TABLE II. Comparison of Adenylic Acid Deaminase Activity in Homogenates of Normal Liver, Primary Hepatoma, and Transplantable Hepatomas.

Tissue	Enzymatic activities*
Liver	(4)† .69 ± .07‡
MDAB primary hepatoma	(4) .88 ± .04
MDAB transplanted hepatoma§	(3) 3.00 ± .09
Novikoff hepatoma	(7) 1.12 ± .05

Experimental conditions described in methods section.

* Enzymatic unit: See Table I.

† No. of observations.

‡ Stand. dev.

§ This tumor has been carried for 52 transplant generations.

tumors may have been contributed by muscle or other nonneoplastic tissues.

Since Greenstein(2) reported that mouse hepatomas deaminated yeast and thymus nucleic acids to a greater extent than normal livers, the adenylic acid deaminase activity in primary and transplanted hepatomas and normal livers was compared (Table II). Activity in the 2 transplantable hepatomas (Novikoff and MDAB) was significantly higher ($P < .01$)§ than normal liver; however, activity in a primary hepatoma (MDAB primary hepatoma) was equivalent to that observed in normal liver. In other studies in which hepatoma and liver were compared(6,7), the activities of several purine-metabolizing enzymes (5'-nucleotidase, nucleoside phosphorylase, guanase, adenase, xanthine oxidase and uricase) were markedly reduced, while adenosine deaminase activity was unchanged(6) or increased(7).

§ Group comparison "t" test.

Our data suggested at least 2 explanations concerning the effect of azo dye carcinogenesis upon adenylic acid deaminase activity of liver. (a) Deaminase activity of liver was not altered during carcinogenesis but subsequent transplantation of primary hepatomas resulted in significant elevation; (b) Since it has been reported that more than one kind of tumor may be induced by azo dyes(8), the 3 hepatomas studied (Table II) may have been different neoplasms.

Summary. 5'-adenylic acid deaminase activity of a spectrum of transplantable rat tumor homogenates was determined, and all tumors studied possessed deaminase activity. Activity in the primary hepatoma induced by 3'-methyl - 4 - dimethylaminoazobenzene was equivalent to that of normal liver, while the Novikoff hepatoma and a transplanted hepatoma (MDAB) had significantly higher deaminase activity than either the primary hepatoma or normal liver.

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Serum Spheroplasts of *Shigella dysenteriae*. (24649)

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We reported(1) on isolation of strains of *Shigella dysenteriae* with inherently different resistance to the bactericidal effects of "normal" human serum and on the inverse correlation between resistance to serum and resistance to penicillin. In view of the latter relationship, which agrees with the assumption that both penicillin and serum affect synthesis and/or integrity of cell-wall components(*cf.*, 2,3), an effort was made to de-

TABLE I. Survival of *Shigella dysenteriae*, Strain S_3, Organisms following Exposure to Serum (Dilution 1:3) and Penicillin (2,000 U/ml) in Absence and Presence of 20% Sucrose.

Treatment with:	Viable count at: 0	30	60 min. (colonies/0.1 ml)
Serum	426	255	60
" + sucrose + Mg^{++}, Ca^{++}	418	372	356
Penicillin	466	76	6
" + sucrose + Mg^{++}, Ca^{++}	397	380	360
" + serum	414	46	12
" + " + sucrose + Mg^{++}, Ca^{++}	427	71	20

termine whether, as in the case of penicillin (4), a hypertonic medium might interfere with the bactericidal effects of serum. With penicillin and certain other wall-damaging agents(5), it is now well established that a hypertonic medium, such as 20% sucrose, is capable of protecting sensitive bacteria from lysis by these agents, because the hypertonic environment can assume the "corsetting" functions normally provided by the intact bacterial cell wall. The resulting viable spheric structures have been referred to as *protoplasts* when essentially all wall material has been removed, as in the case of lysozyme-treated gram-positive bacteria, or as *spheroplasts** when the wall-deficiency was incomplete, as in the case of penicillin- or glycine-treated gram-negative organisms(*cf.*, 5). The present report confirms prior observations on the protective effects of sucrose on serum lysis(6) and demonstrates that the resulting viable structures represent unique spheroplasts. Somewhat similar observations have been made independently but simultaneously by Muschel *et al.*(7).

Methods. Strains S_1 and S_3 of *Sh. dysenteriae*(1) were employed. The bacteria were grown on tryptose agar for 18-24 hours at 37° C and exposed to bactericidal concentrations of serum (usually obtained from one human donor) either in tryptose broth or ABA buffer (8) supplemented with 0.05% $CaCl_2$, 0.05% $MgCl_2$, and various concentrations of carbohydrates. Prior to use, all serum samples were stored at −40°C. Depending upon serum susceptibility of the bacterial strain, final serum concentration was either 1:2.5 or 1:6. After various periods of exposure at 37°C, samples of the bacterial suspensions were plated, in appropriate dilutions, on tryptose agar. Aliquots of the bacterial suspensions were also examined microscopically under the phase microscope; an alternate technique for microscopic studies of cell structures employed fixation with 1% formaldehyde and subsequent staining with methylene blue.

Results. Addition of 20% sucrose to usually bactericidal serum dilutions resulted in complete protection, *i.e.*, survival, of the bacteria. A similar protection was not produced by lactose, starch, galactose, or glucose, added in 10 or 20% concentrations. Microscopic observations on the viable elements in serum-sucrose suspensions supported the assumption that sucrose protection involved a prevention of lysis of wall-deficient cells; spheroplasts were indeed formed under the influence of normal serum in the presence of 20% sucrose. Fig. 1a shows the appearance of untreated *Shigella* S_3 cells and Fig. 1b shows typical protoplast-like spherical cell structures obtained after 3 hours of treatment. The illustrated material is from stained preparations but comparable observations were also made on living cells, both for *Sh. dysenteriae* and *E. coli* strains, with the aid of phase microscopy. Thus, normal serum, in the presence of an appropriate, hypertonic environment, can lead to spheroplast formation of gram-negative bacteria comparable to that reported in cases of treatment with penicillin, lyso-

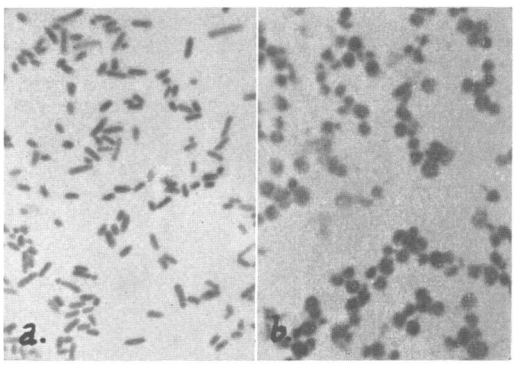

FIG. 1. *Shigella dysenteriae*, fixed with 1% formaldehyde and stained with methylene blue, before (a) and after (b) treatment with normal human serum in the presence of 20% sucrose.

* A term proposed by K. McQuillen.

zyme, glycine, and certain other agents(5) affecting bacterial cell walls. The failure of lactose and certain other carbohydrates to produce, in the presence of serum, the required protection against lysis of *Sh. dysenteriae* may be ascribed to the cells' possession of permease systems for these sugars. Heat-inactivated (56°C for 30 minutes) sera were found to lack the capacity for spheroplast formation in the presence of sucrose; this heat inactivation paralleled the inactivation of bactericidal factors, indicating involvement of heat-sensitive complement in both reactions. Rate of spheroplast formation by serum + sucrose depended upon serum concentration and was proportional to the inherent level of serum sensitivity of the strain used. Prior absorption of the "normal" sera with *Sh. dysenteriae* organisms significantly reduced both bactericidal and spheroplasting activities, whereas *E. coli* absorption did not, which suggests that the blood of the "normal" donors may actually contain considerable quantities of specific antibodies.

Optimal conditions for obtaining serum spheroplasts differed considerably from those reported for penicillin protoplast and spheroplast formation. In the case of penicillin spheroplasts, cells taken from the exponential phase of growth have been reported to be most suitable; in contrast, for serum spheroplast formation, bacteria obtained from the stationary phase (18-24-hour-old broth cultures) appear to be best. Furthermore, growth is generally required for formation of protoplasts or spheroplasts by penicillin in the presence of sucrose; in contrast, serum spheroplast formation occurred with high efficiency even when serum + sucrose was added to saline suspensions of cells taken from the stationary phase of broth cultures (simultaneous control cultures confirmed that penicillin failed to affect such cells). The latter observation suggests that serum lysis may be, at least partially, enzymatic in nature. The site of attack in the cell wall also appears to be significantly different in the case of penicillin and serum. This is indicated by the fact (Table 1) that 20% sucrose is capable of exerting almost complete protection against the bactericidal effects of either serum alone

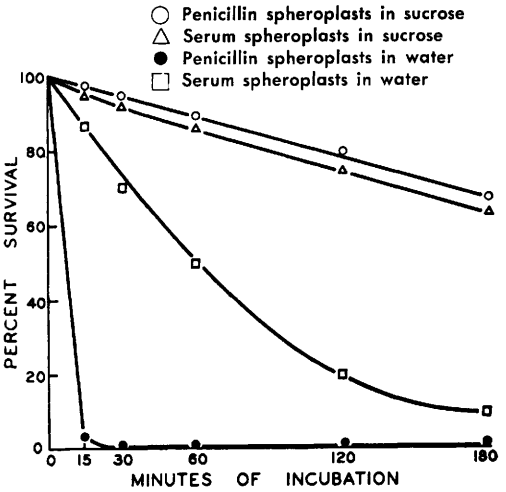

FIG. 2. Comparison of stability of penicillin-induced and serum-induced spheroplasts of *Shigella dysenteriae*.

or penicillin alone, but fails to protect against the combined effects of serum plus penicillin. The latter failure may be explained on the assumption that the combination of serum plus penicillin may produce so many lesions in the cell wall that even the corsetting capabilities of 20% sucrose are insufficient to protect against lysis. However, it may be added that after formation of spheroplasts by exposure to penicillin plus sucrose, such spheroplasts prove resistant to the effects of serum, and, of course, serum spheroplasts, maintained in a non-growing condition in serum plus sucrose fail to be affected by penicillin.

Finally, serum spheroplasts displayed considerably greater resistance than penicillin spheroplasts to osmotic shock produced by resuspending previously formed spheroplasts in large amounts of distilled water (Fig. 2). This differential resistance again is highly indicative of significant differences in the remaining wall structures of penicillin and serum spheroplasts.

Summary. Exposure of *Shigella dysenteriae* organisms to bactericidal "normal" human sera in the presence of 20% sucrose results in survival of the bacteria and formation of wall-deficient spheroplasts. These "serum spheroplasts" differ from "penicillin spheroplasts" in several respects, including their susceptibility to osmotic shock. The relation of these observations to certain aspects of the

phenomenon of bacterial lysis by serum has been discussed.

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Echo Virus Type 13. I. Isolation and Characteristics.*† (24650)

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The first 13 ECHO prototype viruses were described in Dec. 1955 by the ECHO Virus Committee(1). Prototype ECHO-13, Hamphill 2-188-20‡, had been isolated in 1954(2) from rectal swab taken Nov. 30, 1953 from a healthy 21-month-old son of American military family stationed at Clark Air Force Base in the Philippines. During 8 weeks preceding collection of above specimen, this infant yielded poliovirus type III and ECHO type 1. Antibody responses occurred to all 3 viruses. Prior to acceptance as prototype ECHO-13, 2-188-20 virus was reciprocally tested against relatively low-titered antisera for the 12 other ECHO prototype viruses by our laboratory and those of Melnick and Sabin. No cross neutralization occurred. The above tests were conducted with 4th passage of 2-188-20 virus in rhesus kidney cell cultures; the $TCID_{50}$ titer was approximately $10^{-4.0}$/0.1 ml. After 3 additional passages the titer rose to $10^{-7.0}$. Aliquots of passage 7 were sent to Dr. Herbert A. Wenner and Microbiological Associates for preparation of hyperimmune monkey and rabbit serum respectively. Composite neutralization tests with these antisera have been reported(3). These data indicated that there was a cross relationship between ECHO viruses 1, 8 and 13 or that passage 7 of 2-188-20 contained either ECHO-1 or 8 and ECHO-13 viruses. A thorough study was therefore undertaken and pure ECHO-13 prototype strain sought. The findings are reported herein. Epidemiologic and clinical studies on ECHO-13 infections are to be published separately.

Materials and methods. Monolayer cell cultures of trypsinized rhesus monkey kidney (MKCC) were used in isolation, neutralization tests, preparation of virus pools and plaque studies. Virus pools were grown in medium 199 without serum. Neutralization tests were performed in 0.5% lactalbumin hydrolysate in Earle's balanced salt solution containing 0.5% calf serum. All media contained 400 units of penicillin, 0.2 mg of streptomycin, 20 units of polymyxin and 25 units of mycostatin/ml. Neutralizing antibody (NA) and complement fixation (CF) tests were performed as described previously(4). Results of NA tests were expressed as reciprocal of serum dilution calculated by Reed-Muench method(5) to protect 50% of inoculated cell cultures. Monkeys were hyperimmunized by repeated intramuscular injection of emulsion containing equal volumes of Ar-

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‡ 2-188 is number given this child in field study and 20 is number of specimen from which virus was isolated.