# SCIENTIFIC PROCEEDINGS.

## NEW YORK MEETING.

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### 127 (2650)

### Pneumococci cultivation in large amounts.

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The need of large amounts of Pneumococci for certain immunological and chemical investigations led to the devising of the following methods:

The problem divided itself into three phases:

1. The discovery of a simple medium, easy to prepare and relatively inexpensive, which would allow of a vigorous growth of the organisms.
2. The devising of an apparatus permitting growth in bulk.
3. The recovery of the organisms from the culture medium.

#### THE MEDIUM.

The medium finally selected after much experimentation is known as the L. A. P. medium, the letters indicating the initials of the principal ingredients.

The formula is as follows: Tap water q. s.

| | | |
|---|---|---|
| Lactose | 0.5 | per cent. |
| Amnoids (Arlington Chemical Co.) | 0.2 | per cent. |
| Peptone (Bacto) | 0.1 | per cent. |
| NaCl | 0.25 | per cent. |
| Dipotassium phosphate | 0.5 | per cent. |
| Monopotassium phosphate | 0.03 | per cent. |

The ingredients are dissolved in the water with the aid of heat, and the solution sterilized.

The pH of the mixture should be 7.6 as tested cold with phenyl red.

In preparing large quantities of this medium it is necessary to sterilize the lactose separately, as a 10-15 per cent solution in distilled water. This avoids the caramelization of the lactose which acts as a growth retarder.

This medium must be inoculated heavily with a vigorously growing culture of the Pneumococcus. When such conditions are met the subsequent multiplication is very rapid.

Growth of one billion organisms to the cubic centimeter occurs in three hours, two billion in six hours, and from four to six billion in eighteen hours.

## THE APPARATUS

The apparatus employed is so designed that it is used in the preparation of the medium, in its sterilization, and finally acts as an incubator during the growth of the Pneumococci.

It consists essentially of a conical cast iron tank with a capacity of 300 liters. This tank is jacketed and is lined throughout with acid proof enamel as is the tight fitting cover.

The jacket is connected with steam and water lines and a pipe system to ensure circulation of the water content when used as an incubator.

The cover is pierced with openings, for a thermometer, for a compressed air connection (used to stir up the contents of the tank), and an opening for the introduction of the inoculum.

In use, the L. A. P. medium is prepared in the tank in 200 liter amounts by adding all the ingredients with the exception of the lactose to the water. The temperature is raised to 110° C. by introducing steam into the jacket, and is held at this point for 2 hours. The steam is turned off and the tank allowed to remain hot overnight; on the following morning the temperature is reduced to 37° C. by passing water through the jacket. The lactose (previously sterilized in distilled water) is added, and the inoculum introduced.

The temperature is maintained at 37° C. by means of a single Bunsen flame acting on the pipe system spoken of before.

The pipe has its entrance to the jacket at one side of the upper level of the contained water, it is carried around to the opposite side and down to connect again at the lower level of the contained water, so that heat applied to this pipe causes a circulation through the pipe and the water jacket.

After 18 hours incubation the temperature of the culture medium is raised to 60° C. to kill any living Pneumococci, and

the growth of from four to six billion organisms per cubic centimeter is ready for recovery.

### RECOVERY OF THE ORGANISMS.

This is accomplished by piping the growth to a battery of Sharpless super centrifuges, where it is centrifuged at 30,000 revolutions to the minute, which removes practically all the organisms from the medium.

The packed organisms are removed from the bowls of the machine and packed in glass containers, which are then placed at a temperature of minus five degrees C.

Organisms so obtained retain their morphology and staining characteristics, and are as useful for immuniological work as those obtained from the ordinary media employed.

This method has been in constant use for over a year and has proved satisfactory.

## 128 (2651)

## The physiologic properties of some unsaturated hydrocarbons.

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The recent introduction of ethylene and acetylene as anesthetics into medical practice has made it appear desirable that a general study of the physiologic properties of the unsaturated hydrocarbons be undertaken. Studies have therefore been carried out on hydrocarbons of the olefine, diolefine and acetylene series. Each hydrocarbon has been studied from the following points of view: 1. Symptoms produced in experimental animals by the inhalation of various concentrations of each hydrocarbon studied. 2. The toxicity of the various hydrocarbons when administered by inhalation. 3. An attempt has been made to relate quantitatively the anesthetic potency and the toxicity of the various hydrocarbons studied.

In order that these studies might be made as strictly comparable as possible a single strain of white rats of uniform weight was used.