cc of water by stomach tube. The maximum change in electrolyte excretion following cortin injection occurred during the first six hours. Initial injections of cortin (20 cat units) into eight normal dogs produced marked reduction in the sodium and chloride excretion and usually an increased excretion of potassium. The initial response of the different dogs to the same amount of cortin was essentially the same.

Four dogs have been injected repeatedly. With each succeeding injection the response has become smaller until it has entirely disappeared. Table I is typical of the four animals. Two human beings have shown a similar response to repeated cortin injections.

FRANK A. HARTMAN
LENA LEWIS
GWENDOLINE TOBY

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A PLEURAL CANNULA[1]

THE recording of respiration in animal experiments is frequently a source of difficulty to the student and annoyance to the staff. The more accurate methods are beset with so many technical difficulties that either the student fails to obtain a record or the apparatus may be so expensive that the school fails to provide it. In an attempt to simplify our animal experimental methods, we have used many devices for graphically recording respiration. The pleural cannula method seemed to be the most simple, even though it involved a slight amount of surgical procedure, *i.e.*, a short slit in the skin between two ribs, a sharp thrust of the cannula so that the open end lies in the pleural cavity. The outer end of the cannula is connected with a recording tambour, giving essentially a record of variations in intrathoracic pressure during respiration. The most annoying difficulty with this method is the ease with which the cannula is displaced, thereby upsetting the record or requiring re-introduction when the cannula is completely pulled out. This unfortunate accident seems most likely to occur at the most critical moments of an experiment.

The principle of recording respiration by the pleural cannula method seemed sound and very easy to apply. The only difficulty is the instability of the cannula itself. A careless finger catching the rubber tube leading to the tambour resulted in the sudden withdrawal of the cannula; or even the accidental dropping of a light instrument on the rubber tubing may cause the cannula to slip out. It was felt that if we could overcome this difficulty of instability in this method, we would have an easily recording mechanism for respiration.

With this idea in mind, we developed the pleural cannula herein described. Instead of using the ordinary flattened and tapering point on the pleural cannula, we capped the point with a two-pointed barb which was somewhat flattened and pierced with four holes. Fig. 1 is a drawing of the cannula. One of the four holes is seen near the tip of the barb. This cannula is introduced in much the same manner as any pleural cannula, except that the flattened sides of the barb should be introduced parallel with the borders of the ribs. When the cannula enters the pleural cavity, it is immediately given a turn of 90°. This will result in the barb points catching the ribs if you attempt to withdraw it. While firmly holding the cannula in this position, quickly tighten the wing-nut shown at A in Fig. 1. If the wing-nut is very firmly tightened, the cannula will now stay firmly anchored in the pleural cavity and the rubber tubing may be quite carelessly handled without danger of the cannula slipping out. The firm tightening of the wing-nut on the underlying washer effectively seals and prevents loss of negative pressure in the pleural cavity.

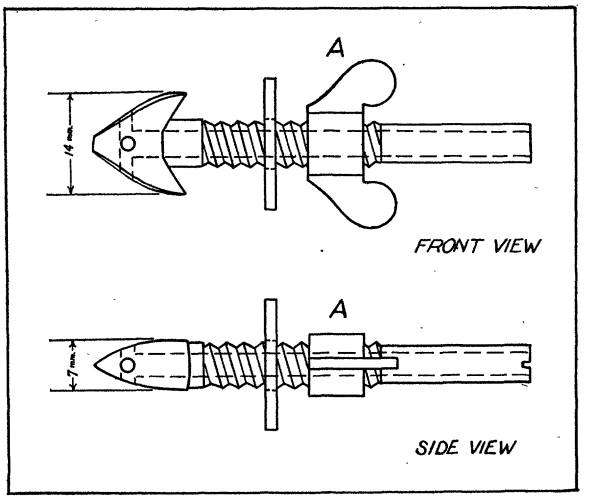

FIG. 1. Pleural Cannula

Certain precautions, if taken, will give better results. For example, it is best to insert the cannula where the rib borders lie in closest approximation; otherwise the barb will fail to engage the ribs and will come out. We usually choose the right side of the chest so that the heart will not be interfered with. The site chosen is about the midaxillary line and fairly high up on this line. It is best to cut the hair very short over the chosen site. It should be removed from a space about

[1] From the Department of Pharmacology of Boston University School of Medicine and the Evans Memorial Hospital.

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.

WALTER L. MENDENHALL

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."

SAM MORELL
GREGORY SHWARTZMAN
LABORATORIES OF THE MOUNT SINAI HOSPITAL
NEW YORK

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

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4 "Cellophane" No. 600 was used.

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