Bull. Inst. Zool., Academia Sinica **3**: 45-46 (1964)

Preliminary Reports and Notes

NOTE ON A MODIFIED METHOD FOR THE PREPARATION OF AXENIC CULTURE OF CILIATES

MOU-LIANG CHEN[1] AND TZU LIN[2]

Received for publication May 12, 1964

Axenic growth in chemically defined medium is generally essential in nutritional and metabolic studies on ciliated protozoa. The V-tube technique of Glaser and Coria (1) and Glaser(2) and Claff's migration-dilution technique(3) have currently been employed for the preparation of bacteria-free and pure cultures of protozoa. Difficulties are usually encountered with some fast moving ciliates where repeated migration and skillful manipulation are necessary. With the attempt to use a ciliate of the native strain, *Tetrahymena pyriformis* MC, isolated in this laboratory for the screening of protein quality and the studies on metabolic changes of the ciliates in media of different nitrogen sources, both techniques were used to prepare axenic culture of the ciliates. Nevertheless, these methods showed difficulty to achieve such purposes. In V-tube technique, it was usually unable to wash the ciliates completely free from contaminated micro-organisms by repeated migration in a relatively short distance. With migration-dilution apparatus, results showed that only a very narrow margin of bacteria-free collection could be obtained with extreme care in operation.

In our later experiments, satisfactory results of bacteria-free collections could be obtained by using a modified migration-dilution apparatus. In the modified system (*Fig. 1*), an extra inlet tube was introduced to the neck of each of the six migration flasks about an inch below the top and led to a second reservoir. The filling of the system, sterilization, and injection of concentrated ciliate suspension into the lower part of the first migration flask were essentially the same as the original method. As the migrated ciliates accumulated and reached in the upper part of the first flask, instead of using the first reservoir led to the lower connection tube, fluid was admitted from the second reservoir by opening the upper inlet tube and desired number of ciliates was pushed into the lower part of the second flask. Similar operations were carried out in the subsequent pushings and a series of overflowing fluid was obtained at the end of the system. Such an arrangement and operation permitted a steady flow of fluid in the system and minimized turbulence in the contents of the lower part of flasks during each pushing, and thus reduced the possibility of carrying along of contaminated bacteria supposed to be stationary in the lower part of flasks. With the modified method, results showed that bacteria-free cultures up to five (6th to 10th) 2-ml collections could be obtained from the overflowing fluid at the end of the system, whereas with unmodified system, one collection could be occasionally

1 Assistant Professor, Department of Biochemistry, National Defense Medical Center, Taipei, Taiwan.
2 Assistant, Department of Biochemistry, National Defense Medical Center, Taipei Taiwan.

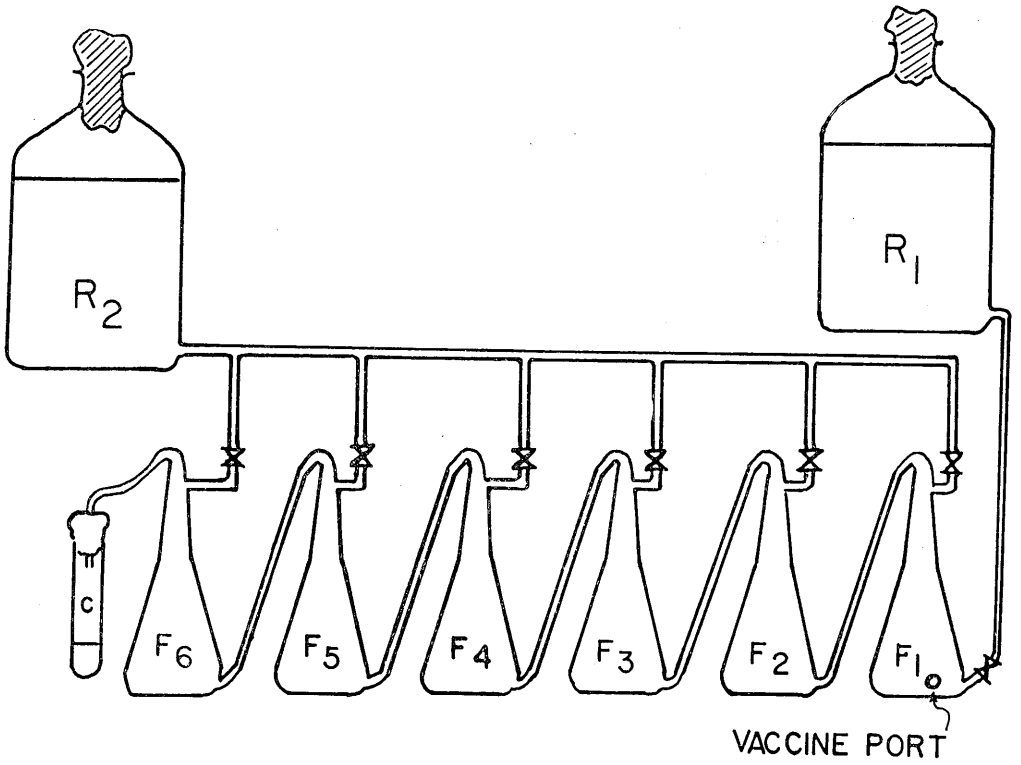

Fig. 1. Modified migration-dilution apparatus. R_1 and R_2 represent the first and second reservoirs respectively, F_1 to F_6 stand for the first to the sixth flasks. C is the collecting tube.

bacteria-free only with extreme care in operation, such as injection of ciliates into the system and very slow rate of pushing. Therefore, for preparing axenic cultures of fast free-moving ciliates, the present modification is considered more convenient than the original one.

LITERATURE CITED

1. GLASER, R. W. and N.A. CORIA. 1930. Methods for the pure culture of certain protozoa. *J. Exptl. Med.* **51**: 787-806.
2. GLASER, R. W. 1943. *The germ-free culture of certain invertebrates.* in *"Micrurgical and Germ-Free Methods"* (J. A. Reyniers, ed.) Charles C. Thomas, Springfield, Illinois. pp 164-187.
3. CLAFF, C. L. 1940. A migration-dilution apparatus for the sterilization of protozoa. *Physiol. Zool.* **13**: 334-341.