Lytic Property of Human Cord Blood Serum. (24799)

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During investigation on neonatal jaundice Mitchell(1) found that 5 out of 23 samples of cord blood sera possessed hemolytic activity in infants' red cells. Goldbloom and Gottlieb(2), however, did not confirm this finding. The following report presents results of a study of lytic properties of normal human cord sera.

Materials and methods. One hundred and one cord blood sera were examined. One hundred and twenty-six peripheral blood sera served as controls. The latter were obtained as follows: 20 from parturient women simultaneously with corresponding cord sera; 41 from women in 2nd and 3rd trimester of pregnancy and 65 from medical students. Sera were examined on day of collection or kept at -20°C until used. Cord blood was obtained either by venipuncture or immediately after cutting the cord with scissors. Human erythrocytes from adults of Group I Rh+ and O Rh- taken on day of examination were used. Care was taken to exclude Rh incompatibility. Normal and papainized erythrocytes were examined parallel throughout. Papain treated erythrocytes were prepared by adding 0.25 ml of washed erythrocytes and 0.5 ml of 1% papain solution to 4.25 ml of normal saline. After incubation at 37°C, 20-30 minutes, the cells were washed 3 times in saline and a 5% suspension prepared. *Estimation of degree of hemolysis.* 0.5 ml of cell suspension added to 0.5 ml of serum was incubated at 37°C for 10 hours and occasionally shaken. All glassware, reagents and blood samples were sterile and care was taken to avoid bacterial contamination during procedure. The supernata were removed by centrifugation and diluted with normal saline to 3.5 ml. Degree of hemolysis was determined in Klett photocolorimeter with a 56 filter. As control, representing 100% hemolysis, a saline suspension of red blood cells of same concentration was repeatedly frozen and thawed. The zero point for each serum tested was determined at same dilution but without added red blood cells. Only hemolysis greater than 20% was considered positive. In some cases the following additional procedures were performed and lytic activity of the serum reexamined. Eighty-three cord sera were heated to 56° for 30 minutes. Ten cord sera, which showed lytic activity at a titer of at least 1:10, were diluted to 1:4 and 1:8 with normal saline and heated to 80°C or 100°C for 30 minutes. 2 ml of cord serum were dialyzed against 1000 ml of physiological salt solution for 12-16 hours at 6°C. Twelve cord sera were used. *Osmotic fragility of erythrocytes.* Ten drops of normal packed erythrocytes were added to 2 ml of cord serum and incubated for 3 hours at 37°C with occasional shaking. The serum was then separated by centrifugation and one drop of erythrocytes added to each of 10 solutions of NaCl from 0.50% to 0.32%.

Results. Sixty-eight of 101 cord sera examined showed hemolytic activity of papainized erythrocytes (Table I). Normal erythrocytes were not affected. The 126 control sera lysed neither normal nor papainized red cells.

Heating of cord sera to 56°C for 30 minutes intensified the lytic effect. As shown in Table II, 17 negative samples became positive and previously positive samples showed higher degree of hemolysis after heating. It then seems possible that some heat labile factor protecting the papainized erythrocytes was

TABLE I. Lytic Effect of Cord Sera on Papain Treated Erythrocytes.

	No. of cord sera	Positive	No. of control sera	Positive
Papain treated erythrocytes	101	68	126	0
Normal erythrocytes	101	0	126	0

TABLE II. Influence of Heating to 56°C for 30 Min. on Lytic Effect of Cord Sera.

	No. of sera	21-40% hemolysis	41-70% hemolysis	Over 70% hemolysis	Total
Unheated	83	36 (43.37%)	13 (15.65%)	7 (8.43%)	56 (67.3%)
Heated	83	20 (24.09%)	33 (39.75%)	20 (24.09%)	73 (87.7%)

present in non-heated cord serum. Forty-five similarly treated blood sera from normal adults developed no hemolytic activity. Heating of cord sera to 80°C or 100°C for 30 minutes either diminished or abolished lytic activity. When some sera which gave negative results, were reexamined with different samples of erythrocytes, positive results were obtained in a few cases. The role which the erythrocytes may play in lytic process is under investigation.

After dialysis against physiological salt solution 10 out of 12 cord sera showed complete disappearance of lytic properties. This indicates that the responsible factor is a small molecule.

Normal (*i.e.*, non-papainized) erythrocytes which had been incubated with cord sera showed increased fragility. Such cells when incubated with 8 cord sera showed hemolysis beginning at 0.46% NaCl and complete at 0.38% NaCl. When incubated with normal sera, fragility ranged 0.44% to 0.34% NaCl.

Summary. Sixty-eight of 101 cord sera possessed ability to lyse papainized red blood cells. Heating of cord sera to 56°C enhanced lytic activity and revealed hemolytic properties in some of previously negative sera. This lytic property could be eliminated by heating sera to 80° - 100°C for 30 minutes, and in most cases by dialysis against a physiological salt solution. Incubation of normal erythrocytes in cord serum caused an increased osmotic fragility.

1. Mitchell, J. M., *Am. J. Dis. Child.*, 1928, v36, 486.

2. Goldbloom, A., Gottlieb, R., *ibid.*, 1929, v38, 57.

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Effect of Sickle Cell Erythrocytes on *in vitro* Growth Characteristics of Certain Bacteria. (24800)

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In our study of *in vitro* effect of homozygous sickle cell blood plates (S-S plates) on growth of certain Salmonella species and other pathogenic micro-organisms we demonstrated a bacterial-erythrocyte relationship previously unreported which was deemed of sufficient interest to warrant publication of preliminary findings. This report deals with demonstration of real growth differences of 3 organisms on normal human blood agar plates (A-A hemoglobin cells) as compared with sickle cell blood agar plates (S-S hemoglobin cells). This work in progress on Salmonella and other species will be reported later.

Method and material. Fresh blood from 4 homozygous sickle cell patients were collected under sterile conditions in Baxter-Alsever (A.C.D.) solution. Blood was then washed 3 times with sterile .85% saline solution, and washed, plasma-free cells resuspended in physiological saline solution to an hematocrit of 40%. Analysis of blood by filter paper electrophoresis showed them to be homozygous

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