



**Laboratory of the Army Medical School, Showing Preparation of  
the Culture Medium  
to be used in Raising Typhoid and Paratyphoid Bacilli**

The laboratory at Uncle Sam's Army Medical school in Washington, working at top speed, has prepared and shipped enough typhoid and paratyphoid vaccine to inoculate every man in the army against these diseases and in addition it has made all the vaccines used by the navy since April 1, 1917. At the time the first contingents of the National army were being mobilized in the various cantonments, the army school was turning out 3,000 quarts of typhoid vaccine a month. The cost of this monthly output in material alone, not to mention labor, equipment and overhead expense, was \$150,000. Its commercial value was five or six times that amount.

Throughout the process of making the vaccine is closely guarded against contamination. It is been stored in seal the

vessels and lock refrigerators to which only two officers in charge have keys; and none of these vessels is ever moved except in the company of one of these two officers--Col. Eugene R. Whitmore, in charge of the laboratory, and his assistant, Maj. C.G. Snow. In more than six months of large-scale production not a trace has been found of any contamination.

The United States army uses the "Rawlings" strain of the typhoid bacillus, isolated by the British army from a case of typhoid at Netley, England, in 1900. The stock from which the typhoid vaccine is made is composed of lineal descendants of these germs isolated 17 years ago and propagated in artificial media ever since. The paratyphoid vaccines are a combination of four strains, two American and two British.

## **The process of cultivation**

First the "stock cultures," from which the bacilli for the vaccines are to be propagated are tested. If, for example, typhoid vaccine is to be made, germs are taken from the stock, allowed to propagate, and tested by several methods to determine, first, whether they are pure strain of typhoid bacillus and nothing else, and, second, that they are affected in the desired way by blood previously inoculated against typhoid, showing that this particular strain of typhoid bacillus is neither too strong nor too weak.

The next step in the process, after determination that the stock cultures are right, is propagation. First a few colonies are started by swabbing over with stock bacilli the surface of a gelatinous substance made from agar-agar (Japanese seaweed), which is used as the culture medium. After incubation the colonies which appear are washed off with a liquid medium called "broth." This broth is used for swabbing the agar-agar surfaces in a large number of flasks, the desired quantity of the bacilli for vaccine being obtained by incubating the cultures in these flasks.

### **Every care against contamination**

This swabbing operation is done in rooms whose walls are washed with anti-septic each morning, with doors and windows tightly closed, and in a very moist atmosphere of high temperature. It is often above 100°F. in these rooms last summer it is known to have reached 124° on one occasion. The workers where sterilized coats and rubber gloves, rarely speak, make as few and as slow movements as possible, so that will be little air circulation, and in every way minimize the possibilities of contamination.

After the flask swabbed with "broth" have incubated, the billions of bacteria in each are washed off with a salt solution and this emulsion heated to 53°C. (127.4 degrees F.) This kills a large number of the bacilli. One-fourth of 1 per cent of tricresol, a coal-tar product, is added, which kills all the rest of them and also acts as a preservative.

## **Test cultures made**

After the emulsion of broth and bacilli has been heated, the test culture is made to see that it is still uncontaminated. After the tricresol has been added, other test cultures are made, both in air and in a vacuum, to determine whether incubation will disclose any contaminating micro-organisms. (Certain dangerous germs will not incubate if exposed to the air.) This is done in spite of the fact that there are a few bacteria, either harmless or injurious, which the tricresol will not kill.

If the test show absolute sterility of the emulsion, animal tests are made. A mouse, a guinea pig and a rabbit are inoculated. If a bit too much tricresol has been added, the mouse will be killed. If tetanus germs are present, both the mouse and the guinea pig will be killed. If the emulsion is as it must be, to be used, neither animal is killed and the guinea pig and rabbit not visibly affected by the inoculation. The rabbit test is for "anti-bodies" – that is, to make certain that inoculations with the vaccine causes the blood to produce in satisfactory abundance the several kinds of substances hostile to the disease it is to combat.

### **Counting the bacilli**

The number of bacilli in the vaccine is ascertained by mixing it with an equal quantity of blood and determining by count under the microscope the proportion of bacilli to red blood corpuscles. Simple mathematical computation does the rest.

After the vaccine has passed all the tests it is mixed with other similarly made and tested vaccine if the final product is to be "double" or "triple" vaccine. After mixture further test cultures, both in air and in vacuum, are made. If that the tests are satisfactory, the vaccine is released to be put into sterilize glass tubes each holding 1, 5, 10 or 25 cubic centimeters. Immediately each glass tube is filled, it is sealed by melting the glass of the tube until the hole is closed. All glassware is sterilized on the day it is used.

Lastly, from each lot of vaccine sealed tubes are taken at random and again test cultures are made both in air and in vacuum. If anyone of these does not show sterility, the entire lot is thrown out. If satisfactory, small boxes of the sealed tubes, pack in sawdust, are put in larger cases and are ready for shipment.

A time limit of four months from the time of the bacilli are washed off with the broth is set, beyond which the vaccine may not be used.