two inches in diameter so that the hair will not become entangled in the cannula when the wing-nut is tightened. Having removed the hair, cut a slit about a half inch long between and parallel to the borders of adjacent ribs. You may then thrust the cannula quickly through the muscle tissue between the ribs, turn it through 90° quickly and then fasten the wing-nut. We have found it more satisfactory, however, to incise the intercostal muscles and parietal pleura because occasionally the cannula point strips the parietal pleura from the chest wall and pushes it ahead of it. By this method, the cannula slips in easier and since it is so quickly done, no respiratory distress results in the animal, even if the lung collapses. You can quickly restore the intrathoracic pressure by suction through a T-tube placed in the rubber tubing connecting the cannula with the recording tambour.

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THE USE OF DIALYSIS IN THE PREPARATION AND PURIFICATION OF IMMUNOLOGICALLY ACTIVE BACTERIAL PRODUCTS*

THE problem of purifying active bacterial products is frequently complicated by the presence of non-specific ingredients derived from the nutrient medium. We have recently employed a purely physical operation, dialysis, to overcome this difficulty. The particular bacterial products investigated were those capable of eliciting the phenomenon of local skin reactivity to bacterial filtrates;[1] the method described, however, appears generally applicable to other bacterial products which are non-diffusible through Cellophane.

It was reported in a former communication[2] that the active principles of the phenomenon of local skin reactivity to bacterial filtrates are retained by Cellophane membranes. The observations of McClean[3] on production of staphylococcus toxin in fluid media diffused through Cellophane suggested the possibility of preparing active filtrates free from non-specific ingredients, as described below.

A diffused broth medium is prepared by immersing Cellophane[4] bags, containing saline, into nutrient broth. Sterilization is accomplished by autoclaving. After standing at room temperature for 24 hours, the inner contents are inoculated and the apparatus incubated. During the abundant growth in the bags, observed thus far with many microorganisms, the outside broth remains sterile. The production of potent culture filtrates under these conditions is consistent. The function of the outside broth is to supply diffusible nutrient substances to the growing bacteria. After various periods of incubation, the cultures are removed and filtered. The filtrates obtained in this manner are then redialyzed against saline, in Cellophane bags.[2] Diffusible substances which have not been rearranged to specific bacterial products are thus removed. In many cases, practically water-clear preparations are obtained. The final dialyzed solutions usually contain about 2 mgms total solids (ash free) and 0.02 mgms total nitrogen. The method is very practical, and large quantities of excellent starting materials for chemical investigations are readily prepared. They are considerably lower in total solids and nitrogen than most of preparations formerly analyzed.[2]

The filtrates give abundant precipitation with specific immune sera, thus apparently containing a considerable amount of antigenic material. The active principles of the phenomenon present in these preparations are of considerable potency. In the case of meningococcus, the reacting titer approximates one half of that of "agar washings" filtrates. It may be noted that filtrates of meningococcus cultures in fluid media without the use of Cellophane have ordinarily a potency of 1/40 to 1/20 of the "agar washings." These principles are specifically neutralized by immune sera in "multiple proportions."

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* This investigation has been aided by a grant from Eli Lilly and Co., Indianapolis.

1 G. Shwartzman, "Phenomenon of Local Tissue Reactivity and its Immunological, Pathological and Clinical Significance." Paul B. Hoeber, Inc., Medical Book Department of Harper and Brothers, New York, 1937.

2 G. Shwartzman, S. Morell and H. Sobotka, *Jour. Exp. Med.*, 65: 323, 1937.

3 D. McClean, *Jour. Path. and Bact.*, 44: 47, 1937.

4 "Cellophane" No. 600 was used.

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