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Two Kinds of Culture Methods for the Early Detection of the Tubercle Bacilli.

First Report. Cultivation from Sputa.

By

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Introduction.

It may be one of the weak points of the culture method on egg slants that more or less long period of time is necessary to see the development of the tubercle bacilli. Many workers tried, using various methods, to obtain the culture as fast as possible. Above all the method of BERRY and LOWRY[1] reported recently in U. S. A. is thought to be interesting. It is, however, rather difficult to carry out this method and one is still obliged, even through this method, to spend from one week to ten days to wait for the the development of the tubercle bacilli. KIMURA and MIZUNO[2] reported recently in our country a similar method which involves also the same difficulties as the former.

Two kinds of culture methods which enabled us to observe the tubercle bacilli just beginning to develop were described below. Through these methods one could detect the tubercle bacilli after only two or three days of incubation.

Experiments.

1. The Slide Culture Method in the Moist Chamber.

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Materials and methods.

70 samples of sputa were examined, of which the smears stained with the Ziehl-Neelsen showed none of acid-fast bacilli. About 1 cc of each sputum was put into the centrifuge tube and was homogenized with five volumes of 4% solution of sodium hydroxyde. After being kept for 20 minutes in an incubator, the tube was centrifuged strongly for 20 minutes. The supernatant fluid was poured off, and then certain volumes of sterile destilled water was added to the sediment and shaken strongly. The tube was centrifuged again and the supernatant fluid was poured off. Equal or double amount of liquid medium of Kirchner was mixed to the washed sediment.

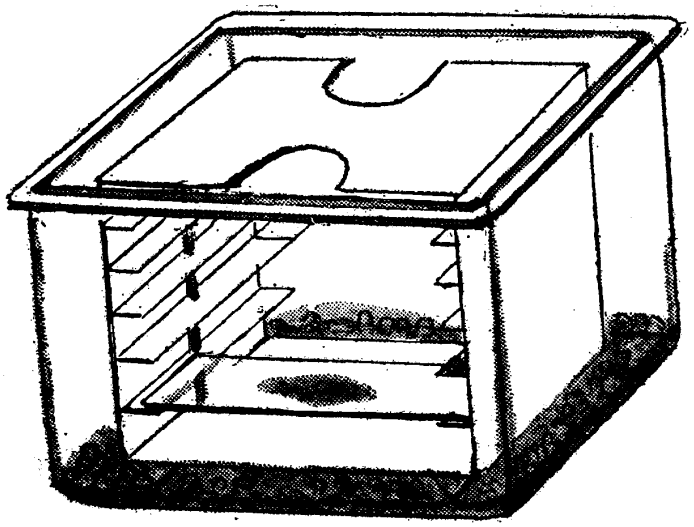
A drop of the mixed fluid was placed on six sterile slides respectively. The slides thus prepared were mounted on the shelf in the moist chamber and incubated. After 1, 2, 3, 4, and 5 days of incubation respectively, the slides were taken out from the moist chamber and dried, being kept in an incubator. The dried slides were fixed, being passed through the flame and stained with the Ziehl-Neelsen method. 100 microscopic fields of each slide were observed.

In order to compare with the above mentioned method, a loopful of the washed sediment was inoculated on two egg slants respectively and incubated.

The moist chamber.

The moist chamber is composed of a glass box (10 cm long, 8 cm wide and 7 5 cm deep) with a plain glass cover and a metal shelf (8 cm long, 5,5 cm wide and 6 cm high) having five stairs on both sides as shown in Fig. 1.

Fig. 1. The moist chamber.



A small amount of cotton being spread previously at the bottom of the glass box was moistened with 60 to 100 cc of destilled water and the metal shelf place on it. The glass box with the glass cover thus prepared was sterilized in vapor. The slides having a drop of the culture material on them as described above were mounted on each stair of the metal shelf. The box was covered and kept in an incubator. Much attention was paid to keep the glass box in a horizontal position so that the culture material did not spread over the slide and flow down from it.

Results.

Amoung 70 samples of sputa examined 6 showed bacilli in the smears made with their washed sediments. Results obtained from remaining 64 samples were as follows.

Table 1. Numbers of sputa showed bacilli when cultivated in the moist chamber.

	Days of incubation					Total
	1	2	3	4	5	
Numbers of positive sputa	3	22	9	0	0	34
Numbers of negative sputa						30

Table 2. Numbers of colonies found in each 100 microscopic fields after two days of incubation.

	Numbers of colonies			
	Less than 10	More than 10	More than 20	More than 30
Numbers of sputa cultivated	16	6	2	1

Table 3. Comparison of the culture in the moist chamber with that on egg slants.

Numbers of sputa	In the moist chamber	On egg slants
33	+	+
27	−	−
1	+	−
3	−	+

Table 4. Days necessary to detect the bacilli.

Culture methods	Numbers of sputa examined	Numbers of positive sputa	Days necessary to detect (average)
In the moist chamber.	64	34	2,2
On egg slants.	64	36	20,6

The above mentioned culture method showed almost equal numbers of tubercle bacilli in sputa when compared with those in which the culture on

egg slants gave positive results. It must be noted, moreover, that the bacilli were detected, in this method, in few numbers of sputa after only one day of incubation, in large numbers after two days and in the remaining few numbers after three days. In this method, numbers of days which were necessary to detect the bacilli were shortened remarkably: only 2,2 days on an average were necessary in this method while an average of 20,6 days were required in the culture method on egg slants.

2. *The culture method in the centrifuge tube.*

Materials and methods.

89 samples of sputa were examined, of which the smears stained with the Ziehl-Neelsen showed none of acid-fast becilli. About 1 cc of each sputum was put into three centrifuge tubes respectively and shaken thoroughly with 5 cc of 4% solution of sodium hydroxyde. After being kept for 20 minutes in an incubator, the tubes were centrifuged The sediment was washed once with sterile destilled water. Equal or double amount of liquid medium of Kirchner was mixed with the washed sediment. After being sealed, the tubes were kept in an incubator. After 2, 3, and 7 days of incubation respectively, the tubes were centrifuged again and a loopful of the sediment was smeared on a slide. The slide was dried and fixed in the flame and then stained with the Ziehl-Neelsen method. 100 microscopic fields of each smear were observed.

Results.

Table 5. Numbers of sputa which showed bacilli when cultivated in the centrifuge tubes.

	Days of incubation			Total
	2	3	7	
Numbers of positive sputa	37	6	0	43
Numbers of negative sputa				46

Table 6. Comparison of the culture in the centrifuge tubes with that on egg slants.

Numbers of sputa	In centnifuge tubes.	On egg slants.
37	+	+
43	−	−
6	+	−
3	−	+

Table 7. Days necessary to detect the bacilli.

Culture method	Numbers of sputa examined	Numbers of positive sputa	Days necessary to detect (average)
In centrifuge tubes.	89	43	2,2
On egg slants.	89	40	20,1

Tubercle bacilli were demonstrated in more numbers of sputa through the culture method in the centrifuge tubes than through the culture method on egg slants. It must be noticed, moreover, that the bacilli were detected, through the culture method in the centrifuge tubes, in large numbers of sputa after two days of incubation and in remaining few numbers after three days. In this method, days which were necessary to detect the bacilli were as short as the slide culture method in the moist chamber. Only 2,2 days on an average were necessary in this method while it required an average of 20,1 days in the culture on egg slants.

Discussion.

The tubercle bacilli can be detected from sputa through these two kinds of culture methods described above within 2 or 3 days. This is remakably earlier than the ordinary culture method on egg slants and a little earlier than the slide culture mehod of BERRY and LOWRY. This is, after all, because one can observe, in these culture methods described above, the tubercle bacilli just beginning to develop.

However, one should always pay attention to the contamination of the saprophytic acid-fast bacilli which may come from saliva or other materials. We did not encounter such difficulties in the above observations. In order to make sure, we examined, however, several cultures of the saprophytic acid-fast bacilli. It became certain that the differentiation of such saprophytic bacilli from tubercle bacilli was probably possible, if one examined thoroughly the morpology and staining behavior of the bacilli and at the same time the largeness of their colonies, namely, the fastness of the development of the bacilli. After 2 or 3 days of incubation the colonies of the tubercle bacilli were consisted of less tan 10 bacilli, generally 2 or 3. On the contrary, the colonies of the saprophytic bacilli were consisted generally of more bacilli than the former. Within these colonies one could find often non acid-fast forms together with acid-fast ones. Such difference as described above between tubercle bacilli and saprophytic bacilli concerning the morphology and also the fast-

ness of the development became more distinct after 5 or 7 days of incubation. It seemed, therefore, possible to distinguish saprophytic bacilli from tubercle bacilli, supposing that the culture were contaminated by them.

Summary.

By means of those two kinds of culture methods as described above, namely, "the slide culture method in the moist chamber" and "the culture method in the centrifuge tubes", the detection of the tubercle bacilli from sputa showing none of bacilli through the staining method was possible already after two or three days (2,2 days on an average) of incubation.

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