the father of the child. Going back to the original specimen from the father, and using enrichment media and subsequent testing of "approximately 200 colonies," Juenker found four different Salmonella types.

In this report, a fecal specimen from a child with severe gastro-enteritis was plated on the usual isolation media and seeded into enrichment broths. Colonies picked from the primary plates yielded cultures which were H_2S negative on triple sugar iron agar and reacted in Salmonella 6,7 O-antisera and cultures which were H_2S positive and reacted in 6,8 O-antisera. Colonies from plates inoculated from the enrichment broths yielded only H_2S positive cultures which could be separated into O group 6,7 and 6,8 types.

At this time, it was apparent that we were dealing with either two types and an H_2S negative variant of one of these types or with three distinct types. The cultures were identified by Dr. Saphra at the New York Salmonella Typing Center as *Salmonella bareilly* (H_2S negative), *Salmonella muenchen-oregon*, and *Salmonella montevideo*.

Fecal specimens from the child and from the other members of the family were examined periodically for a period of 4 months. In the third month, a fourth type appeared in the feces of a male and female sibling who had previously, excreted one or more of the first three types found. These new isolates, also H_2S negative, were serologically in group E of the Kauffman-White schema and were identified in our laboratory as *Salmonella senftenberg*.

With certain few well-known exceptions, Salmonella cultures produce abundant H_2S on media such as triple sugar iron agar. Kauffman (*Enterobacteriaceae*, 1954) does not list H_2S negative variants of *S. bareilly*. A biochemical variant of *S. senftenberg*, designated "newcastle," is shown as H_2S negative. About the time Dr. Saphra received our cultures he had identified similar cultures of *S. bareilly* received from the Boston area. Using the more sensitive lead acetate paper strip method, H_2S production could be detcted in our *S. bareilly* and *S. senftenberg* isolates and in stock cultures of *Salmonella paratyphi A* and *Salmonella abortus equi*, the latter two classically considered as H_2S negative or variable Salmonella types.

In table 1 we have charted the results of the repeated fecal examinations of the family. It can be seen that the *S. senftenberg* type appeared after the other types could no longer be found. This may have been the result of a "new" infection.

Except for the original case, the members of the family have remained asymptomatic throughout the entire study period. Investigation has not revealed the source of the infection with multiple Salmonella types in a family living under good environmental conditions.

APPARENT MUTAGENIC EFFECT OF THYMINE DEFICIENCY FOR A THYMINE-REQUIRING STRAIN OF *ESCHERICHIA COLI*

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Cohen and Barner (Proc. Natl. Acad. Sci., U. S., **40**, 885–893, 1954) reported that when a thymine-requiring strain ($15T^-$) of *Escherichia coli* is incubated in the absence of thymine but with glucose as the sole carbon source, rapid death ensues. This appears to be due to a blocking of deoxyribonucleic acid synthesis without blocking of ribonucleic acid or protein synthesis. For our experiments strain $15T^-$ was grown for 8 hr at 37 C in Roux bottles containing nutrient agar (Baltimore Biological Laboratory, Baltimore). Cells were harvested from Roux bottles, washed in 0.85 per cent NaCl solution, and incubated for 4 hr at 37 C on minimal agar, described by Cohen and Arbogast (J. Exptl. Med., **91**, 619, 1950), containing 0.7 or 0.9 μg thymine per ml. The technique used by Matney (J. Bacteriol., **69**, 101, 1955) for determination of frequency of streptomycin-resistant mutants in a population of cells on a millipore filter was used with modifications. Minimal agar adjusted to pH 7, and containing 0.7 μg thymine per ml and 1.0 mg streptomycin

per ml was used to recover streptomycin resistant mutants. The number of cells impinged on each millipore filter generally was adjusted to yield 10^9 to 10^{10} cells at the time of exposure to streptomycin. Prior to such exposures, the filters were placed on soft (0.75 per cent) minimal agar containing 10 mg glucose per ml plus varying concentrations of thymine. The media always were adjusted to pH 8.2, the pH at which lethal effects of thymine deficiency can be observed consistently (Weinberg and Latham, *unpublished data*).

The frequency of recovered streptomycin-resistant mutants has been expressed in terms of the average number of mutant cells in relation to the total number of viable cells at the time of exposure to streptomycin. The protocol of a typical experiment is shown in table 1. The frequency of recovered streptomycin resistant mutants ranged from 2.7×10^{-10} to 6.7×10^{-10} after growth of cells in Roux bottles with agar containing 0.9 or 0.7 μg thymine per ml (table 2, 0 hr counts). However, following incubation of cells on millipore filters for 8.5 hr without thymine, mutant frequencies rose consistently to values ranging from 25×10^{-10} to 29×10^{-10}. In contrast, following 8.5 to 12.5 hr of incubation on streptomycin-free media containing 1 or 2 μg thymine per ml, consistently lower mutant frequencies of from 1.6×10^{-10} to 3.0×10^{-10} were obtained (table 2). Mutant frequencies ranged from 12×10^{-10} to 15×10^{-10} when cells on millipore filters were incubated for 3 hr without thymine, or with 0.4 μg thymine per ml for 8.5 hr (table 2). The mutant frequency remained at from 9×10^{-10} to 13×10^{-10} when 8.5 hr of thymine starvation on millipore filters was followed by 4 hr of incubation on 1 or 2 μg thymine per ml (table 2).

Streptomycin-resistant mutants obtained from experiments in which increased mutant frequencies had been observed were mixed with streptomycin-sensitive cells, impinged on millipore filters, and incubated for 8.5 hr without thymine. There was no noticeable selection for or against streptomycin-resistant mutants (table 3). This led us to the tentative conclusion that the increase in mutant frequency observed upon

TABLE 1

Protocol of an experiment with Escherichia coli to test the effects of thymine deprivation on the frequency of streptomycin-resistant mutants

No. of Viable Cells Impinged per Millipore Filter	Duration of Subsequent Exposure to Thymine	Thymine	Viable Cells per Millipore Filter*	Mutant Frequency per 10^{10} Cells†
	hr	*μg per ml*		
1.2×10^{10}	0.0	—	1.2×10^{10}	6.7
2.4×10^{9}	0.0	—	2.4×10^{9}	1.3
2.3×10^{8}	0.0	—	2.3×10^{8}	no mutants recovered
1.0×10^{8}	8.5	2.0	2.2×10^{9}	2.9
1.2×10^{10}	8.5	0.0	3.0×10^{8}	29
1.2×10^{10}	8.5	0.0	—	—
	4.0	2.0	2.2×10^{9}	13

* At time of exposure to streptomycin.

† Based on cell counts at time of exposure to streptomycin.

TABLE 2

The effect of thymine deficiency upon the frequency of streptomycin resistant mutants recovered from Escherichia coli

Thymine	Duration of Exposure	Mutant Frequency $\times 10^{-10}$
μg per ml	*hr*	
0.0	8.5	29
0.0	8.5	25
0.0	3.0	15
0.4	8.5	12
0.0	8.5	—
2.0	4.0	13
0.4	8.5	—
1.0	4.0	9.0
0.9	0.0	6.7
0.9	0.0	2.7
1.0	8.5	1.6
2.0	8.5	2.9
2.0	12.5	3.0

TABLE 3

Tests on the survival of streptomycin-resistant and -sensitive Escherichia coli cells after 8.5 hr of incubation without thymine

Resistants $\times 10^{-6}$ Initially Present	Per Cent Killing	
	Streptomycin-resistant cells	Streptomycin-sensitive cells
1.9	72	65
2.1	61	52
1.8	54	70
47	70	46

incubation of strain 15T⁻ without thymine may result from an increased rate of mutation from streptomycin sensitivity to streptomycin resistance, presumably as a result of significantly altered deoxyribonucleic acid metabolism.

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A PROCEDURE FOR ADSORBING VIRUS NEUTRALIZING ANTIBODIES ON PAPER DISKS[1]

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Stapp and Berks (Phytopathol. Z., **25**, 47, 1948) described a technique for drying neutralizing antibodies for a potato virus on blotting paper without appreciably impairing their activity. A similar method was found to have advantages in handling serum samples to be tested for antibodies which neutralize vesicular stomatitis virus.

Paper disks with a diameter of 15 mm and a thickness of 0.75 mm were cut from white, commercial grade blotting paper. Three disks were dipped into each serum sample, soaked until saturated, removed, and air dried. To each disk was added 0.6 ml of nutrient broth, to bring the neutralizing antibodies back into solution, and 0.6 ml quantities of one of the three serial dilutions (10^{-4}, 10^{-3}, and 10^{-2}) of the virus suspension to be neutralized. The mixtures were shaken and incubated at 25–30 C for 30 min. Six 10-day-old chicken embryos received each of the dilution mixtures in 0.1-ml quantities in the allantoic chamber. Incubation was at 37–38 C. Death of the embryo was determined by candling. The neutralization titers of the eluted serums were calculated from the difference between the embryo lethal dose of the virus alone and in the presence of antibody. More than 40 positive and negative serum samples were tested by the paper adsorption neutralization method, and the titers obtained corresponded with the titers obtained by the fluid neutralization method.

The dried antibody was quite stable. Sera capable of neutralizing 10^5LD_{50} of virus before adsorption neutralized more than 10^4LD_{50} after the serum impregnated disks had been held for 7 days at 37 C, and for 1 hr at 56 C. The disks adsorbed approximately 0.15 ml of serum when immersed in a serum sample. This was equivalent to about 17 mg of serum solids. Approximately 65 per cent of the adsorbed serum solids went back into solution on the basis of dry weight when diluent and virus-containing allantoic fluid were added. An average loss of 0.9 ± 0.2 of a log value of neutralizing activity observed following paper adsorption is actually less when adjustment is made for the 1:4 dilution inherent in the reconstitution of 0.15 ml to 0.6 ml. Although a serum with a low titer might be undetected by the paper adsorption method, low titer sera have been rare and have not contributed information of significance to the present epizootiological study of vesicular stomatitis (Karstad, Adams and Hanson, J. Am. Vet. Med. Assoc., **129**, 1956). When serum was adsorbed by the disks, the heat labile nonspecific neutralizing substances for vesicular stomatitis virus present in some unheated hog sera (neutralization indices of 5 to 50) were eliminated or irreversibly bound. The nonspecific neutralizing substance for Newcastle disease virus present in unheated human serum (neutralization indices of 100 and greater) also was eliminated by paper adsorption in the one trial conducted.

The paper adsorption method was found to be applicable to serological survey work. It eliminated many of the problems (such as bacterial spoilage, chemical denaturation, or breakage of glass vials) associated with the handling and shipping of fluid serum samples.

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