must subsequently undergo a heat sterilization which usually causes some increase in particle size.

Conclusion. Supersonic radiation appears to be the most suitable method yet studied for preparing fat emulsions, suitable for intravenous use in man.

8866 C

Enlarged Tissue Cultures of European Typhus Rickettsiae for Vaccine Production.

HANS ZINSSER AND ATTILIO MACCHIAVELLO.*

From the Department of Bacteriology and Immunology, Harvard University Medical School, Boston, Massachusetts.

Our methods of producing typhus vaccines with the murine strains of Rickettsiae has depended upon the inoculation of rats in which resistance had been reduced by a variety of procedures, the most consistently successful of which has been preliminary X-ray radiation. These methods have persistently failed to give adequate results with the European virus. This fact in itself constitutes further strong evidence that the two types of Rickettsiae are distinct and biologically fixed varieties. After much effort to apply the "rat" methods to the organism of the classical European disease we were finally persuaded that other methods of approach must be sought for obtaining accumulations of the European Rickettsiae adequate for practical purposes of immunization.

In a paper, now in press, the writers have reported the results of experiments in which the active immunization of guinea pigs against the European typhus virus was accomplished, both with the use of formalinized tissue culture vaccines and by methods of sero-vaccination. While this work was going on, Kligler and Aschner[1] published observations on a method of successful animal immunization with formalinized tissue culture vaccine, similar in all important principles to the method which we are using.

Since the development of a tissue culture technique for producing vaccines in amounts adequate for practical purposes appeared the most hopeful direction of effort, we have tried for a long time to improve the technique of making such cultures, particularly in regard to increasing the volumes of the tissue cultures themselves.

*Guggenheim Fellow.

1 Kligler, I. J., and Aschner, M., *Brit. J. Exp. Path.*, 1934, **15**, 337.

In studying the general problem of tissue cultures, one of the writers (Zinsser) in collaboration with Schoenbach, turned his attention to observations on what may be called the physiology of tissue cultures in general, particularly as regards changes in pH, oxygen consumption and oxydation-reduction potentials.† This work is far from complete and will not be reported until more extensive studies have been made. But as far as its preliminary results influenced our conception of Rickettsiae cultivation, it appears safe to make the following statements: In tissue cultures set up in the ordinary manner with guinea pig serum-tyrode solution and tunica vaginalis material from medium-sized guinea pigs, the oxygen consumption reaches its maximum and flattens out at the end of 40 to 46 hours. The potential distinctly and consistently rises, also reaching its maximum at about 40 hours. The pH usually shows a gradual change toward the acid side from an initial pH of about 7.8 to 7 to 7.2 at the end of 6 or 7 days. Thus, although the changes in the factors mentioned slow down and become more or less stabilized in such cultures before the end of the first 48 hours, the most active growth of viruses usually occurs after the 2nd day and that of Rickettsiae does not seem to take place until after the 5th to the 7th days. It would seem, therefore, that the conditions favoring growth depended upon the preliminary establishment of some sort of an equilibrium. It will not, of course, be possible to formulate any conception of what this equilibrium is or whether the measured changes have any direct significance, for the growth of Rickettsiae or virus agents, until far more extensive work along the lines mentioned has been carried out with cultures in which Rickettsiae and virus are actively growing. Even then physico-chemical interpretation may be impossible in systems so complicated.

Meanwhile, however, it seemed logical, in endeavoring to increase the volume and consequently the yield of typhus tissue cultures, to take these matters into consideration and to try to adjust culture volumes to air volumes in the larger flasks in a proportion comparable to that usually successful in the small 30 cc. flasks. In these, with 3 cc. of culture the cubic volume of fluid to the closed air space above it, is about 1 to 10. At the same time the amounts of tunica vaginalis tissue, while not actually measured were kept approximately proportionate to the larger amounts of fluid. In this

† Since in the ordinary smaller cultures the total oxygen consumption rarely exceeded 130 cubic millimeters, indicating a maximum of not over 800 in the larger cultures, it is most likely that the respiratory activity of the tissue is of less importance than the relative volumes of fluid and air placed in the sealed space.

way it is hoped that we might, by experimental trial and error, achieve an optimum at which the eventual ratio of oxygen and carbon dioxide in the culture fluid would favor growth. It may be stated that in earlier experiments, with large flasks in which we had paid no attention to these relations and had used only enough culture fluid to cover the bottoms in thin layers, either no growth or inadequate multiplication of Rickettsiae occurred.

The method as it is now successfully practiced is carried out in special flasks so constructed that the bottoms are flattened and the necks narrowed, the latter feature making it easier to seal and to avoid contamination. They are easily made from ordinary 250 cc. pyrex Erlenmeyers. Such a flask is represented in the figure. The volume of the altered 250 cc. flask is approximately 235 cc. not including the narrow necks.

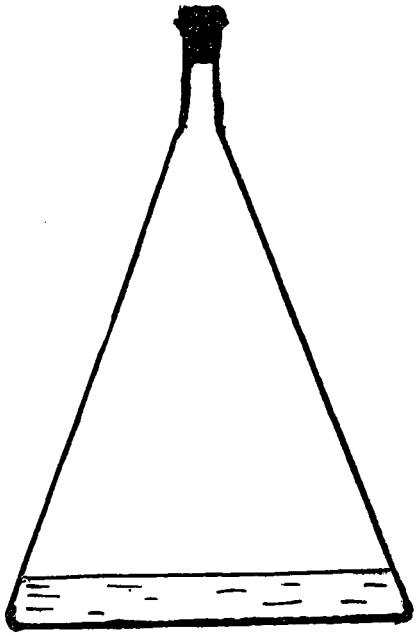

FIG. 1.
Modified 250 Erlenmeyer flask for Typhus Tissue Culture.

Experiment has shown that the optimum amount of culture fluid for these flasks is about 20 cc. which gives a volume ratio of fluid to air space of 1 to 11 or 1 to 12 in different flasks. It is of the greatest importance to avoid an excess of tissue and to mince the tissue in such a manner that individual bits do not much exceed the size of a pin head. The tissue need not be accurately measured, but the proportion should be kept more or less as this is adjusted in the

ordinary Maitland cultures in the 30 cc. flasks as recommended by Nigg and Landsteiner.[2]

By using this method we are obtaining with reasonable regularity, considerable yields of European Rickettsiae in these large flasks. We have had positive results not only when the culture fluid consisted of guinea pig serum, one part to 3 or 4 of Tyrode's solution, but also when human serum or horse serum was mixed before filtration in proportions of 1 to 4 with the Tyrode solution. Indeed we seem to be getting our best Rickettsiae yields in guinea pig tissue with the horse serum Tyrode solution preparations. The best time for "harvesting" is on the 8th or 9th day of incubation at 37°C. As the cultures are going at present it should be possible to obtain 5 or more doses of vaccine from each of these flasks. And in view of observations that such tissue culture vaccines, treated with 0.1% formalin produce active immunization of guinea pigs against the European strain of typhus virus, the method offers practical possibilities for the mass production of such vaccines for trial on man.

8867 C

Serum Phosphatase in Cats with Total Bile Stasis.*

A. CANTAROW, H. L. STEWART AND S. G. MCCOOL.

From the Laboratory of Biochemistry, Jefferson Medical College Hospital, and the Department of Pathology, Jefferson Medical College, Philadelphia.

The observation by Roberts of an increase in serum phosphatase activity in patients with jaundice has been confirmed by a number of investigators. Similar results have been obtained in dogs with experimentally produced obstructive and nonobstructive hepatic jaundice[1, 2, 3] and elevated values have been reported in portal cirrhosis.[4, 5] In view of the uniformity of the previously reported data, particularly in obstructive jaundice, and the importance of this

2 Nigg, C., and Landsteiner, K., *J. Exp. Med.*, 1932, **55**, 563.

* Aided by the Jefferson Hospital Tumor Clinic and the Martin Research Fund.

1 Bodansky, A., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1179.

2 Hartman, F. W., and Schelling, V., *Arch. Path.*, 1934, **18**, 594.

3 Armstrong, A. R., and King, E. J., *Canad. Med. Assn. J.*, 1934, **31**, 14.

4 Greene, C. H., Shattuck, H. F., and Kaplowitz, L., *J. Clin. Invest.*, 1934, **13**, 1079.

5 Herbert, F. K., *Brit. J. Exp. Path.*, 1935, **16**, 365.