Biochemically Aberrant Salmonella enteritidis ser. newington from Human Sources in Connecticut

GEORGE O. CARRINGTON,* PORTER CLEVELAND, JR.,† ALEXANDER VON GRAEVENITZ,‡ AND W. DEAN RUPP§

*Division of Microbiology, Department of Pathology, Hartford Hospital, Hartford, Connecticut 06115; †Clinical Laboratories, Bridgeport Hospital, Bridgeport, Connecticut 06602; ‡Department of Laboratory Medicine, Yale University School of Medicine, and Clinical Laboratories, Yale-New Haven Hospital, New Haven, Connecticut 06504; and §Departments of Therapeutic Radiology and Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06510

Received February 7, 1975

Three isolates of a lactose-fermenting, xylose-negative variety of Salmonella enteritidis ser. newington, identical in biochemical and serological reactions and in the antibiogram, were recovered from three patients in different areas of Connecticut in January 1974. Hydrogen sulfide production was not visible in Salmonella-Shigella agar, in triple sugar iron agar, and in Kligler iron agar but was noticed in lysine iron agar and on XLD agar, among others. The amount of fermentable carbohydrates present was found to correlate with failure to show hydrogen sulfide production (pH effect). In contrast to lactose-fermenting Salmonella strains reported by other authors, we could not elicit a direct transfer of the lac⁺ character at frequencies above 10⁻⁴. An epidemiological follow-up remained unsuccessful. Recommendations for the recognition of similar strains are presented.

Salmonella enteritidis ser. newington (henceforth called S. newington) was first isolated by Leo F. Rettger from ducks in Newington, Connecticut in 1937 (1). It belongs to group E₂ of the Kauffmann–White scheme (2). In various series, S. newington strains comprised only 0.3 (3), 0.4 (4), 2.1 (5), and 6.9% (6) of human Salmonella strains isolated. The 6.9% figure (37 of 532 strains for the period between 1934 and 1941) includes 31 carrier strains, which also figure large in other series (4, 7, 8). Reports of human disease due to S. newington have been infrequent (9, 10); two institutional outbreaks of gastroenteritis have been described (11, 12). In the last quarter of 1973, only eight S. newington isolates (of a total of 7686 Salmonella isolates) were reported by the Center for Disease Control for the United States, two of them from Connecticut (13).

In their main biochemical characteristics, S. newington strains resemble the majority of S. enteritidis serotypes (1, 2). In particular, they fail to ferment lactose and sucrose, ferment xylose, and produce hydrogen sulfide (H₂S) in iron chloride gelatin, Kligler iron agar (KIA), and triple sugar iron agar (TSIA).

Three biochemically and serologically identical isolates of S. newington that ferment lactose (lac⁺) but fail to ferment xylose and to produce H₂S in some common laboratory media were observed in January 1974 in three different Connecticut hospitals. Similar human strains have only been reported from one case each of meningitis (10) and gastroenteritis (9); more often, they have been found in dried milk (14). A lac⁺, H₂S-producing strain originating from dried milk was responsible for one institutional outbreak (12). Most lac⁺ Salmonella strains have been able to transfer the lac⁺ character to strains of Escherichia coli and/or Salmonella species as part of an extrachromosomonal sex factor called F₀-lac (15), either directly (15, 16) or after mobilization by another plasmid (17).
We report on clinical, biochemical and genetic studies of the three lac⁺ S. newington isolates.

MATERIALS AND METHODS

Clinical data. The first isolate originated from the appendiceal pus of a 25-year-old Stratford woman who was admitted to Bridgeport Hospital for appendicitis. An identical isolate was recovered from her stool a few days postoperatively and 2 weeks after discharge. The second isolate originated from the cerebrospinal fluid of a 14-day-old male infant from Willimantic who had been transferred to Hartford Hospital because of meningitis. The isolation was repeated twice during his illness which lasted for 3 weeks; identical isolates were recovered from the patient’s feces four times and from his blood once. The third isolate originated from the stool of a 19-year-old New Haven man who had been admitted to Yale-New Haven Hospital for septic arthritis of a finger and who developed diarrhea with fever one day after admission. Salmonella was not isolated from the joint. All primary isolations were made within a period of one week.

Isolation and Identification. The appendiceal and spinal fluid strains were selected on MacConkey agar, the stool isolates on XLD agar and on Salmonella-Shigella (SS) agar at 37°C. Further biochemical work-up followed methods described elsewhere (18). Production of H₂S was investigated in a variety of media. Lysine iron agar (LIA) was adjusted to several pH values before inoculation and was also supplemented with 0.5% glucose, 1.0% glucose, 1.0% sucrose, and 1.0% lactose, respectively.

Antimicrobial sensitivity was determined by the Kirby–Bauer disk method (19).

Genetic transfer studies. One of us (W. D. R.) investigated a possible transfer of lac⁺ to the F–lac⁻ E. coli K 12 strain KL 320 (20) which is resistant to streptomycin and nalidixic acid (Str⁻ Nal⁺). The method of Falkow and Baron (15) was followed by adding 1 ml of the S. newington culture to 9 ml of the E. coli culture, each culture containing 2 × 10⁶ – 4 × 10⁸ cells/ml. After 2 hr incubation at 37°C, the mixture was plated on minimal medium (21) plates containing proline, methionine, histidine, tryptophane (required by E. coli K 12 KL 320), streptomycin (200 μg/ml) and nalidixic acid (50 μg/ml), and scored for the lac⁺ Str⁻ Nal phenotype. All three S. newington isolates were checked separately. The F⁻ lac⁺ E. coli strain Q served as a control donor strain, the recipient being again E. coli K 12 KL 320.

RESULTS

Epidemiology. The three patients recovered but were lost to follow-up. A search for a common source proved futile as a retrospective investigation was started only after it had become known that the three isolates had been sent to the Connecticut State Department of Health. The patient from Bridgeport Hospital had used powdered milk before admission; it was cultured and found negative for Salmonella. A check of 30 employees of Hartford Hospital failed to turn up any fecal Salmonella from personnel in contact with the infant in January 1974. No further cases of lac⁺ S. newington have since been reported to the Connecticut State Department of Health. No contact between the patients could be elicited.

Cultural characteristics. On MacConkey agar, deeply pink, flat colonies with an entire margin grew in 24 hr. The 24-hour growth on XLD agar showed, beside

*BBL, Cockeysville, MD.
TABLE 1
Biochemical Reactions of Three Strains of *Salmonella enteritidis* ser. *newington* (24-48 hr at 37°C)\(^a\)

| Reaction                  | Strain 1 | Strain 2 | Strain 3 |
|---------------------------|----------|----------|----------|
| Indole                    | -        | Glucose acid + | +        |
| Urease                    | -        | Glucose gas + | +        |
| Methyl red                | +        | Lactose + | +        |
| Voges-Proskauer           | -        | Sucrose - | -        |
| Citrate (Simmons)         | +        | Mannitol + | +        |
| Motility                  | +        | Dulcitol + | +        |
| Gelatin (22C)             | -        | Salicin - | -        |
| Lysine decarboxylase      | +        | Adonitol - | -        |
| Arginine dihydrolase      | +\(^d\)  | Inositol - | -        |
| Ornithine decarboxylase   | +        | Sorbitol + | +        |
| Phenylalanine deaminase   | -        | Arabinose + | +        |
| Malonate                  | -        | Raffinose - | -        |
| Deoxyribonuclease         | -        | Trehalose + | +        |
| Beta galactosidase        | +        | Rhamnose + | +        |
|                           |          | Xylose -   |          |

\(^a\) +, positive; -, negative; d, delayed (more than 48 hr); w, weak reaction after 48 hr.

typical coliform colonies, those with black centers on a yellow base. On SS agar, lac\(^+\) colonies without blackening were seen after 48 hr of growth; some colonies, however, showed an intensely pink color.

Colonies from MacConkey agar as well as the unusual lac\(^+\) colonies from SS agar and the H\(_2\)S-positive ones from XLD agar were studied further (Table 1). The negative indole test coupled with H\(_2\)S production and decarboxylation in LIA suggested the presence of either Arizona or *Salmonella* sp. The three isolates gave identical reactions and agglutinated in *Salmonella* group E antiserum.\(^2\)

TABLE 2
Visible Production of Hydrogen Sulfide by Three Strains of *S. newington* in Different Media (24-48 hr at 37°C)\(^a\)

| Tube medium                  | Initial pH | Final pH |
|------------------------------|------------|----------|
| Lead acetate (strip (19))    | +          | Salmonella-Shigella agar - | |
| Triple sugar iron agar       | -          | Bismuth sulfite agar + | |
| Kligler iron agar            | -          | XLD agar + | |
| SIM medium                   | +          | Hektoen enteric agar +w | |
| Neutral Red-lysine-Iron-cystine broth (26) | + | Sulfite-dulcitol agar (15) + | |

| Tube medium                  | Initial pH | Final pH |
|------------------------------|------------|----------|
| Lysine iron agar             | 4.0        | 4.0      | -        |
| + 0.5% glucose               | 5.2        | 7.4      | +        |
| + 0.5% lactose               | 5.8        | 7.4      | +        |
| + 1.0% glucose               | 6.0        | 7.4      | +        |
| + 1.0% sucrose               | 6.3        | 7.4      | +        |
| + 1.0% lactose               | 6.4        | 7.4      | +        |
| + 1.0% sucrose               | 6.7        | 7.4      | +        |

\(^a\) Source for media or constituents (25, 26): BBL, Cockeysville, MD. See Table 1 footnote.
analysis yielded the formula (3, 15): e,h:1,6\(^2\) and thus established the isolates as \textit{S. newington}. Atypical were the reactions for lactose and xylose fermentation while \(H_2S\) production varied considerably between different media (Table 2). The TSIA and KIA butts and slants were acid; there was also gas production.

\textit{Antimicrobial sensitivity.} All three isolates were fully sensitive to ampicillin, carbenicillin, cephalothin, chloramphenicol, colistin, gentamicin, kanamycin, nalidixic acid, nitrofurantoin, and tetracycline.

\textit{Genetic transfer.} No recombinants between each of the \textit{S. newington} strains and \textit{E. coli} K 12 KL 320 were found at frequencies higher than \(10^{-6}\). In the control experiment with \textit{E. coli} K 12 Q as the donor, the transfer frequency to \textit{E. coli} K 12 KL 320 was greater than 1 after mating for only 1 hr.

\section*{DISCUSSION}

\(\text{Lac}^{+}\) strains of \textit{Salmonella} are very rare (0.8\% of all \textit{Salmonella} strains (18)) and have been observed mainly in the \textit{S. enteritidis} serotypes \textit{S. tennessee} (14, 18, 22), \textit{S. anatum} (14), \textit{S. newington} (9, 10, 12, 14), and, recently, \textit{S. typhimurium} and \textit{S. oranienburg} (17). Of 552 \textit{Salmonella} strains from dried milk and milk-drying plants, however, 86 (15.6\%) were found to be \text{lac}^{+} (14). Failure to ferment xylose is commonly observed only in the \textit{S. enteritidis} bioserotypes \textit{S. paratyphi A} and \textit{S. sendai} (18). One of the \text{lac}^{+} \textit{S. newington} strains that was described earlier (10) was also xylose-negative.

\(\text{Lac}^{+}\) \textit{Salmonella} strains often fail to show \(H_2S\) production in some media (17), notably on SS agar (10) and in TSIA (10, 14, 18, 22). We did not observe \(H_2S^{+}\) variants on SS agar (10, 22), but after several subcultures on Trypticase Soy Agar\(^1\) a \text{lac}^{-} \(H_2S^{+}\) variant was recovered from the first isolate, similar to findings of earlier authors (9, 22).

A similar failure to show \(H_2S\) production in TSIA has been reported for sucrose-positive strains of \textit{Citrobacter freundii} and \textit{Proteus vulgaris} and was thought to be due to products of fermentable carbohydrates (23). Also, \text{lac}^{+} \textit{C. freundii} and \textit{Arizona hinshawii} strains, while showing blackening in glucose (0.1\%–)lactose (1.0\%)–\(H_2S\) medium, failed to blacken the medium when 0.9\% glucose was added; however, this medium with 1.0\% glucose (and 1.0\% lactose) showed blackening upon secondary alcalinization (24). A plausible explanation was that, although \(H_2S\) was actually formed, an iron sulfide precipitate did not show at a low pH since the solubility of FeS increases with decreasing pH (molar concentration of Fe\(^{2+}\) at pH 5 is \(3.7 \times 10^{-6}\); at pH 7, it is \(3.7 \times 10^{-10}\)) (24).

Taking a different approach, we tried to find out whether a similar pH drop is responsible for the absence of FeS precipitation in some identification media inoculated with our \textit{S. newington} strains. The amount of substrates from which \(H_2S\) could be produced is higher in TSIA and in KIA than in LIA, and higher in SS agar than in XLD agar or in the sulfite-dulcitol agar of Padron and Dockstader (25) (Table 3). Thus, no correlation could be found between substrate quantity and visible FeS production. The total amount of carbohydrates is higher in TSIA and KIA than in LIA or in SIM medium;\(^1\) on the other hand, it is higher in XLD agar, which showed FeS production, than in SS agar, which failed to show it. The amount of lactose is highest in the media in which visible FeS was not formed but there is no evidence that \text{lac}^{-} \textit{Salmonella} strains form \(H_2S\) less often in these media than in

\(^2\)We thank the Laboratory Division of the Connecticut State Department of Health, Hartford, Connecticut, for the serological analysis and the confirmation of our biochemical results.
| Ingredient                | Triple sugar agar | Kligler iron agar | SIM medium | NR-lysine-iron-cystine broth (26) | Lysine iron agar | SS agar | Bismuth sulfite agar | XLD agar | Hekt enter agar | Sulfur dulc agar (25) |
|---------------------------|-------------------|-------------------|------------|----------------------------------|------------------|--------|----------------------|---------|----------------|----------------------|
| Initial pH                | 7.3               | 7.4               | 7.3        | 6.2                              | 6.7              | 7.1    | 7.5                  | 7.4     | 7.6            | 7.5                  |
| Glucose                   | 1.0               | 1.0               | –          | 1.0                              | 1.0              | –      | 5.0                  | –       | –             | 1.5                  |
| Lactose                   | 10.0              | 10.0              | –          | 5.0                              | –                | 10.0   | –                    | 7.5     | 12.0          | –                    |
| Sucrose                   | 10.0              | –                 | –          | –                                | –                | –      | 7.5                  | 12.0    | –             | –                    |
| Salicin                   | –                 | –                 | –          | 1.0                              | –                | –      | –                    | –       | 2.0            | –                    |
| Dulcitol                  | –                 | –                 | –          | –                                | –                | –      | –                    | –       | 5.0            | –                    |
| Kylose                    | –                 | –                 | –          | –                                | –                | –      | 3.5                  | –       | –             | –                    |
| Cystine and methionine    | 0.5               | 0.5               | 0.65       | 0.24                             | 0.13             | 0.12   | 0.25                 | –       | 0.28          | 0.02                 |
| Ferrous (ammonium) sulfate| 0.2               | –                 | 0.2        | –                                | –                | –      | 0.3                  | –       | –             | –                    |
| Sodium thiosulfate        | 0.2               | 0.5               | 0.2        | 0.1                              | 0.04             | 8.5    | –                    | 6.8     | 5.0           | –                    |
| Bismuth sulfite           | –                 | –                 | –          | –                                | –                | –      | 8.0                  | –       | –             | –                    |
| Sodium sulfite            | –                 | –                 | –          | –                                | –                | –      | –                    | –       | –             | 3.0                  |
| Bismuth citrate           | –                 | –                 | –          | –                                | –                | –      | –                    | –       | –             | 0.4                  |
| Ferric (ammonium) citrate | –                 | 0.5               | –          | –                                | –                | –      | 0.8                  | 1.5     | –             | –                    |
| Yeast extract$^b$ (or beef extract) | –               | –                 | –          | 3.0                              | 3.0              | 5.0    | 5.0                  | 3.0     | 3.0           | –                    |

$^a$Data from BBL Manual of Products and Laboratory Procedures, 5th edition, 1968; and from publications (25) and (26). The cystine and methionine contents were computed from the BBL Peptones table.

$^b$Contains 16.6% carbohydrates and 3.6% cystine and methionine.
others. The initial pH of the H$_2$S-indicating media was, with two exceptions, above 7.0 (Table 3). The exceptions, LIA and neutral red–lysine–iron–cystine broth (26), contain 1% lysine which is decarboxylated by *S. newington* with a resulting alkaline pH. The sulfite-dulcitol medium of Padron and Dockstader (25), which contains no lysine (except for the amount present in the peptone) fails to exhibit FeS production in lac+ and lac– Salmonella species if the initial pH is below 6.0 (25). Thus, there seems to be a correlation between visible FeS production and the final pH in the medium.

Our experiments with LIAs showed that, indeed, initial pH values above the lower limit for lysine decarboxylase activity (27) did not prevent blackening, and that under these circumstances the final pH (after decarboxylation) always rose to 7.4. However, if the final pH was at 6.4 or below, which happened only when fermentable sugars like glucose or lactose (but not sucrose) were added, FeS production was not visible. We conclude, therefore, that the amount of fermentable carbohydrates, whose catabolism resulted in a sustained (rather than in a mere initial) pH drop not overcome by decarboxylation or other countervailing processes, is of paramount importance for visible FeS production in our strains, which means H$_2$S production as well. We can, however, not explain why other lac+ strains of Salmonella (14) and *C. freundii* do show FeS production in TSIA and KIA.

In previously reported lac+ Salmonella strains, the transfer frequency of lac+ ranged from $3 \times 10^{-2}$ to $1 \times 10^{-5}$ to *E. coli* (15) and from $2 \times 10^{-3}$ to $6 \times 10^{-5}$ to *S. typhi* (16). Only in one of seven strains of *S. anatum* was no transfer detected (16). Our strains behaved similar to this *S. anatum* strain. Two possibilities cannot be excluded: 1) that the lac+ character is located on a plasmid or on a chromosome with a transfer frequency of less than $10^{-6}$; and 2) that the lac+ character can be transferred only after mobilization with other plasmid(s) (17). Experiments are in progress to study these possibilities.

Should the incidence of lac+ Salmonella increase to a point where there is a reasonably high possibility of their being present in clinical specimens, particularly feces, the clinical laboratory would be faced with a serious diagnostic challenge. In order to detect such strains, we suggest 1) that stools be routinely plated on XLD agar which gives a high recovery rate for Salmonella and is superior to SS agar in the recovery of Shigella (28, and 2) that lac+ indole-negative colonies be checked for H$_2$S formation on media with a low content of fermentable carbohydrates, such as SIM medium or LIA. The latter medium, however, is recommended for lysine decarboxylation only in Salmonella and Arizona (18). The use of lead acetate strips for routine diagnostic purposes is not advisable since they are overly sensitive (29).

Whether the intensity of the pink color displayed by our strains on MacConkey agar and SS agar had any diagnostic significance is unclear; further isolations of lac+ Salmonella would have to bear this out.

**ACKNOWLEDGMENT**

This work was supported by United States Public Health Service Grants CA 06519 and GM 18587.

**REFERENCES**

1. Edwards, P. R., New *Salmonella* type possessing hitherto undescribed nonspecific antigen. *J. Hyg.* 37,384–387 (1937).
2. Kauffmann, F., “The Bacteriology of *Enterobacteriaceae*.” E. Munksgaard, Copenhagen, 1966.
3. MacCready, R. A., Reardon, J. P., and Saphra, I., Salmonellosis in Massachusetts. A 16-year experience. *N. Eng. J. Med.* 256,1121–1128 (1957).
4. Seligmann, E., Saphra, I., and Wassermann, M., Salmonella infections in USA: A second series of 2000 human infections recorded by the New York Salmonella Center. J. Immunol. 54,59-87 (1946).
5. Schneierberg, S. S., Herschberger, C., and Honigsberg, R., Salmonellosis at the Mount Sinai Hospital: A ten-year survey (1953–1953). J. Mt. Sinai Hosp. 31,1–9 (1964).
6. Edwards, P. R., and Bruner, D. W., The occurrence and distribution of Salmonella types in the United States. J. Infect. Dis. 72,58–67 (1943)
7. Saphra, I., and Winter, J. W., Clinical manifestations of salmonellosis in man. An evaluation of 7779 human infections identified at the New York Salmonella Center. N. Eng. J. Med. 256,1128–1134 (1957).
8. Seligmann, E., Mass invasion of Salmonellae in a babies' ward. Ann. Paed. 172,406–408 (1949).
9. Saphra, I., and Seligmann, E., Coliforms with complete Salmonella antigens, or lactose-fermenting Salmonellae? J. Bacteriol. 54,270–271 (1947).
10. Seligmann, E., and Saphra, I., A coliform bacterium with the complete antigens of Salmonella newington. J. Immunol. 54,275–282 (1946).
11. Neva, F. A., Nelson, R. J., and Finland, M., Hospital outbreak of infections with Salmonella newington. N. Eng. J. Med. 244,252–255 (1954).
12. Pickett, G., and Agate, G. H., Outbreak of Salmonellosis due to a lactose-fermenting variant of Salmonella newington. Salmonella Surveillance Report No. 57, Center for Disease Control, Atlanta, GA, 1967.
13. Center for Disease Control, Salmonella Surveillance October–December 1973. DHEW Publication No. (CDC) 74–8219, 1974.
14. Blackburn, B. O., and Ellis, E. M., Lactose-fermenting Salmonella from dried milk and milk-drying plants. Appl. Microbiol. 26,672–674 (1973).
15. Falkow, S., and Baron, L. S., Episomic element in a strain of Salmonella typhosa. J. Bacteriol. 84,-581–589 (1962).
16. Easterling, S. B., Johnson, E. M., Wohlhieter, J. A., and Baron, L. S., Nature of lactose-fermenting Salmonella strains obtained from clinical sources. J. Bacteriol. 100,35–41 (1969).
17. Le Minor, L., Coynault, C., and Pessoa, G., Déterminisme plasmidique du caractère atypique "lactose positif" de souches de S. typhi-murium et de S. oranienburg isolées au Brésil lors d'épidémies de 1971 à 1973. Ann. Inst. Pasteur 125A,261–285 (1974).
18. Edwards, P. R., and Ewing, W. H., “Identification of Enterobacteriaceae,” third ed. Burgess Publishing Co., Minneapolis, MN.
19. Bauer, A. W., Kirby, W. M. M., Sherris, J. C., and Turck, M., Antibiotic susceptibility testing by a standardized single disk method. Amer. J. Clin. Pathol. 45,493–496 (1966).
20. Birge, E. A., and Low, K. B., Detection of transcribable recombination products following conjugation in Rec-, RecB-, and RecC- strains of Escherichia coli K 12. J. Mol. Biol. 83,447–457 (1974).
21. Adelberg, E. A., and Burns, S. N., Genetic variation in the sex factor of Escherichia coli. J. Bacteriol. 79,321–330 (1960).
22. Bulmash, J. M., Fulton, Mcd., and Jiron, J., Lactose and sulfide reactions of an aberrant Salmonella strain. J. Bacteriol. 89,259 (1965).
23. Bulmash, J. M., and Fulton, Mcd., Discrepant tests for hydrogen sulfide. J. Bacteriol. 88,1813 (1964).
24. Véron, M., and Gasser, F., Sur la détection de l'hydrogène sulfuré produit par certaines Enterobacteriaceae dans les milieux dits de diagnostic rapide. Ann. Inst. Pasteur 105,524–532 (1963).
25. Padron, A. P., and Dockstader, W. B., Selective medium for hydrogen sulfide production by Salmonellae. Appl. Microbiol. 23,1107–1112 (1972).
26. Hargrove, R. E., McDonough, F. E., and Reamer, R. H., A selective medium and presumptive procedure for detection of Salmonella in dairy products. J. Milk Food Technol. 34,6–11 (1971).
27. Gale, E., The bacterial amino acid decarboxylases. Adv. Enzymol. 6,1–36 (1946).
28. Taylor, W. E., and Schelhart, D., Isolation of Shigellae. VIII. Comparison of Xylose Lysine Deoxycholate Agar Hektoen Enteric Agar, Salmonella-Shigella Agar, and Eosin Methylene Blue Agar with stool specimens. Appl. Microbiol. 21,32–37 (1971).
29. Costin, I. D., Bemerkungen zur Praxis der biochemischen Identifizierung der Darmbakterien in der Routinarbeit. Zentralbl. Bakteriol. Abt. I Ref. 198,385–463 (1965).