vated, filtered, human plasma or distilled water in place of horse serum, and from 0 to 5,000 μg/ml of methicillin. After incubation under aerobic conditions at 37 °C for 2 to 5 days, typical L colonies developed. These were identified microscopically by Dienes' methylene blue staining procedure (Dienes and Weinberger, Bacteriol. Rev. 15:245, 1951).

Figure 1 shows a typical L colony derived from a penicillin G-resistant strain of Staphylococcus aureus containing numerous large bodies and granular elements. Although all strains produced L-type colonies, induction varied with the concentration of methicillin, antibiotic sensitivity of the strains, and presence of serum. In general, as the concentration of methicillin increased in serum-containing medium, the confluent bacterial growth was replaced by large and small discrete bacterial colonies mixed with, in some instances, a few L colonies and finally only L colonies. Induction of penicillin G-sensitive strains was observed at methicillin concentrations of 1 to 5,000 μg/ml; of penicillin G-resistant strains from 10 to 5,000 μg/ml; and of methicillin-resistant strains from 50 to 5,000 μg/ml. When serum was omitted from the medium, very sparse or no growth of L colonies was observed at any concentration of methicillin, with the exception of those from the penicillin G-sensitive strains. At some inducing concentrations of methicillin, L colonies from these strains appeared to grow even better in the absence of serum.

Induction of L-type colonies from methicillin-resistant and penicillin G-resistant strains was not observed on medium containing 600 to 6,000 μg/ml of penicillin G.

L-type colonies from all but one methicillin-resistant strain have been maintained successfully for ten or more transfers on inducing medium containing 500 μg/ml of methicillin. Gram-positive cocci were observed again in two L cultures (one penicillin G-sensitive and one penicillin G-resistant strain) after two or three passages on methicillin-free maintenance medium.

A FERRICHROME-REQUIRING ARTHROBACTER WHICH DECOMPOSES PUROMYCIN AMINONUCLEOSIDE

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An organism, presumably Arthrobacter flavescens Lochhead (Lochhead, Arch. Mikrobiol. 31:163, 1958), which decomposes puromycin aminonucleoside [6-dimethylamino-9-(3'—amino-3'—deoxy-β-D-ribofuranosyl)] purine] and requires ferrichrome (Neilands, J. Biol. Chem. 206:647, 1953), or its equivalent, for growth has been isolated. [This organism, used by Burnham and Neilands (J. Biol. Chem., 236:564, 1961) for studies on the metabolism of ferrichrome compounds, has previously been designated Arthrobacter JG-9.]

The isolation of this organism is being reported for three reasons. (i) The otherwise conventional enrichment techniques were complicated by the uncommonly observed requirement for ferrichrome. (ii) This organism provides the only biological system known to decompose puromycin aminonucleoside (PAN); a nonspecific riboside hydrolyase from Lactobacillus delbrückii failed to decompose this compound (Takagi and Horecker, J. Biol. Chem. 226:77, 1957). (iii) Finally, the tentative identification of the organism as A. flavescens is complicated by the fact that authentic A. flavescens does not decompose the nucleoside.

The enrichment medium was tap water buffered with 0.05 m potassium phosphate (pH 7.0), containing 0.1% PAN and 0.1% yeast extract. The initial culture (100 ml) was inoculated with about 1 g of a mixed soil sample and incubated in a shallow layer with agitation at 30 °C. The decomposition of PAN was followed by observing the decrease in absorbancy at 270 mμ, the absorption maximum of PAN, in samples suitably

1 This work was done while the senior author was a member of the staff of the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service, and a Senior Research Fellow of the National Science Foundation. Present address: Palo Alto Medical Research Foundation, Palo Alto, Calif.
diluted in 0.1 x HCl. After an initial delay of several weeks, the PAN began to disappear from the enrichment culture, and eventually it was decomposed completely. When a transfer was made to fresh medium, 90% or more of the PAN was decomposed in 48 to 72 hr. The crude enrichment culture could be transferred by serial passage every 3 days, or kept viable without transfer for as long as 1 month at 4 C.

The isolation of the PAN-decomposing organism was made difficult by two factors. (i) In the absence of yeast extract, the decomposition of PAN occurred at an almost imperceptible rate. The yeast extract needed to accelerate the decomposition of PAN also supported the growth of organisms not primarily responsible for the decomposition of PAN. (ii) The PAN-decomposing bacterium is unable to form visible colonies on the PAN-yeast-extract-agar medium unless ferrichrome is added or unless another organism is present that produces ferrichrome or a nutritionally equivalent substance. Since this requirement was not recognized for some time, many attempts to isolate the PAN-decomposing bacterium by streaking plates of a PAN-yeast-extract-agar medium were unsuccessful. Eventually, the active organism was identified as a slowly growing bacterium that formed small satellite colonies close to a large colony of another organism. The PAN-decomposing species was then readily isolated by first streaking a plate of the above medium with a satellite colony and then restreaking the plate at right angles to the first streaks with the growth factor-producing organism. After several days of incubation, satellite colonies of the PAN-decomposing species attained a considerable size and could be isolated without difficulty.

Before the growth factor was identified, it was found that the PAN-decomposing organism would grow in pure culture in a yeast-extract medium with or without PAN when one of the following materials was added: "centrifuge cake" or "SS-69," fermentation products of Hiram Walker Co.; liver concentrate or factor "S" (Eli Lilly & Co.); or blood meal. A variety of known growth factors were tested for ability to support the growth of the organism. The only compounds found to have activity were ferrichrome and coprogen (Hesseltine et al., J. Am. Chem. Soc. 74: 1362, 1952); these compounds stimulated observable growth, measured turbidimetrically in the enrichment medium over 48 hr, at a level of 0.1 μg per ml, and produced maximal growth at 5 to 10 μg per ml. Several related compounds and hemin were later shown by Burnham and Neilands to be able to replace ferrichrome.

The PAN-decomposing organism was identified on the basis of morphology as an Arthrobacter: small, gram-variable rods or cocci, some club-shaped, some curved. M. P. Starr was kind enough to perform the appropriate diagnostic tests, confirming that the organism is an Arthrobacter, probably A. flavescens. In the opinion of A. G. Lochhead, the two organisms belong to the same species. However, authentic A. flavescens does not decompose PAN. The two organisms may be genetic variants of a single species.

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**Salmonella Elsiesrivier, A New Serotype (16:Z4:1,6)**

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This new _Salmonella_ serotype was isolated in February, 1961, from the feces of a 4-month-old suckling child (Cape-colored ethnic group), suffering from diarrhea, who had been bottle-fed by the mother after she had developed a breast abscess. The infant was admitted to hospital soon afterwards.

The isolate had the characteristics shown in Table 1. The biochemical reactions are those of the _Salmonella_ subgenus II.