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.

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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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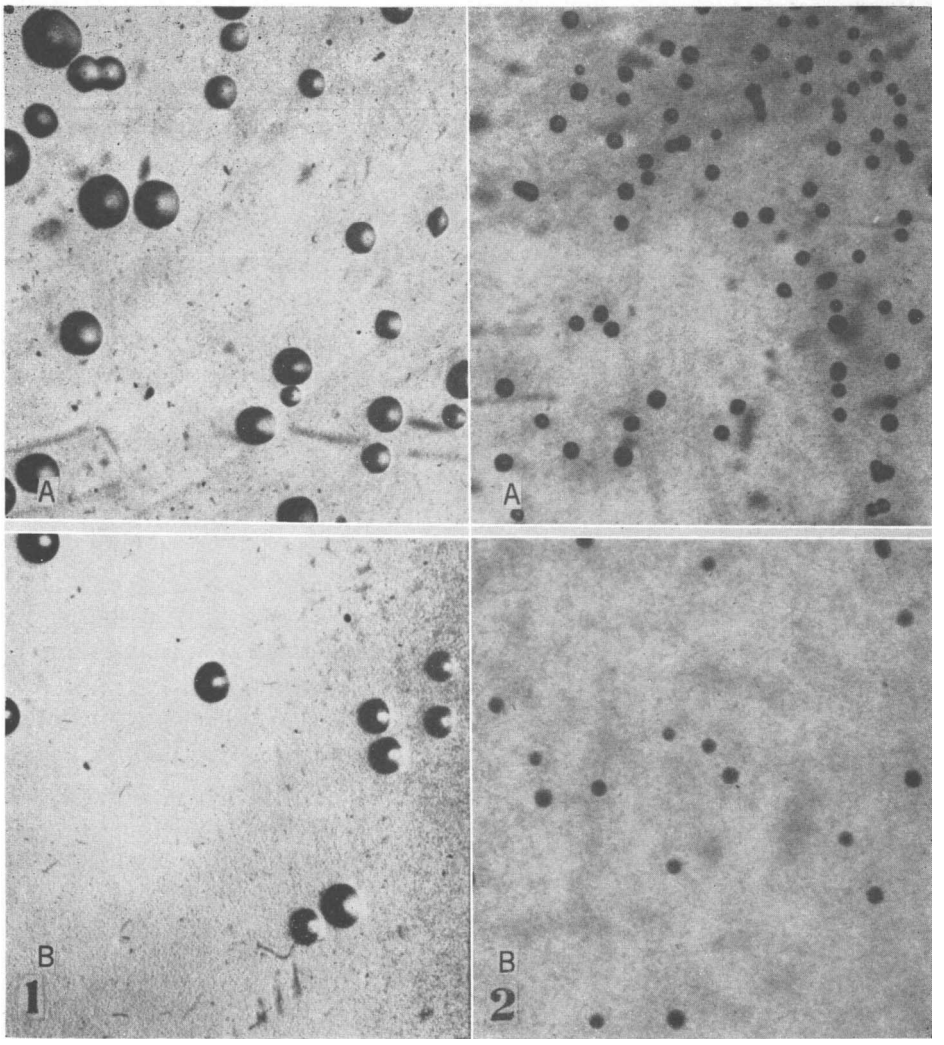

FIG. 1. 1:100 dilution of *Hemophilus influenzae* on (a) A-A plates and (b) on S-S plates.
FIG. 2. 1:100 dilution of *Streptococcus salivarius* on (a) A-A and (b) S-S plates.

hemoglobin S. Alkali denaturation studies for fetal hemoglobin content were carried out on all sickle cell bloods. Samples ranged in fetal (F) hemoglobin content from 3.2 to 6.7%. Blood from 5 normal donors was treated similarly. An aliquot of these samples was also analyzed by filter paper electrophoresis. Electrophoresis was carried out in Beckman Spince Model R paper electrophoresis system, using veronal buffer with pH 8.6 and ionic strength 0.05M. Hemoglobins were run 12 hours at 7 milliamps. Trypticase soy agar (B.B.L.) was used to prepare blood plates. Plates were prepared using known homozygous A hemoglobin cells (A-A plates) and homozygous S hemoglobin cells (S-S plates). Original rough procedures for screening possible differences in erythrocyte effects were carried out with organisms of the following 6 genera: *Salmonella*: *S. typhosa, S. paratyphi, S. schottmueller*; *S. cholerae sius, S. newport, S. enteritidis, S. montevedio; Hemophilus*: *H. influenzae; Bordetella*: *B. pertussis; II. Streptococcus*: *Strep. pyogenes, Strep. salivarius; Diplococcus*: *D. plococcus pneumoniae Type III; and Staphylococcus*: *Staph. pyogenes Var. Aureus.* These organisms were chosen as common members of 3 groups, Salmonella,

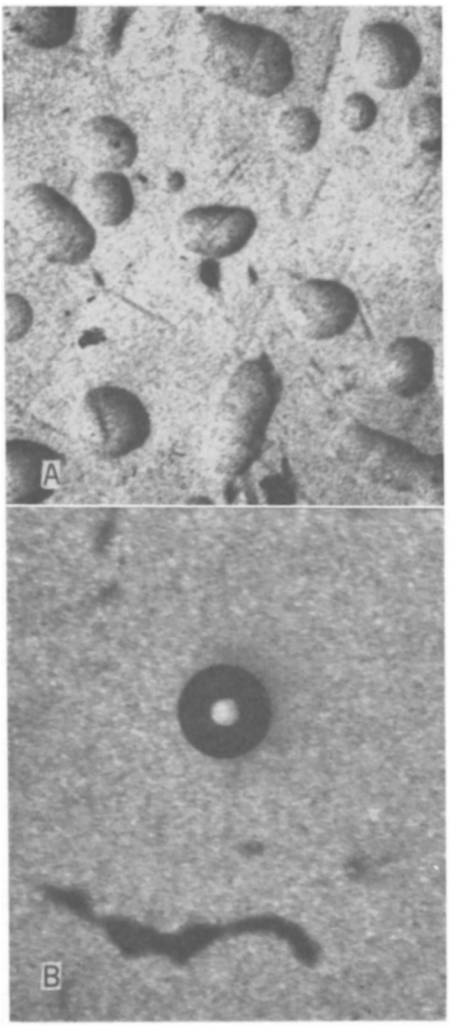

FIG. 3. 1:100 dilution of *Diplococcus pneumoniae* Type III on (a) A-A plates and (b) S-S plates.

because of reported association with osteomyelitis(1,2,3), Hemophilus as a hemophilic group, and the remainder because of their characteristic hemolysis of human blood. Initial screening was accomplished by inoculation of 1:10 serial dilutions of organisms made from 18-hour broth culture or broth suspension of 18-hour surface plate growth onto both A-A and S-S plates. Screening procedure was repeated *in toto* on 2 occasions using different blood donors with similar results. This procedure showed obvious differences in only 3 of above organisms, findings in the Salmonella group being conspicuously negative. These 3 organisms were restudied as follows: 18-hour growth of *Pneumococcus Type III*, *Hemophilus influenzae* and *Streptococcus salivarius* was harvested from surface inoculated plates (Trypticase soy blood agar) with sterile cotton swabs and suspended in saline to approximate turbidimetric equal of Kingsbury-Clark 40 mg/100 ml. Serial 1:10 dilutions of each organism were made to 10^3. A sterile glass spreader was employed in distribution 0.1 ml of each dilution over surface of agar plates employing (1) 4% washed homozygous A (A-A), (2) 4% washed homozygous S (S-S), and (3) 4% control unwashed homozygous A (A-A) red blood cells. All inoculations were performed in duplicate. Colony numbers of all 3 organisms in washed A-A and unwashed control A-A plates were within limits of standard error.

Results hemophilus influenzae: This organism grew less profusely on S-S hemoglobin plates than on A-A plates. Colonial morphology appeared indistinguishable on the 2 hemoglobins (Fig. 1a, 1b).

Streptococcus salivarius. Colonies were more numerous and smaller on A-A plates and less numerous and larger on S-S plates (Fig. 2a, 2b). No difference in hemolysis was noted.

Diplococcus pneumoniae. Colonies grew normally on A-A plates, maintaining tendency to produce watery colonies. S-S plates failed to produce growth except on heavy inoculation. These colonies that did grow rarely, produced typical watery colony (Fig. 3a, 3b). No difference in hemolysis was noticed where growth did occur. Two strains of Type III pneumococci showed similar results.

Discussion. This work serves to demonstrate that S-S red blood cells differ in ability to react to and to supply the needs of one hemophilic and 2 hemolytic organisms. This effect appears to be all-or-none with regard to individual organisms in the inoculum. Colony size appeared unaltered (smaller sizes in *Streptococcus salivarius* on A-A plates was probably due to competitive inhibition) whereas colony numbers were obviously affected. At present we can offer no explanation of this phenomenon.

It is of interest that *Hemophilus influenzae*, a hemoglobinophil, is somewhat inhibited by

S-S red blood cells. It is accepted that heme constitutes X growth factor for this organism (4), but the 2 hemoglobins under study (A-A and S-S) are thought to differ only in their globin fraction(5).

As multiple blood donors from unrelated families were used and experiments were run on 2 to 4 separate occasions with similar results, we believe our findings to be highly significant.

Summary. Homozygous sickle cell (S-S) red blood cells were less capable of fostering surface growth of *Hemophilus influenzae*, *Diplococcus pneumoniae*, and *Streptococcus salivarius* in Trypticase soy agar than were normal adult (A-A) red blood cells.

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Cortisone Effect on Exogenous Cholesterol Metabolism: Isotope Observations in Cholesterol-Fed Rabbit. (24801)

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It is firmly established(1-5) that cortisone retards development of atherosclerosis in cholesterol fed rabbits. Although aortal findings were correlated with several kinds of physico-chemical alterations in blood lipid composition, none of these appeared directly responsible for the observed inhibitory phenomenon(1,2,4,5-7). Since cholesterol deposition in aortic intima is primary to development of atheroma in the experimental animal and cortisone exerted an influence upon this process, an investigation was undertaken of the effect of cortisone on cholesterol metabolism. In this paper is reported an experiment comparing fate of meal containing cholesterol-4-C^{14} in untreated and cortisone treated rabbits fed a high cholesterol diet for 2 weeks.

Materials and methods. Eight, female, New Zealand white rabbits of pedigreed stock were used. A morning ration of 35 g of ground food pellets mixed with 1 g of crystalline cholesterol (U.S.P., Merck†) was given daily to each rabbit and 6 hours later an additional 100 g of unground stock chow. The morning meal usually was completely ingested in 3 hours. Tap water was available *ad lib.* At 1:30 daily, 10 mg of cortisone acetate was injected into a thigh muscle of 4 rabbits while others were injected with equal amount (1 ml) of physiological saline. On 12th day a meal containing 1 g of labeled cholesterol (10 μc cholesterol-4-C^{14}) was substituted for the morning ration. After labeled cholesterol meal, heart blood (5 cc) was taken at 6, 10 and 24 hours. A 1 ml plasma aliquot of these blood samples was extracted with 25 ml of 1:1 alcohol-acetone and adequate amounts of extract were taken for cholesterol chemical and radioactivity determinations. A terminal heart blood sample (15 cc) was taken 48 hours after labeled cholesterol meal and the animals killed with an overdose of intravenously administered EVIPAL (n-methyl-cyclo-hexenyl-methylbarbituric acid). The entire liver, the aorta, and 5 g approximately of uppermost section of ileum were excised. The latter 2 tissues were cleaned of adherent fat, opened longitudinally, and rinsed with ice-cold 0.85% saline solution. The lipids of

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