NOTES

UNUSUAL *SALMONELLA* TYPE WITH THREE "NORMAL" FLAGELLAR ANTIGENS

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A number of *Salmonella* serotypes that contain three or more separable flagellar components have been recognized. Some of these display multiple reversible H phases of uncomplicated constitution (Edwards et al., Japan. J. Med. Sci. Biol. **15**:111, 1962), and others are diphasic forms in which both phases are unusually complex (Edwards, McWhorter, and Douglas, J. Bacteriol. **84**:95, 1962; Douglas and Edwards, J. Gen. Microbiol. **29**:367, 1962). The latter types may be divided into two categories, one of which contains only well-known and long-accepted *Salmonella* flagellar antigens, such as *S. salinatis* (*4,12:d,e,h:d,e,n,z*$_{15}$) and *S. montgomery* (*11:d,a:d,e,n,z*$_{15}$), while the other contains organisms which possess newly recognized antigens (*z*$_{43}$, *z*$_{45}$, etc.) as the major component of each of the two phases. Kauffmann (*Die Bakteriologie der Salmonella Species*, Munksgaard, Copenhagen, 1962) considers these newly found antigens as "R phases" and groups them with the long-recognized "induced" phases (*j,z*$_5$, etc.) which emerge when "normal" or "S phases" are cultivated in homologous antisera. The organism described here contains only "normal" phases.

The *Salmonella* culture 5888-61 was isolated by L. A. Page of the University of California from a mealy worm (*Tenebrio mollitor*), which was one of a lot being propagated as food for striped plateau lizards (*Sceloporus undulatus virgatus*). Examination of the worms was occasioned by continued deaths among the lizards. The lizards apparently were infected with Arizona serotype *20:13,14*, which was isolated from the livers of several of the reptiles at autopsy but was not found in the mealy worms. So far as is known, the *Salmonella* type had no deleterious effect upon the worms. Its ultimate source was not discovered but it may well have been blood meal used to feed the worms.

Culture 5888-61 was a member of *Salmonella* subgenus I (Kauffmann, Acta Pathol. Microbiol. Scand. **49**:393, 1960). The organism possessed the biochemical properties characteristic of subgenus I, with the exception that it failed to utilize mucate. The culture was a member of *Salmonella* O antigen group B, contained O antigens *4,12* and in absorption tests removed all O agglutinins from *S. abortivoequina* antiserum. When received, the organism was flocculated to the titer of *S. typhi* H (*d*) serum but not by diagnostic dilutions of other *Salmonella* H sera. When the culture was placed in tubes of semisolid medium which contained *d* serum for the isolation of phase 2, no migration through the medium was noted until after 48 to 72 hr of incubation. From the spreading growth a form was isolated which was agglutinated only by *S. paratyphi* A H (*a*) serum. Such forms, when placed in semisolid medium containing *a* serum, were immobilized. Ten single colonies of the *a* form, each obtained from a different colony of the original *d* form, were transferred serially four times in tubes containing *a* serum. On the fourth transfer, growth in two of the ten tubes spread rapidly throughout the medium, and from these tubes forms were recovered which were agglutinated to the titer of *S. bredeney* phase 2 H (*1,7*) serum. From these results, it was evident that a *Salmonella* culture was involved in which the change from typical phase 1 antigens [*d;a;* or *d,(a)*] to typical phase 2 antigens was accomplished only with difficulty; i.e., the rate of phase variation was extremely low. This also was borne out by failure to find naturally occurring phase 2 colonies in the original culture, despite diligent search.

The antigenic changes observed in the culture may be summarized as follows.

$$4,12:d \xrightarrow{d} 4,12:a \xrightarrow{a} 4,12:1,7 \xrightarrow{1,7} 4,12:a \quad (1)$$

$$4,12:d \xrightarrow{d+a} 4,12:j \xrightarrow{j} 4,12:a \xrightarrow{a} 4,12:1,7 \xrightarrow{1,7} 4,12:a \quad (2)$$

The symbols on the arrows indicate antisera added to the semisolid medium in which the organism was cultivated. In no case was the *d* antigen recovered once the flagellar antigens were changed to *a* or *1,7*. The change from antigen *d* to antigen *j*, when the organism was placed in a combination of *d* and *a* sera, is a reflection of the very low rate of phase variation that exists in the culture, and the difficulty of obtaining the *1,7* phase. Antigen *j* is an "induced" or "artificial" form which, under appropriate conditions, can be obtained with relative ease from any *Salmonella* type which contains antigen *d*. It is noteworthy that further progressive changes in the H antigens were not influenced by the change from *d* to *j*.

Although they were agglutinated to titer by the corresponding sera, neither the *d* nor *a* antigens of 5888-61 were identical respectively with the *d* and *a* antigens of *S. typhi* and *S. paratyphi* A. On absorption, a residue of 5 to 10% of the original titer for the homologous organism remained in each serum. On the contrary, the *1,7* phase effected a complete removal of H agglutinins from *S. bredeney* phase 2 serum.

The results obtained in the study of 5888-61 are entirely compatible with the hypothesis that the organism is comparable to *S. salinatis* and *S. montgomery*. In these types, antigen *d* is the major component of both phase 1 and phase 2, and both phases undergo variation resulting in the irreversible loss of the *d* factor from each phase, thus giving rise to simpler forms corresponding to known *Salmonella* serotypes of unexceptional antigenic constitution. Since the low rate of phase variation in 5888-61 precluded the isolation of phase 2 without resort to selection through growth in antisera and consequent loss of antigen *d* from phase 1, it was not possible to isolate phase 2 components in the natural state. Nevertheless, it is believed that the culture represents a type having the antigenic formula *4,12:d,a:d,1,7* and that it may be regarded as a complex form of *S. arechavaleta* (*4,12:a:1,7*).

TECHNIQUE OF ANAEROBIC CULTURE ELIMINATING REDUCING AGENTS IN THE MEDIUM[1]

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A method for obtaining and maintaining strict anaerobiosis in individual culture tubes has long been a serious problem for the bacteriologist. The method described here has been found to work successfully with obligate anaerobes isolated in this laboratory.

The extent of the exclusion of oxygen was measured by using methylene blue (Society of American Bacteriologists, *Manual of Microbiological Methods*, p. 122. McGraw-Hill Book Co., Inc., New York, 1957). The ability to maintain the dye in the leuco state was the accepted degree of anaerobiosis. This degree of anaerobiosis could be maintained by using a mineral oil layer 1 cm deep and a nitrogen atmosphere. The layer of oil was kept thin so it would not be an obstruction to inoculation with a loop or needle.

Portions (10 ml) of a freshly prepared liquid

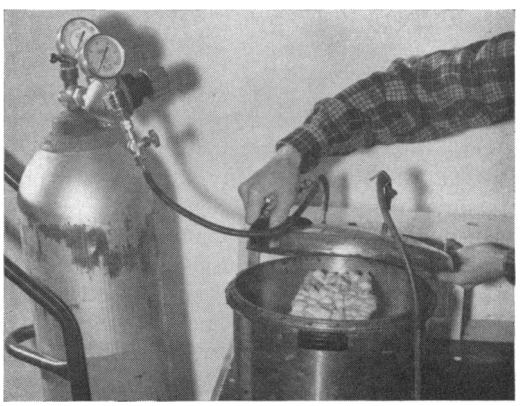

FIG. 1. *Modified pressure cooker used as an anaerobic chamber.*

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