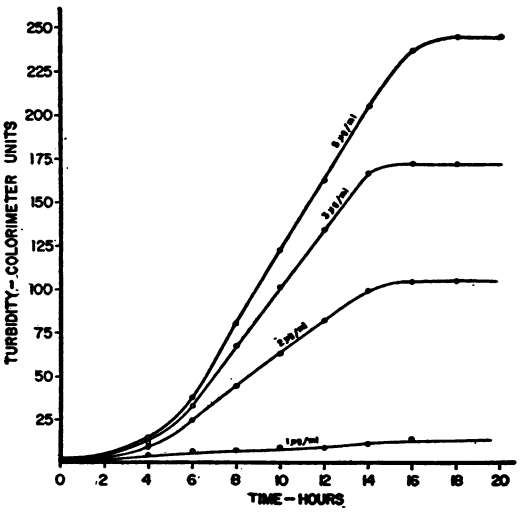

Figure 1. The growth response of *Escherichia coli* strain D-2 to various concentrations of dihydrouracil. Experimental conditions: see table 1.

Lagerkvist *et al.* (Cancer Research, **15**, 164, 1955) reported that dihydrouracil was not utilized as an efficient precursor of the nucleic acid of Ehrlich ascites tumor in mice. The data presented in this report do not indicate whether dihydrouracil serves as a nucleic acid pyrimidine precursor in *E. coli* strain D-2. However if dihydrouracil does serve as a nucleic acid pyrimidine precursor in *E. coli*, then the inability of recognized pyrimidine precursors to replace this compound would indicate that dihydrouracil may be metabolized by a hitherto unrecognized biosynthetic pathway. Alternatively, the requirement for dihydrouracil by *E. coli* strain D-2 may not reflect utilization of this substrate as a metabolite for a biosynthetic reaction, but rather an ability to counteract or to regulate an internal inhibitor. The latter may be produced by the organisms as a result of genetic alteration, and may involve, possibly, a compound of somewhat similar structure.

CLARIFICATION OF SERUM FOR STORAGE IN LIQUID OR LYOPHILE STATE

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Past experience has indicated that lyophilization of streptococcus grouping and typing antiserum is impracticable because of cloudiness of the reconstituted product. This cloudiness or precipitate can be removed only with great difficulty by repeated centrifugation. Furthermore, serum cleared in this manner frequently reprecipitates during subsequent refrigeration. Since crystal-clear reagents are required for the tests, the lyophile technique for the preservation of streptococcus antiserum has not been advocated.

For several months a chloroform extraction procedure, developed in this laboratory, has been used to clarify cloudy or chylous serum which, untreated, had precipitated repeatedly on standing in the refrigerator. Serum treated in this manner remains clear for prolonged periods of refrigeration. The technique is as follows:

One volume of serum is added to 1¼ volumes of chloroform and mixed thoroughly with an electric beater (Updyke and Conroy, J. Bacteriol., **66**, 239, 1953) for approximately 30 sec. After overnight refrigeration the milky suspension is cleared by centrifugation at 6000 rpm (Servall centrifuge) and the serum decanted. Centrifugation may be done immediately after mixing, but separation is facilitated somewhat by overnight refrigeration. The used chloroform can be reclaimed by filtration through paper (Whatman #1) and can be reused as long as it remains clear and essentially colorless. The following modifications of the technique must be avoided since they frequently result in appreciably smaller yields and products which are not uniformly clear: 1) mixing by hand shaking or pipetting; 2) centrifugation at lower speeds; and 3) use of smaller aliquots of chloroform.

In view of the effectiveness of this extraction process, lyophilization of the chloroform-treated

serum was attempted. The reconstituted product proved to be clear and entirely satisfactory for use in the precipitin test.

Since the streptococcus grouping and typing tests are qualitative rather than quantitative, titrations of serum before and after treatment and lyophilization have not been made, but rough comparisons indicate that there is no appreciable decrease in antibody content.

Addendum. Since working with the chloroform extraction method, our attention has been called to the report of Bryan *et al.* (Poultry Science, **29**, 167, 1950) on the use of ethylene dichloride for clarifying serum for use in the pullorum disease tube agglutination tests. The method involved mixing equal parts of serum and reagent (specific details not given), allowing the mixture to stand at room temperature for 12 hr and removing the supernatant with a pipette. As noted above, with chloroform this technique does not consistently clarify adequately for precipitin tests. However, preliminary tests using an electric mixer and high speed centrifugation indicate that chloroform and ethylene dichloride give comparable results.

LACTOSE UREA AGAR; A NEW DIFFERENTIAL TUBE MEDIUM FOR THE DETECTION OF SALMONELLA

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A major objection to the double and triple sugar iron agar media used in the primary identification of gram negative enteric pathogens is that a separate urease test medium has to be employed to exclude organisms with urease activity. Although this objection was overcome by the medium of Rappaport and Stark (Am. J. Clin. Pathol., **23**, 948–950, 1953), preparation is tedious and the addition of urea to the sterilized medium before solidification necessitates preinoculation incubation to test sterility. The demonstration by Tidwell *et al.*, (J. Bacteriol., **69**, 701–702, 1955) that the extent of urea hydrolysis by heat was decreased by proper buffering has led to the development of a differential tube medium which not only indicates urease activity and lactose fermentation, but is simple to prepare and can be sterilized in the autoclave.

Lactose-urea agar consists of heart infusion agar (Difco) 40 g, urea 20 g, lactose 10 g, KH_2PO_4 (anhydrous) 7.5 g, Na_2HPO_4 (anhydrous) 5.8 g, phenol red $\leq$0.02 g, and distilled water 1000 ml. The phenol red indicator is added as 10 ml of a 0.2 per cent stock solution (0.4 g phenol red added to 22 ml N/20 NaOH, volume brought up to 200 ml, pH adjusted to 7.0 with sufficient N/20 NaOH and preserved with 1 ml of chloroform). The medium is heated to aid solution, dispensed into tubes, and autoclaved for 15 min. A hydrogen sulfide indicator is not included in lactose-urea agar as the urea and phosphates present either delay or prevent the reaction.

A number of gram negative enteric organisms isolated from poultry (except ATCC cultures) were used to test this new medium. Fifteen Salmonella species representing five serological groups were used as urease negative lactose nonfermenting test organisms. Four serologically different "O" antigen strains of *Escherichia coli* were used as the lactose fermenting test organisms. These cultures were typed by Dr. P. R. Edwards of the Communicable Diseases Center at Chamblee, Georgia. In addition to freshly isolated strains of Proteus, ATCC cultures (numbers 9916, 9918, 9920, and 9921) of the four recognized species were also used as urease positive lactose nonfermenting test organisms. Cultures belonging to the Klebsiella-Aerobacter and Arizona groups were also included. All test organisms were checked for purity and biochemical reactions.

Salmonella species are easily recognized on lactose-urea agar; the reaction remains unchanged. Other members of the Enterobacteriaceae tested (except the Arizona group) altered

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