250-ml Florence flasks. The mature cultures were centrifuged and the cells resuspended in 1 to 2 ml of the supernatant fluid. Except for the tubes in which the suspensions were dried, the drying technique was then carried out as with leptospirae. Briefly, small volumes (0.04 ml) of equal parts of the treponemal suspension and 20% glucose were dried from the liquid state in vacuo over $P_2O_5$. The suspensions were dried in long narrow tubes (150 × 8 mm) which were felt to be more suitable than conventionally shaped ampules for the subsequent recovery of the organisms. The suspension dried as a coarse foam in the bottoms of the tubes.

Recently, nine tubes from three separate experiments stored at room temperature (approximately 20 C) for 12 months were opened and rehydrated as follows. The tube was opened just above the cotton-wool plug and some freshly prepared medium was pipetted gently in to a depth of approximately 8 cm, care being taken to disturb the desiccate at the bottom of the tube as little as possible. The plug was pushed a little way into the tube and a rubber stopper inserted. The tubes were incubated at 37 C, and all showed flourishing growths in 4 to 5 days. When first rehydrated, the treponemes, as Hampp previously observed, show no animation. However, many active forms begin to appear after incubation for a day or two.

In preliminary work, fluid thioglycolate media (Baltimore Biological Laboratory) was used both for growing batches of the organism for drying and as a recovery medium. The agar content of this medium made harvesting of the organisms somewhat difficult, and it was later replaced by spirolate broth (Baltimore Biological Laboratory), which is agar free and was especially designed for growing the Reiter treponeme. The fluid thioglycolate medium has, however, been retained as a recovery medium, in which role its agar content is of no disadvantage and may even be beneficial. No comparison of the two media for recovery purposes has yet been made. The rehydration procedures have been empirical and based on a consideration of the anaerobic metabolism of the organism.

In previous work (Annear, Austral. J. Exptl. Biol. **36**:211, 1958) it was observed that the recoveries of *Salmonella ndolo*, dried from the liquid state in a mixture of peptone and glucose, varied with the physical form of the desiccates; higher recoveries were obtained when the desiccate was foamy than when it dried as a glassy film. A similar observation was made by Haskins (Can. J. Microbiol. **3**:477, 1957). Although it is unwise to generalize too widely on this observation, it is worth recording that the desiccates of the treponeme in these studies were consistently foamy; this was probably owing to the shape of the tube and to the composition of the drying medium.

## BIOCHEMICAL CHARACTERISTICS OF LACTOSE-FERMENTING *PROTEUS RETTGERI* FROM CLINICAL SPECIMENS

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A number of lactose-fermenting gram-negative rods which appear to be *Proteus* have been found by our laboratory. A few reports of these variants are scattered through the literature. A lactose-fermenting variant of *Proteus* X19 causing false positive Weil-Felix reactions was reported by Welch and Poole (J. Bacteriol. **28:** 523, 1934). Rustigian and Stuart (J. Bacteriol. **49:** 419, 1945) reported one *Proteus vulgaris* and one *P. mirabilis* fermenting lactose. Other reports include a lactose-fermenting *P. rettgeri* (Singer and Bar-Chay, J. Hyg. **52:** 1, 1954) and three lactose-fermenting *P. inconstans*; two by Shaw and Clark (J. Gen. Microbiol. **13**:155, 1955), the third by Proom (J. Gen Microbiol. **13**:170, 1955). The high incidence reported in this paper raises the question of whether these strains are more prevalent than heretofore reported. Our findings may very well be the result of an intensive screening and identification of lactose-fermenting enteric bacteria. They may also be an indication of the existence of a resident or endemic strain within the rather

limited environment of this hospital, since most of them were from urine specimens of patients suspected of urinary tract infection, subsequent to instrumentation of the urinary tract. They occurred in numbers greater than 100,000 bacteria/ml of urine and are considered significant infective agents.

A total of 286 lactose-fermenting variants of *Proteus* species were isolated during a 2-year period. They were distributed as follows: *P. rettgeri*, 258; *P. inconstans*, 11; *P. vulgaris*, 7; *P. morganii*, 6; and *P. mirabilis*, 4. During the same period, 1,527 strains which did not ferment lactose were isolated. These were identified as *P. rettgeri*, 361; *P. inconstans*, 105; *P. vulgaris*, 74; *P. morganii*, 60; and *P. mirabilis*, 927. Table 1 indicates the sources of these bacteria. They were isolated from Levine's eosin methylene-blue agar. Colonies indicating fermentation of lactose were selected for identification. They were classified on the basis of reaction in Kligler's iron agar, utilization of citrate in Simmon's citrate agar, production of phenylpyruvic acid from phenylalanine agar (Ewing, Davis, and Reavis, Pub. Health Lab. **15**:153, 1957), production of indole in 1% tryptone broth, and hydrolysis of urea using Christensen's urea agar. Production of phenylpyruvic acid and hydrolysis of urea were observed after 4 to 6 hr incubation at 37 C. Other reactions were observed after 24 to 48 hr incubation at 37 C.

Because the incidence of lactose-fermenting

TABLE 1. *Source of Proteus isolations*

| Specimen* | Species isolated | | | | | |
|---|---|---|---|---|---|---|
| | *mirabilis* | *rettgeri* | *inconstans* | *vulgaris* | *morganii* | Total |
| Urine | | | | | | |
| $L^+$ | 4 | 240 | 9 | 5 | 5 | 263 |
| All | 604 | 553 | 106 | 55 | 39 | 1357 |
| Wound | | | | | | |
| $L^+$ | 0 | 10 | 0 | 2 | 1 | 13 |
| All | 252 | 44 | 4 | 20 | 24 | 344 |
| Sputum | | | | | | |
| $L^+$ | 0 | 1 | 0 | 0 | 0 | 1 |
| All | 52 | 4 | 0 | 3 | 2 | 61 |
| Blood | | | | | | |
| $L^+$ | 0 | 7 | 2 | 0 | 0 | 9 |
| All | 23 | 18 | 6 | 3 | 1 | 51 |
| Total | | | | | | |
| $L^+$ | 4 | 258 | 11 | 7 | 6 | 286 |
| All | 931 | 619 | 116 | 81 | 66 | 1813 |
| Percentage | | | | | | |
| $L^+$ | 0.4% | 40% | 9% | 9% | 9% | 16% |

* $L^+$ = lactose fermenting.

TABLE 2. *Comparison of biochemical reactions of Proteus rettgeri*

| Strains | Investigators | | | | | |
|---|---|---|---|---|---|---|
| | Sutter (Lactose Pos) | Sutter (Lactose Neg) | Ewing et al. (1960)* | Singer and Bar-Chay (1954) | Shaw and Clark (1955) | Rustigian and Stuart (1945) |
| Total no. | 53 | 23 | 58 | 30 | 9 | 78 |
| Producing: | | | | | | |
| $H_2S$ | 0 | 0 | 0 | 0 | 9 | 0 |
| Indole | 53 | 23 | 58 | 30 | 8 | 78 |
| Phenylpyruvic acid | 53 | 23 | 56 | 30 | 9 | — |
| Utilizing: | | | | | | |
| Citrate | 53 | 23 | 56 | 30 | 8 | 78 |
| Hydrolyzing: | | | | | | |
| Urea | 53 | 23 | 58 | 30 | 9 | 78 |
| Gelatin | 0 | 0 | 1 | 0 | 0 | 0 |
| Producing acid from: | | | | | | |
| Glucose | 53 | 23 | 58 | 30 | 9 | 78 |
| Lactose | 53 | 0 | 0 | 1 | 0 | 0 |
| Sucrose | 53 | 22 | 35 | 27 | 9 | 75 |
| Maltose | 0 | 0 | 1 | 0 | 0 | 0 |
| Mannitol | 53 | 23 | 52 | 29 | 9 | 78 |
| Adonitol | 53 | 23 | 54 | 29 | 9 | — |
| Xylose | 53 | 13 | 9 | 6 | 0 | — |

* Ewing, W. H., I. Saussuna, and I. R. Suassuna. The biochemical reactions of members of the genus *Proteus*. Public Health Service Communicable Disease Center, Atlanta, Ga., 1960.

*P. rettgeri* was exceptionally high, some of the isolates of *P. rettgeri* were further characterized by hydrolysis of gelatin and production of acid from carbohydrates. Liquefaction of gelatin was determined with nutrient gelatin incubated at room temperature from 1 to 30 days. Fermentation of carbohydrates was determined utilizing phenol red broth base with 0.5% carbohydrate and 0.5% agar. These were incubated for 30 days at 37 C before being discarded as negative (Table 2). All of the 53 strains tested produced acid from glucose, lactose, sucrose, mannitol, adonitol, and xylose. Acid was not produced from maltose; gelatin was not hydrolyzed. With the exception of the production of acid from lactose, these results are in agreement with those of other workers.

## FURTHER EVIDENCE FOR THE PRODUCTION OF PYOCYANINE BY NONPROLIFERATING SUSPENSIONS OF *PSEUDOMONAS AERUGINOSA*

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Formation of pyocyanine by nonproliferating suspensions of *Pseudomonas aeruginosa* was reported in an earlier communication (Grossowicz, Hayat, and Halpern, J. Gen. Microbiol. **16**:576, 1957), showing that the synthesis of pyocyanine does not depend upon over-all cellular growth. More recently, Frank and DeMoss (J. Bacteriol. **77**:776, 1959) presented data, of a pattern similar to ours, on the synthesis of pyocyanine by growing cells. However, these authors failed to obtain pyocyanine formation by resting cells, and therefore questioned the nonproliferating nature of the system employed by us; they felt that "it would seem unlikely that so similar results could be obtained in two systems, when only one of them was complicated by cellular growth."

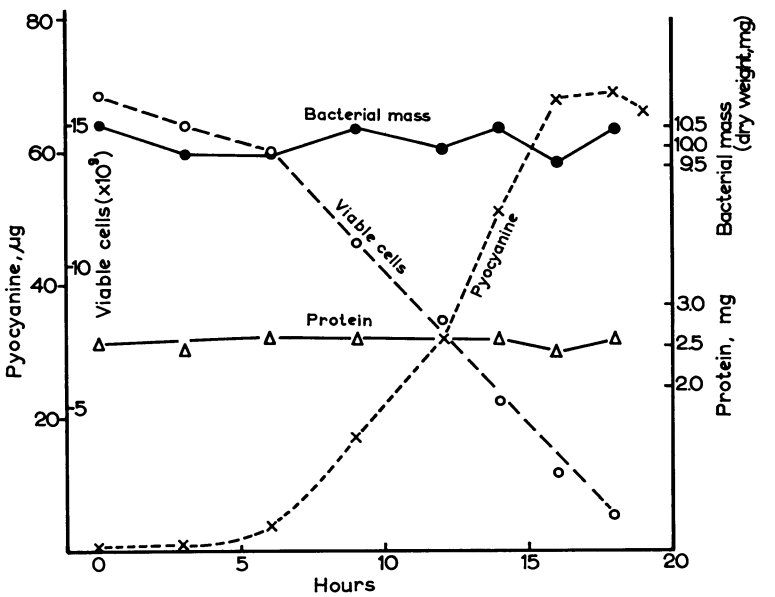


FIG. 1. *Time-course of pyocyanine formation, protein concentration, bacterial mass, and viable-cell count.*

The purpose of the present note is to give further evidence showing that in our system pyocyanine biosynthesis can be entirely dis-