate Division of Cornell University Medical College, and Pathology Laboratories, Memorial Center for Cancer and Allied Diseases, New York City

During routine screening of compounds for anti-tumor effects, 3, 3-dimethyl-1-phenyltriazene was found to inhibit Sarcoma 180 in mice (1). The agent was also shown to be teratogenic in chick embryos(2) and to prolong the survival of mice bearing Leukemias 82 and 5471(3). Since this triazene was representative of a new series of compounds not previously known to be active against experimental tumors, it became of interest for possible clinical trial. For this reason pharmacological studies were undertaken in our laboratories.

Procedure. The kinds of animals used and the procedures employed have recently been described(4,5). The triazene, a liquid, was dissolved in peanut oil and 0.5 ml of the solution per 100 g of body weight was given intraperitoneally to mice and rats. For oral administration to rodents the agent was suspended in 0.9% NaCl containing 0.5% carboxymethylcellulose (CMC). For intravenous injections the compound was dissolved in propylene glycol while for intramuscular injection it was diluted with peanut oil. Dogs received the agent orally in gelatin capsules. LD_{50} values were calculated according to Litchfield and Wilcoxon(6) after administration of a sufficient number of doses, decreasing in series by a factor of ½, to obtain both 100 and 0% mortality during 14 days of observation. At least 10 mice, 6 rats, 2 cats, and 2 dogs were employed per dose.

Results. Toxicity in rodents. Table I is a summary of toxicity data. The compound was somewhat more toxic to mice and rats when given intraperitoneally than when given orally. Within 2 hours after 500 mg/kg intraperitoneally or 1000 mg/kg orally the righting reflexes of mice became impaired; a progressing depression ensued and death occurred between 3 and 20 hours. Following single doses of 250 mg/kg intraperitoneally or 500 mg/kg orally mice became depressed, dyspneic, and ataxic within 3 to 4 hours. A few died within the first day; most of the remainder between the second and eleventh days. The majority of those alive at 24 hours moved about in circles and their heads bobbed continuously from side to side. This syndrome, presumably neurologic in origin, was further characterized by the inability of the mice, when placed in water, to swim in a well-coordinated manner. The disturbance seemed similar to that seen in congenitally abnormal

* This investigation was supported in part by a grant from Natl. Cancer Inst., U.S.P.H.S., and by funds from the Elsa U. Pardee Fn.