For reading, the syringe is detached from the vial and the scale read by sighting across the flat end of the plunger and estimating to the nearest fifth or quarter of a division, again using a lens.

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Improved Technique for Enumeration of *Escherichia coli* on Black Walnut Meats[1]

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The enumeration of microorganisms found on solid materials is, in the main, based on their removal by washing a known weight of the solids with a known volume of washing fluid and then making serial dilutions from this fluid prior to the use of one of the common modes for determining numbers.

In the course of an investigation on the effectiveness of pasteurization for killing *Escherichia coli* on inoculated black walnut meats, it was found that washing the meats to remove the bacteria was not satisfactory when the Halvorson-Ziegler (*1*) method of determining the "most probable number" (MPN) of bacteria was used. The number of positive tubes found in the 10 replicate tubes of each of the three lowest dilutions gave codes which, when applied to the probability table, were equivalent to such "MPN" values as to make it evident that the seeding of the solid itself was needed if more accurate codes were to be secured.

Accordingly, an attempt was made to use the meats themselves in the replicate plantings, so that one member of the resultant code would be of no dilution. This was accomplished

TABLE 1

Test	MPN/gram	
	Washed meats	Blendor-treated meats
1	0	0.7
2	0.9	275.0
3	0	240.0
4	0	1.6
5	0	7.9
6	0	16.9
7	3.2	130.0

by cutting a known weight of the meats into small particles in a sterile Waring blendor container. After a homogeneous mass was secured and 10 replicate 1-gram samples had been removed for planting into lactose broth fermentation tubes, a known volume of sterile water was added to the container and the mixture further agitated until a creamy mass was secured. This material could be pipetted easily in making the 10 replicate plantings from this dilution, and it could also serve as the starting point for making subsequent dilutions.

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The results of typical tests made on inoculated meats which had received various heat treatments are shown in Table 1. This table contrasts the "MPN" of *E. coli* per gram of meats when washing was used for the removal of these organisms with the "MPN" gained by the use of the Waring blendor procedure. Meats from the same batch were used when comparing the procedures.

TABLE 2

Test	MPN/gram	
	Washed meats	Blendor-treated meats
1	6,220	11,600
2	24,400	39,900
3	2,900	4,930
4	3,990	9,200
5	11,400	32,900
6	1,960	4,840
7	7,420	34,900

The superiority of the Waring blendor procedure is shown also in Table 2. Inoculated nut meats which had not been heat treated served as source material for contrasting the two modes of removal. Meats from the same batch were used when comparing the procedures.

The results of these tests suggest that, where the solid lends itself to subdivision, the use of the Waring blendor for this process permits the seeding of homogeneous, undiluted solid material into the tubes of broth. This procedure, together with the actual "carrying over" of the solid into subsequent dilutions, makes for a greater recovery of the organisms than that secured by washing the solid materials.

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Cultivation of Microorganisms With the Aid of Cellophane Membranes

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This brief report describes the use of thin sheets of cellophane for the cultivation of bacteria and fungi. We first used cellophane for this purpose during the German occupation, when Petri dishes were unobtainable.

A sheet of cellophane was rolled around a rod and sterilized by autoclaving. It was then unrolled on a table, and melted agar medium was poured out over the sheet. The surface was inoculated by spraying, and the agar immediately covered by a second cellophane sheet. To save space, the whole culture was finally rolled up again loosely around the rod and incubated.

Recently we have taken up the problem from another side, using the cellophane sheets not only as a substitute for glass but as a dialysing membrane. These sheets are impermeable to bacteria and viruses, but they allow the passage of water and solutes (except those of very large molecular weight). Thus, colonies of bacteria can be grown on one side of the cellophane,

nourished by fluid, which dialyses through from the opposite side of the membrane.

In our first experiments we used cellophane tubes (available as sausage skins), as shown in Fig. 1.

For the production of thick, slimy cultures on the inner side of the cellophane tube (Fig. 1a), the tube (1) is pulled over the ends of two glass tubes (2) of the same diameter and fastened to them at each end by collodion. Through the tube is passed a very slow current of air under a slight pressure, sufficient to keep the tube rigid. Through the narrow space between the cellophane tube and an outer, enveloping glass tube (3) is circulated a slow stream of the nutrient solution. All the tubes are fitted tightly in rubber stoppers (4) as shown. The culture is now growing on the inner surface of the cellophane tube, nourished through the cellophane but not

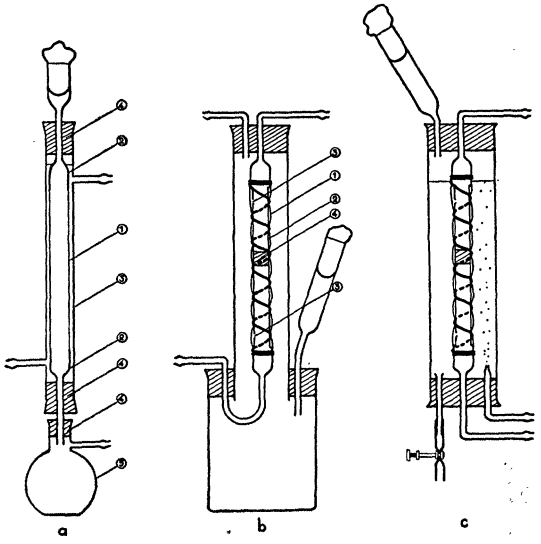

FIG. 1. The three main arrangements for experiments with cellophane tubes.

contaminating the broth. As soon as the growth becomes luxurious, the culture drips down along the tube and is collected in a container (5).

With the apparatus shown in Fig. 1b the same end is attained in a slightly altered manner. Here the culture grows upon the outer surface of the cellophane tube (1), while the nutrient broth circulates between the cellophane and a narrower glass tube (2) inserted in the cellophane tube. To prevent the cellophane from sticking to the surface of the inner glass tube, a string is wound spirally around the cellophane tube. The current is thus forced to circulate spirally around the inner tube. The nutrient solution is fed and drained off through the inner tube, which has two small openings (3) near the top and the bottom but is closed by a rubber stopper (4) between these openings.

In the third type of apparatus, shown in Fig. 1c, the culture grows not upon a cellophane surface in an air-filled space but in a nutrient broth with aeration only by a stream of filtered air, this gives a fluid, but finally very thick, culture.

This method of cultivation is of use when large amounts of bacteria (free from substrate protein) are required (e.g. rhizobia for the inoculation of leguminous plants). It has the added advantage that the culture can be grown without interruption and, if the nutrient broth is renewed from time to time, for an unlimited period. Furthermore, this principle may also prove to be of use for the study of bacterial products, which can thus be obtained free from substrate protein in the dialysate.

Our first experiments proved that the method was sufficiently productive. The apparatus shown in Fig. 1 (a or b) gave within 10 days, at 29° C., 15 grams of a rather stiff, slimy culture of *Rhizobium* of a quality similar to that derived from agar-plate culture, the total surface of the cellophane tube being 100 cm.², while the surface of agar plates for production of the same amount in similar circumstances would be nearly 150 cm.².

We do not know whether others have used cellophane in culturing bacteria or studying their metabolism; recently we have found a short communication (*1*) on the interaction of different bacteria suspended in nutrient solutions and separated from each other by collodion membranes.

The above-described method, of course, does not require that cellophane membranes be used solely. Other types of membranes may be even more suitable for some purposes.

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Laboratory Test of Aviator's Ejection Seat

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Emergency escape from high-speed aircraft is dangerous and sometimes impossible. It is not only difficult for the pilot to leave the cockpit against the force of the wind stream, but he may fail to clear plane structures as he is swept backwards along the axis of flight. In spins he may be unable to move, and at low altitudes there is often insufficient time to abandon the plane in an emergency. The most serious hazard is collision with the tail surfaces after leaving the cockpit.

Development of an ejection seat which instantaneously catapults the pilot from the plane was started in Germany during the last war (*2*) and has been continued and improved by the British (*1*). The U. S. Army and Navy are adapting and developing such devices for service aircraft from which escape is at present dangerous. Each of these groups of workers has successfully ejected aviators from planes in flight under experimental conditions.

Catapulting the seat from the plane provides an escape possibility limited by the dimensions and motion of the plane at the time of ejection and by the tolerance of the aviator to ejection. The primary restriction is the vertical distance in the cockpit available for imparting velocity and direction to the catapulted seat and pilot.

[1] The opinions expressed in this article are those of the authors and are not to be considered as reflecting those of the Navy Department. Appreciation is expressed to A. T. Kornfield and D. Weiss for the design of the velocity meter and pressure pickup and the operation of the instrumentation for these experiments.