I. H., *J. Lab. Clin. Med.*, 1951, v38, 213.
2. ———, *A. M. A. Arch. Path.*, 1952, v53, 217.
3. Masson, G. M. C., del Greco, F., Corcoran, A. C., and Page, I. H., *A. M. A. Arch. Path.*, 1953, v53, 23.
4. ———, *Am. J. Med. Sci.*, 1953, v226, 296.
5. Masson, G. M. C., Plahl, G., Corcoran, A. C, and Page, I. H., *A. M. A. Arch. Path.*, 1953, v55, 85.
6. Edman, P., *Arch. f. Kemi. Minerologi o Geolgi*, 1945, v22, 1.
7. Masson, G. M. C., del Greco, F, Corcoran, A. C., and Page, I. H., PROC. SOC. EXP. BIOL. AND MED, 1953, v83, 631.
8. Hughes-Jones, N. C., Pickering, G. W., Scarborough, L. H., and Vanderbroucke, J. J., *J. Physiol.*, 1949, v109, 288.

Received July 16, 1953. P.S.E.B.M., 1953, v84.

A Closed-Vacuum System for Harvesting Infectious Infant Mouse-Brain Material. (20619)

C. P. A. STROME.* (Introduced by J. Casals.)

From U. S. Naval Medical Research Unit No. 3, Cairo, Egypt, Department of Virology; operated jointly by the U. S. Navy and the Division of Medicine and Public Health of Rockefeller Foundation.

The method developed by Casals, Olitsky, and Anslow(1) for obtaining high-titer complement-fixation antigen from infected suckling mouse brain has demonstrated the advantages of fewer anti-complementary and anti-mouse brain reactions in the serological study of certain neurotropic viruses. The method of antigen production, using a benzene extraction, has been adapted in this laboratory for the serological screening of human and vertebrate animal blood collected in Egypt and the Sudan (Taylor(2)) for immunity to West Nile and Ntaya viruses (Smithburn *et al.* (3); Smithburn and Haddow(4)).

The procedure, however, requires the tedious, time-consuming, and exposed harvesting of infectious mouse-brain material by laboratory personnel in order to obtain a sufficient quantity of material for extraction. These disadvantages have been partially overcome by a closed-vacuum, infant mouse-brain harvester developed in this laboratory.

Apparatus. The apparatus consists of a bluntly beveled 15-gauge needle attached by rubber tubing to a specimen vial and vacuum system. The needle is inserted into one end of a 6-inch section of catheter tubing; the other end of the tubing is attached to a right-angle glass tube that passes through one hole of a 2-hole rubber stopper placed in a specimen vial. The other hole of the stopper is connected to a vacuum system with an intervening trap flask containing chlorine solution. The needle and the rubber attachments are sterilized separately, and are connected with the specimen vial and vacuum system immediately before use. To assure instantaneous chilling and freezing of the brain material, the specimen vial is immersed in a bath of alcohol and CO_2 ice.

Technic. The mice whose brains are to be harvested are placed under deep ether or chloroform anesthesia and, if desired, exsanguinated. The head is cleansed with alcohol and the needle passed through the occiput of the skull. The vacuum, which is controlled by pressing the catheter tubing over the end of the needle attached to the tubing, is then applied by releasing the pressure, and the brain matter is sucked through the needle and tubing and deposited in the specimen vial. The aspiration of the brain is evidenced by the collapse of the skull. The vacuum is then closed until the next brain is harvested. A hundred mouse brains can be harvested completely in less than an hour by this method. Not only does it serve quickly and efficiently to collect all brain material, but it prevents open exposure of virulent material to persons harvesting highly infectious virus. The above technic has been used successfully in this laboratory to harvest brain material from infant mice up to 12 days of age.

* U. S. Naval Medical Research Institute, Bethesda, Md.

Summary. A safe, time-saving, closed-vacuum harvesting technic is described. The advantages and safeguards of this technic are enumerated.

1. Casals, J., Olitsky, P. K., and Anslow, R. O., *J. Exp. Med.*, 1951, v94, 123.

2. Taylor, R. M., 1953 Ann. Rep. to The Rockefeller Foundation of Dept. of Virology, U. S. Naval Med. Research Unit No. 3, Cairo, Egypt, Unpublished.

3. Smithburn, K. C., Hughes, T. P., Burke, A. W., and Paul, J. H., *Am. J. Trop. Med.*, 1940, v20, 471.

4. Smithburn, K. C., and Haddow, A. J., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 130.

Received August 5, 1953. P.S.E.B.M., 1953, v84.

Improved Method for Determination of Plasma Polysaccharides with Tryptophan.* (20620)

JACQUES BADIN,† CLIFFORD JACKSON, AND MAXWELL SCHUBERT.
(Introduced by Morris Ziff.)

From the Department of Chemistry and the Study Group on Rheumatic Diseases, New York University College of Medicine, New York City.

There is currently an active interest in the determination of the polysaccharide content of plasma and other biological fluids as related to different diseases. The tryptophan reaction proposed by Shetlar, Foster, and Everett(1) has, for clinicians, certain advantages over the orcinol reaction of Sorensen and Haugard(2). The stock tryptophan solution can be kept for several days on ice; the production of the color at 100°C does not call for the greater care required in the orcinol reaction which must be run at 80°C; and the color produced is less affected by impurities in the reagents. As described, the tryptophan reaction has the serious failing of giving colored products whose absorption spectra differ widely with different sugars. For instance, glucose gives a yellowish-brown color while mannose gives a pure violet. It will be shown that when the reaction is carried out in the presence of boric acid this failing is diminished. The best results are obtained with the highest possible concentration of boric acid.

Reagents. 1) Absolute ethanol. 2) Boro-sulfuric acid, containing sulfuric acid (770 ml), water (230 ml) and boric acid (50 g). For comparison, a sulfuric acid solution is prepared omitting the boric acid. 3) Tryptophan (1 g) dissolved in boiling water (100 ml). The L-form is preferable to the DL-form because it is more soluble and solutions kept on ice do not crystallize. 4) Standard mannose solution (50 to 150 μg/ml). This can be kept on ice for several weeks in the presence of a few drops of toluene.

Procedure. The reaction is best carried out in carefully cleaned matched tubes that are to be used in the photometer. Serum (.05 ml) is introduced, and then absolute alcohol (about 10 ml). After 15 minutes the tube is centrifuged at 2500 rpm for 10 minutes, the alcohol is decanted, water (1 ml) is added and the precipitate is dispersed. From a burette, boro-sulfuric acid solution (7 ml) is added and the contents of the tube are immediately mixed and chilled in ice water for 5 minutes. After leaving the tube at room temperature for 15 minutes, the tryptophan solution (1 ml) is added. The contents of the tube must be mixed immediately. Because of the high density of the sulfuric acid solution this is best done by bubbling air through it. The tube is again chilled in ice water. At the same time a series of 6 or 8 tubes is similarly prepared containing different amounts of the standard mannose solution, each made up to 1 ml before addition of the boro-sulfuric acid. All the tubes are heated in a boiling water bath for 20 minutes, and the contents again mixed once or twice during this time. The tubes are then

* This work was supported by research grant from the National Institute of Arthritis and Metabolic Diseases, of the National Institutes of Health, Public Health Service, and by the Masonic Foundation for Medical Research and Human Welfare.

† Aided by a Fulbright Smith-Mundt Fellowship.