ter, which is bent into a right angle and the lower end sealed off. The accompanying figure indicates the shape and dimensions of the tube. An important feature is the small shelf projecting into the lumen at the bend. This is made by accentuating the wrinkle produced when the tube is bent, and shaping it in the direction and form indicated. This serves to catch any food the rats might scratch out, and to cause it to drop back into the bulb. The bulb as drawn will hold about 75 gm. of diet. Its size may be altered by blowing it larger or smaller as might be needed for any special purpose. Tubing of the diameter illustrated here may be used with rats weighing up to 250 gm. If larger animals are to be used, a tubing of larger diameter would be desirable.

6872

Experiments on the Use of the K Medium.

EDITH L. SWINGLE AND STEWART A. KOSER.

From the Department of Hygiene and Bacteriology, University of Chicago.

One of the most striking of recent contributions on bacterial variability has been that of Kendall,[1] who reported the preparation of a special medium in which ordinary bacteria developed a filterable or "virus-like" stage. A number of the commoner bacteria, when inoculated into this medium, were converted within a short time to a form which passed Berkefeld filters and which could not be cultivated on ordinary media. Not only was the "K" medium effective in promoting such a change, but it was claimed to be effective also in bringing about the opposite process, namely reversion from the filterable form to the usual visible organism.

Since such a method, if effective, would place in our hands a valuable tool for the study of many debated problems on bacterial variability, an investigation of the K medium was undertaken in this laboratory.

Several different lots of this medium were employed. One was prepared from hog intestine according to Kendall's directions. After the alcohol and benzene extractions, the preparation was dried, ground and kept in a stoppered container. At the time of use, the proper quantity of the powder was placed in test tubes or flasks

[1] Kendall, *Northwestern University Bull.*, 1931, **32**, No. 5, 8.

and Tyrode solution added to give a 2% suspension. The pH of the medium was adjusted to 7.4. After autoclave sterilization the medium was somewhat turbid with considerable sediment. In addition to our own preparation, 2 experimental lots prepared by the Difco laboratories were used. The first of these, also prepared strictly according to Kendall's directions, produced a medium quite similar in appearance to our own. The second had been subjected to a more thorough extraction and washing and resulted in a much clearer medium.

With these different preparations an attempt was made to convert several of the common bacteria to a filterable stage and then, after filtration, to cultivate them in the K medium and again establish growth on ordinary media. Laboratory stock cultures of the typhoid bacillus and of *Staphylococcus aureus* were used.

Three different procedures were employed. In the first series of experiments cultures were inoculated into 150 cc. amounts of K medium in flasks and held at 37°C. Samples were withdrawn for filtration after 48 hours, one week, and after 3 to 4 weeks. In the second series of experiments each culture was subjected to 5 successive transplants at 3-day intervals in the K medium. Following the incubation period each culture was held in the ice box until the series was completed, at which time the last 3 K medium tubes of each series were combined for filtration.

In the last series, repeated transfers in the K medium were also employed but in this instance transfers were made daily for 12 days. After transfer each culture tube was held in the ice box. Three separate filtration tests were made on each of these series of K medium cultures: the first 3 tubes were combined and filtered, then the middle 3 of the series (numbers 5, 6 and 7) and finally the last 3. This gave filtrates of cultures which had been passed through varying numbers of transfers in K medium.

Both *Eberthella typhi* and the *Staphylococcus* developed readily in the several different lots of K medium and produced a distinct clouding within 24 hours. In a number of cases agar plates were made from the K medium for purposes of colony study. The colonies which developed were uniformly smooth colonies. Rough colonies or the G forms of Hadley did not appear.

Microscopic examination of the cultures in K medium likewise failed to reveal any real departure from the usual morphological appearance and staining reactions of these organisms. In one series of experiments dark-field examinations were made of the typhoid

bacillus and the staphylococcus after 12 successive transfers in the K medium. The organisms, however, presented much the same appearance as controls in the nutrient broth. Dark field examinations impressed us as being rather unsatisfactory since many particles of various sizes are to be seen in the turbid K medium, whether sterile or after development of a culture.

For filtration tests, both Berkefeld N and Seitz filters were used. Since the Berkefeld has been the standard type of filter chief significance was attached to it and the Seitz filters were used more as a matter of interest. A preliminary test of all filters was made by applying 24-hour broth cultures of *Serratia marcescens* and filters were not used unless the filtrate proved to be sterile. Altogether, 48 filtrations of K medium cultures were made with Berkefeld N candles and 24 with Seitz filters.

In testing for the presence of filter-passing forms, the K medium filtrates were usually examined as follows: 5 cc., 2 cc. and 0.25 cc. amounts of filtrate were inoculated into separate K medium tubes and also into tubes of dextrose veal infusion broth. A few drops were also placed on the center of an agar plate and the material transferred from plate to plate every 48 hours after the manner of the Hauduroy technic. Each plate was examined with the microscope before transfer was made to the next plate.

Growth could not be detected in any of these media, except on a few occasions when the filters were evidently at fault and the normal form of the typhoid bacillus appeared in all cultures within 24 hours. The tiny dew drop colonies which are supposed to represent an early stage in reversion from the filter-passing form to the normal were not seen in any of our experiments.

Our results, therefore, failed to confirm those of Kendall and we could obtain no evidence that the K medium was effective in promoting the appearance of a filterable stage of the organisms which we employed. This conclusion agrees with several other reports which have recently appeared.[2]

[2] Craig and Johns, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 661. Varney and Bronfenbrenner, *Ibid.*, 1932, **29**, 804. Seastone and Lawrence, *J. Infect. Dis.*, 1933, **52**, 20. Carpenter and Long, *J. Bact.*, 1933, **25**, 241.