2.8 g; KH_2PO_4, 1.8 g; and distilled water, 1000 ml. The effects of potassium nitrate and of various concentrations of sodium lactate between 0.05 M and 0.2 M when added to the basal medium were also determined. The media, pH adjusted to 6.7 ± 0.1, were put into 250-ml screw capped pyrex Erlenmeyer flasks in 150-ml amounts and autoclaved at 121.6 C for 15 min. Ten milliliters of a 24-hr broth culture containing 5×10^9 cells (smooth) of *V. fetus* constituted the inoculum for 150 ml of medium. Incubation after inoculation was at 37 C on a gyratory shaker (New Brunswick model C) at 240 oscillations per min with the flasks tightly stoppered. Samples from all specimens were removed after 36 hr incubation and turbidimetric determinations performed using a spectrophotometer at 650 mμ wave length with 19 by 105 mm round cuvettes. Uninoculated medium served as the blank and results were expressed as optical density. Kjeldahl nitrogen determinations were made on each sample using the method of Wong (J. Biol. Chem., **55,** 431, 1923).

Maximal growth was obtained in swirl cultures which contained basal medium and 0.05 M sodium lactate. Lactate in excess of 0.08 M was toxic for most *V. fetus* strains tested. Lactate has been shown to be a utilizable carbon source for *V. fetus* (Alexander, M. Sc. Thesis, Montana State College, Bozeman, 1954; Alexander, J. Bacteriol., **74,** 168, 1957; Lecce, J. Bacteriol., **76,** 312, 1958). It is probable that lactate is oxidized to pyruvate, which then enters the tricarboxylic acid cycle. The presence of nitrate provides a readily available electron acceptor for lactic dehydrogenase when incubation is under reduced oxygen tension. The addition of 0.1 per cent potassium nitrate to the medium resulted in earlier and more rapid growth; however, as nitrites accumulated the growth rate diminished and this resulted in a lower total yield of cells. The yields obtained for different strains of *V. fetus* varied with their individual characteristics. *Vibrio fetus* UCLA strain 270 when grown in basal medium plus 0.05 M sodium lactate for 36 hr typically will produce a change in optical density of 0.67, 6.02 g wet weight of cells per L of medium, and 148.67 mg cellular nitrogen per L. *Vibrio fetus* grown in this manner showed no variation and remained smooth throughout the incubation period.

EGG YOLK AGAR FOR ISOLATION OF COAGULASE-POSITIVE STAPHYLOCOCCI

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The production of opacity in egg yolk broth has been described by Gillespie and Alder (J. Pathol. Bacteriol., **64,** 187, 1952) and used by Alder, Gillespie, and Herdon (J. Pathol. Bacteriol., **66,** 205, 1953) and others in identifying coagulase-positive staphylococci. Colbeck (Can. Services Med. J., **12,** 563, 1956) reported the use of a solid medium containing egg yolk for the isolation of coagulase-positive staphylococci.

This note reports the results obtained when staphylococcus 110 agar containing egg yolk as suggested by Dr. Herman (Sanitary Engineering Branch, National Institutes of Health, Bethesda, Maryland), was employed for the isolation and identification of coagulase-positive staphylococci. One egg yolk was suspended in 100 ml of sterile brain heart infusion broth (BBL) which was then added to 900 ml of sterile cooled staphylococcus 110 agar (BBL).

Surface colonies of coagulase-positive staphylococci produce a zone of precipitation; however, because subsurface colonies did not produce a visible zone of precipitation, pour plates were not satisfactory. Accordingly, streak and spread plates were employed. When observed with transmitted light the zone of precipitation may occur only below the surface of the colony or may extend 4 to 5 mm beyond the periphery of the colony.

Of 35 stock cultures of coagulase-positive staphylococci (12 of which were known enterotoxin producers), 5 (one an enterotoxin producer)

were negative on egg yolk agar after incubating 48 hr at 35 C. Thirty-one stock coagulase-negative strains of staphylococci did not produce a zone of precipitation on egg yolk agar after 48 hr incubation; however, a few produced a zone after an additional 24 to 48 hr incubation.

Examination of frozen food samples by inoculating appropriate dilutions into tubes of cooked meat media + 10 per cent salt, incubating 48 hr at 35 C, and streaking from those tubes showing growth onto staphylococcus 110 agar with and without egg yolk, resulted in the isolation of coagulase-positive staphylococci from 43 of 122 samples. Thirty-two were positive on both egg yolk agar and on staphylococcus 110 agar. Six samples were positive only on egg yolk agar and 5 only on staphylococcus 110 agar. All but one isolate produced a zone on egg yolk agar.

A series of 48 frozen chicken pot pies were examined in the same manner and in addition spread plates made (0.2 ml of 0.1 and 0.01 dilution) directly on egg yolk agar. Thirty-six pies were shown to contain coagulase-positive staphylococci (table 1) and all isolates produced a zone of precipitation when streaked on egg yolk agar. The total number of coagulase-positive staphylococci, when isolated by all three methods, was essentially the same.

TABLE 1

Number of frozen chicken pot pies containing coagulase-positive staphylococci

Streak Plates from Cooked Meat + 10% Salt		Direct Plates
Egg yolk 110 agar	Staphylococcus 110 agar	Egg yolk 110 agar
21	21	21
2	2	Negative
3	Negative	Negative
5	Negative	5
Negative	Negative	5

Since most coagulase-positive staphylococci produce a reaction on egg yolk agar, their isolation and recovery is enhanced.

SCREENING METHOD FOR MICROORGANISMS ACCUMULATING METABOLITES AND ITS USE IN THE ISOLATION OF *MICROCOCCUS GLUTAMICUS*

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Success in producing certain amino acids from carbohydrates and a source of ammonia by microorganisms opened a new field for the fermentation industry (Kinoshita, Advances in Appl. Microbiol., **1**, 201, 1959). In particular, the production of L-glutamic acid by fermentation on an industrial scale has been in practice for some time. An organism, *Micrococcus glutamicus* Kinoshita, which gives very efficient production of L-glutamic acid was first isolated in early 1956 by a new screening method which is described below.

The principle of the screening method is derived from the bioautographic technique which is here applied directly to the microbial colony on an agar plate. A series of plates containing various, defined test media, and one containing nutrient medium, were all inoculated with bacterial isolates of various origins, each at the same relative position on the plate. The organisms were fully grown, and those present on the test media then killed by subjecting the plates to a strong dose of ultraviolet irradiation. The plates were layered with a basal agar medium for glutamic acid bio-assay containing the assay organism *Leuconostoc mesenteroides* strain P-60, dispersed in the medium. During incubation at 37 C, a halo of growth developed around each colony which had produced glutamic acid during the initial incubation. Thus, the bacteria were selected by picking the area of growth on the nutrient agar plate corresponding to the site where colonies were surrounded by halos on the test plates.

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