at re-examination one year later this glycoside was decomposed after 5 days' incubation, subcultures being salicin-positive after 24 hr.

Slide-agglutination tests in sera of all known *Shigella* types, including members of the *alkalescens-dispar* group, *B. scholtensii*, and certain *Shigella*-like cultures were negative. Strong reaction was seen in an antiserum of strain 1831 described by Wheeler and Stuart (J. Bact., 51, 317, 1946). Confirmatory tube-agglutination tests showed that strain "6a" agglutinated to titer in 1831 serum and vice versa. Reciprocal absorption tests revealed that both strains are O-identical.

When it was learned that Wheeler's strain no. 1831 was a biochemical variant of the *Escherichia* group O-22 (Ewing, personal communication), further comparative tests were performed with a reference strain of this group. This strain is still anaerogenic as described by Knipschildt but differs from strains 1831 and "6a" by its strong motility. Formalized and boiled suspensions of reference culture no. O-22 agglutinated in high dilutions 1831 and "6a"-antiserum and reduced the titer of "6a"-antiserum from 1:2,560 to 1:40 with a trace of agglutination up to a dilution of 1:160. Therefore the anaerogenic mannitol-fermenting strain no. 6a must be regarded as another biochemical variant of the *Escherichia* group O-22.

In their studies on *S. alkalescens*, Stuart *et al.* (J. Immunol., 47, 425, 1943) have shown that an intergrading series of strains connects the typical *B. alkalescens* with aerogenic cultures of the *Escherichia* group. Other members of the *alkalescens-dispar* group are likewise related to certain types of *Escherichia coli* (Ewing, W. H., J. Bact., 58, 497, 1949; Frantzen, E., Acta path. et microbiol. Scand., 27, 236, 1950). The occurrence of several fermentative variants within the *Escherichia coli* group O-22 of Kauffmann and Knipschildt is one more example that organisms closely resembling *Shigella* biotypes can be correctly identified and classified as members of the *Escherichia coli* group by serological methods only.

The results of the fermentation test indicate that the biochemical variants of the same serotype may be regarded as offsprings which arise by loss-mutation from parent strains with the typical characteristics of coliform bacteria during the process of evolution.

A SIMPLE PROCEDURE FOR THE PREPARATION OF EGG MEDIUM FOR MYCOBACTERIUM TUBERCULOSIS

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The following simple procedure for the preparation of Jensen's modification of Lowenstein's egg medium, *Egg malachite green medium (F17)*, which has recently been incorporated in the *Standard Methods* of this Division, (Wadsworth,

Williams and Wilkins Co., 1947, p. 194) should be of interest to workers engaged in the preparation of egg media for the growth of *Mycobacterium tuberculosis*. This technic was adopted only after experimental use over a period of months had demonstrated that medium prepared in this way is adequate for growth of this microorganism. It has the advantage over the procedure usually recommended (Bull. W. H. O., 1950, 2, 705) of economy in time and equipment without sacrificing the nutritive qualities of the medium. Thus a base is prepared by autoclaving the solution of salts and potato flour in a flask containing glass beads at 10 pounds for 10 minutes instead of heating the solution of salts at 56 C for 2 hours and then adding the potato flour and cooking in a boiling water bath with constant stirring for 15 to 30 minutes. The presence of the glass beads ensures a smooth mixture and facilitates the blending of the eggs with the base, making their preliminary homogenization unnecessary in a separate container.

Egg malachite green medium (Lowenstein's medium for M. tuberculosis)

Monopotassium phosphate, KH_2PO_4	1.2	g
Magnesium sulfate, MgSO_4	0.12	g
Magnesium citrate.....	0.3	g
L-asparagine.....	1.8	g
Glycerol, A.C.S.....	0-40	ml
Distilled water to make.....	300	ml
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Potato flour.....	15	g
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Fresh eggs (weighed in shell).....	10 eggs or 500	g
Malachite green, 2 per cent solution.....	10	ml

Make a solution of the first group of ingredients and dispense in 300- or 600-ml amounts in 2- or 3-liter pyrex Erlenmeyer flasks containing enough glass beads (20 to 30 mm in diameter) to cover the bottom surface. Add the required amount of potato flour to each flask and sterilize in the autoclave at 115 to 118 C (10 lbs) for 10 minutes. A supply of this sterile base may be stored in the cold room. As required, warm the base to 50 to 54 C, then add the eggs and brilliant green.

Clean the eggs thoroughly with wet gauze, place in a container, cover with 80 per cent alcohol (by volume), and let stand from 30 to 45 minutes. Remove the eggs and dry on clean filter paper.

To add the egg contents to the base, carefully break the shell in half and drop the whole contents into the flask. When all the eggs have been added, shake the flask vigorously until the egg yolks are completely broken and thoroughly blended with the base. Add the malachite green and again shake until thoroughly mixed. Dispense aseptically through a sterile drain with strainer (see figure 1) 5 to 6 ml amounts in sterile 20- by 125-mm tubes with screw caps. Cap loosely and inspissate at 85 C for 50 minutes. When cool, tighten the caps securely.

The tubes and caps are dry sterilized at 160 to 175 C for 1 hour; the tubes without caps are packed inverted in perforated stainless steel or wire-mesh baskets which hold about

100 tubes each. Then the baskets are enclosed in a heavy brown paper bag; a smaller paper bag contains a corresponding number of the caps.

Figure 1. Assembly for dispensing egg medium

Sterile drain with strainer is inserted, the stopper is tied in securely, and the flask is inverted. The flask and delivery tip then are clamped into position.

The strainer is a resistant metal tube about 1 cm in diameter. The open end extends through the stopper and is connected to the delivery tip by means of a rubber tube; the closed butt extends about 10 cm into the flask and has between 50 and 100 perforations which are slightly smaller than the smallest beads.