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A simple technique for culturing tubercle bacilli*

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Microscopy, traditionally used in peripheral health centres to diagnose tuberculosis, could be supplemented by sputum culture if sputum specimens were inoculated on suitable media and sent to intermediate or central laboratories to be incubated and read. To facilitate this procedure, the authors propose a simplified swab culture method and a modification of Ogawa's egg medium.

The culture technique for isolating *Mycobacterium tuberculosis* is becoming more important because it is highly sensitive and yields a harvest of living microorganisms that provides valuable information on the virulence, drug sensitivity, and other biological properties of tubercle bacilli.

In 1964 and 1967 we visited a number of laboratories in south-east Asia. Most of these laboratories use the sputum swab culture method recommended by WHO (referred to throughout the present paper as the WHO swab culture method) and originally developed by Nassau (1). However, several of the laboratories experienced a high frequency of contamination with that method. Furthermore, the method is difficult to apply in rural areas because it requires fresher specimens than direct smear microscopy does.

The need for a more simple and stable culture technique that could be applied in remote, poorly-equipped laboratories was evident. We therefore carried out a series of studies with a view to developing a better technique, and the results of these studies are presented in this paper.

I. STUDIES ON CULTURE TECHNIQUES

EXPERIMENT 1. COMPARATIVE STUDY OF OGAWA'S ORIGINAL SPUTUM CULTURE METHOD AND THE WHO SWAB CULTURE METHOD

The method developed by Ogawa et al. (2)—the standard method used in Japan—is a simple one: the specimens are inoculated by pipette direct on the medium after treatment with strong alkali. No centrifugation or neutralization is needed. The WHO swab culture method is also fairly simple: the specimens adhering to cotton swabs are treated with acid and then neutralized with weak alkali. This method is widely used in south-east Asia.

Materials and methods

The formulae of the media used in these studies are as follows:

Component	Löwenstein–Jensen	Ogawa	Modified Ogawa (Kudoh)
Monopotassium phosphate	0.4 g	3 g	2 g
Magnesium sulfate	0.04 g	—	—
Magnesium citrate	0.1 g	—	0.1 g [a]
Sodium glutamate	—	1 g	0.5 g
Asparagin	0.6 g	—	—
Glycerol	2 ml	6 ml	4 ml
Distilled water	100 ml	100 ml	100 ml
Egg homogenate	167 ml	200 ml	200 ml
Malachite green (2%)	3.3 ml	6 ml	4 ml

[a] This component is not essential for the detection of tubercle bacilli but improves the viability of the microorganisms.

* The data presented in this paper are largely the same as those published, in Japanese, in *Kekkaku*, **48**: 453-461 and 501-512 (1973).

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After the distilled water has been added, the media are heated at 100°C for 30 min. Once the media have cooled, the egg homogenate and 2% malachite green are added. The Löwenstein–Jensen medium is coagulated at 85°C for 45 min and the other two media at 90°C for 60 min. The final pH of the media is: Löwenstein–Jensen, 6.6; Ogawa, 6.2; and modified Ogawa, 6.4.

The inoculation procedures applied in this experiment were as follows:

WHO swab culture : each specimen was placed on 2 cotton swabs. These were immersed in a 5% oxalic acid solution for 35 min and then kept in a 5% sodium citrate solution for 10 min. The two swabs were then smeared on the surface of a Löwenstein–Jensen slope.

Ogawa's culture : each specimen was added to 4 times its volume of 4% sodium hydroxide solution and mixed well for 2 min. With a pipette, 0.1 ml of the mixture was poured on the surface of each of 2 slopes of Ogawa medium and spread by inclining the tube.

Thai technicians performed both swab culture methods on the same sputum specimens, which had been collected from inpatients at the Central Chest Hospital, Nondhaburi, Thailand, and from outpatients attending the Central Chest Clinic, Yodse, Bangkok.

Results

As may be seen from Table 1, 295 (57.6%) of the 512 sputum specimens were positive by Ogawa's method and 263 (51.4%) by the WHO method; 30 (5.9%) and 132 (25.8%), respectively, were partly contaminated (a small part of the surface of the medium or one of two cultures was contaminated but it was still possible to read the result), and 5 (1.0%) and 50 (9.8%), respectively, were completely contaminated, making the result impossible to read. There was no significant difference between the two methods as regards the positivity rate, but the difference in the contamination rate was statistically significant.

EXPERIMENT 2. COMPARATIVE STUDY OF THE WHO SWAB CULTURE METHOD AND OF THE NEW SWAB CULTURE METHOD WITH OGAWA'S MEDIUM

The results of Experiment 1 prompted us to undertake a further experiment in which sputum specimens treated with strong alkali but not neutralized were inoculated direct on Ogawa's medium by means of a new swab culture method developed by us (see Annex 1). This new method was compared with the WHO swab culture method.

Materials and methods

Sputa collected from inpatients at the Central Chest Hospital were examined by Thai technicians. Swab sticks and swabs were made as described in Annex 1. Three swabs were prepared from each sputum specimen: 2 for the WHO swab culture method and 1 for the new method with Ogawa's medium.

Results

Of the 120 specimens, 40 (33.3%) were positive with the WHO method and 45 (37.5%) by the new method with Ogawa's medium. As shown in Table 2, 49 (40.8%) were partly contaminated and 2 (1.7%) were fully contaminated with the WHO method, the corresponding figures for the new method being 14 (11.7%) and 2 (1.7%). As in Experiment 1, the difference between the two methods as regards the positivity rate was not significant, whereas the dif-

Table 1. Comparative study of the WHO swab culture method and Ogawa's original culture method

Method	No. of sputum specimens examined	Positive cultures		Partly contaminated		Fully contaminated [a]	
		No.	%	No.	%	No.	%
WHO (with Löwenstein–Jensen medium)	512	263	51.4	132	25.8	50	9.8
Ogawa's (with Ogawa's medium)	512	295	57.6	30	5.9	5	1.0

[a] Fully contaminated cultures were regarded as negative.

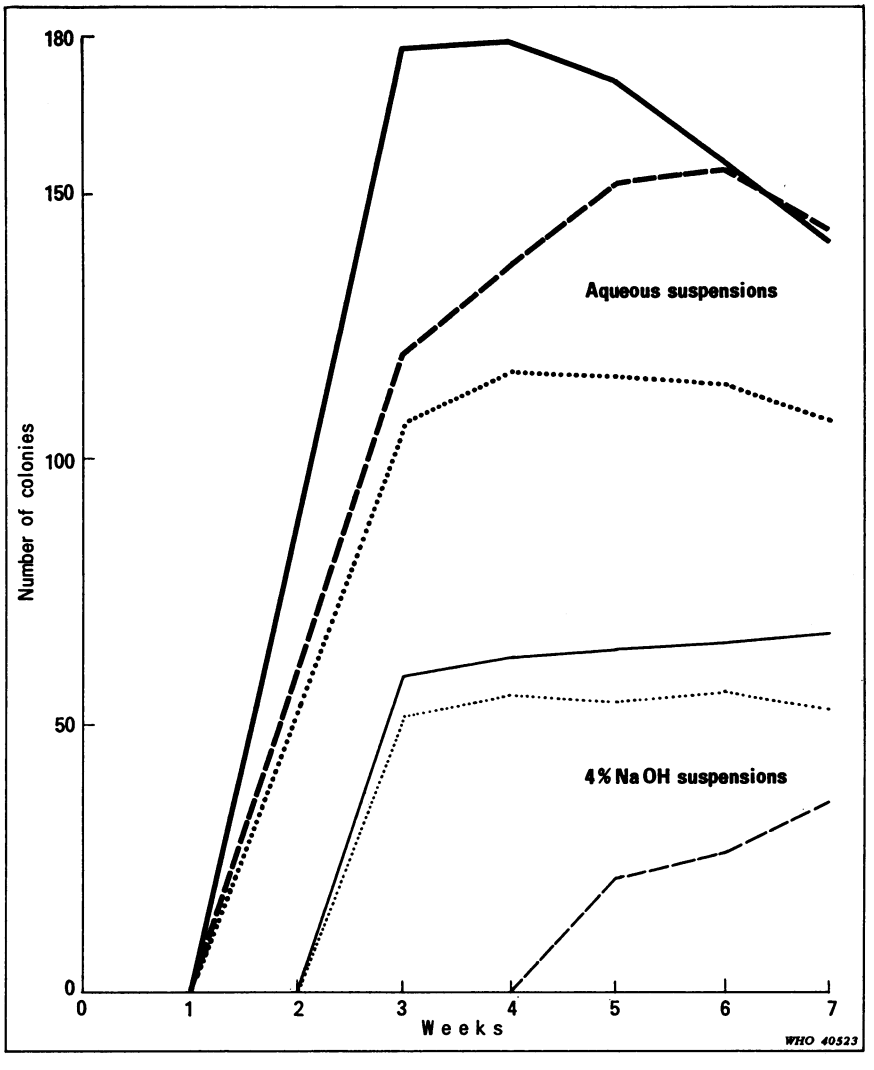

Fig. 1. Colony counts at various intervals after inoculation of aqueous suspensions and of 4% sodium hydroxide suspensions on modified medium (continuous line), Löwenstein–Jensen medium (broken line), and Ogawa medium (dotted line).

ference in the contamination rate was statistically significant.

EXPERIMENT 3. COMPARATIVE STUDY OF LÖWENSTEIN–JENSEN, OGAWA'S, AND MODIFIED OGAWA MEDIA FOR USE WITH THE NEW SWAB CULTURE TECHNIQUE

Since Ogawa's medium had been developed for inoculation with a 0.1-ml specimen treated with strong alkali, we thought that it might be inadequate for use with a smaller inoculum—i.e., one of about 0.05 ml on a cotton swab. We therefore modified the formula of Ogawa's medium (see page 71) and compared the efficacy of the modified medium with that of Löwenstein–Jensen medium and of the original Ogawa medium.

Materials and methods

A culture of *M. tuberculosis*, H37Rv strain, which had been grown on Sauton's liquid medium, was

ground by the crystal bead method and suspended in distilled water and in 4% sodium hydroxide solution at the concentrations of 10^{-2}, 10^{-3}, and 10^{-4} mg/ml. Slopes of Löwenstein–Jensen medium, Ogawa's medium, and modified Ogawa medium were inoculated with 0.1 ml of each aqueous suspension and 0.05 ml of each sodium hydroxide suspension. The numbers of colonies were counted weekly for 7 weeks.

Results

Only the results obtained with bacillary suspensions in which the colonies could be counted exactly were taken into consideration (Fig. 1). The decreasing curve in this figure reflects a confluence of colonies that occurred after 4 weeks or more.

It may be concluded from these results that the modified medium has a buffering activity of wide range for the pH of the inoculum, and that its power to sustain the growth of tubercle bacilli is at least as high as that of Löwenstein–Jensen medium inoculated with 0.1 ml of a 10^{-4} mg/ml aqueous suspension and of Ogawa medium inoculated with 0.05 ml of a 10^{-3} mg/ml 4% sodium hydroxide suspension.

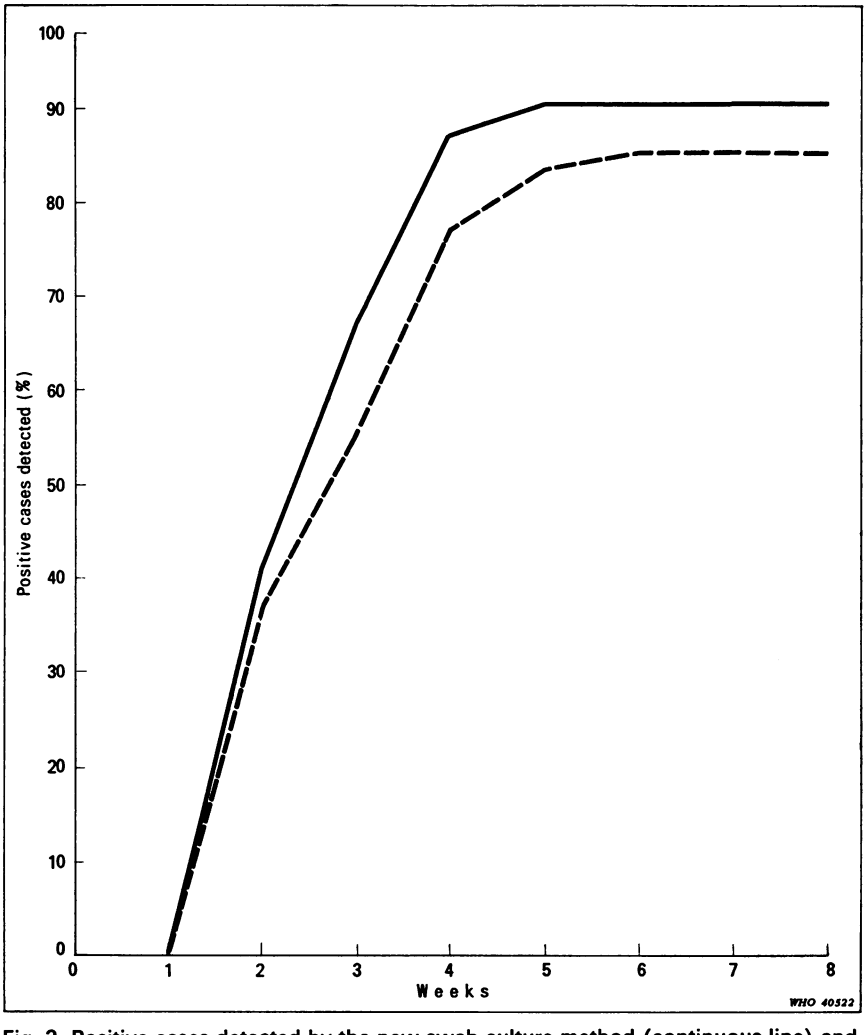

Fig. 2. Positive cases detected by the new swab culture method (continuous line) and the WHO swab culture method (broken line) as a percentage of all cases found positive at any time by either method.

Table 2. Comparative study of WHO and new swab culture methods

Method	No. of sputum specimens examined	Positive cultures		Partly contaminated		Fully contaminated [a]	
		No.	%	No.	%	No.	%
WHO (with Löwenstein–Jensen medium)	120	40	33.3	49	40.8	2	1.7
New (with Ogawa's medium)	120	45	37.5	14	11.7	2	1.7

[a] Fully contaminated cultures were regarded as negative.

Table 3. Comparative study of WHO swab culture method with Löwenstein–Jensen medium and the new method with modified Ogawa medium

Method	No. of sputum specimens examined	Positive cultures		Partly contaminated		Fully contaminated [a]	
		No.	%	No.	%	No.	%
WHO (with Löwenstein–Jensen medium)	302	51	16.9	14	4.6	3	1.0
New (with modified Ogawa medium)	302	54	17.9	17	5.6	2	0.7

[a] Fully contaminated cultures were regarded as negative.

Table 4. Correlation of culture results obtained with the two methods for 302 sputum specimens

		New swab culture method (modified Ogawa medium)						
		0	1–10	11–200	++	+++	++++	Total
WHO swab culture method (Löwenstein–Jensen medium)	0	242	8	1				251
	1–10	6	11	2				19
	11–200		3	12	2		1	18
	++				4	2		6
	+++				2	1	2	5
	++++					1	2	3
	Total	248	22	15	8	4	5	302

EXPERIMENT 4. COMPARISON OF WHO SWAB CULTURE METHOD AND NEW METHOD WITH MODIFIED MEDIUM

Materials and methods

Purulent sputa were selected from among the specimens obtained at routine clinical examinations in our laboratory. Twenty specimens were examined each day, until 362 had been accumulated. Of these, 60 that were completely contaminated when cultured according to the WHO swab method were excluded from analysis. This huge amount of contamination is presumed to have occurred as a result of long

storage of the sodium citrate solution after sterilization (more than one month). The procedures for the two methods are described on page 72 and in Annex 1, respectively.

Results

Table 3 shows that, of the 302 sputum specimens included in the analysis, 51 (16.9%) were positive, 14 (4.5%) were partly contaminated, and 3 (1.0%) were fully contaminated, when cultured according to the WHO swab method. On the other hand, the new method with modified medium produced 54 (17.9%) positive results, with 17 (5.6%) partial and 2 (0.7%) complete contaminations.

The two methods gave similar results as regards the rates of positivity, contamination (Table 3), growth (Fig. 2), and the numbers of colonies (Table 4), except for the 60 accidental contaminations mentioned above.

DISCUSSION

The efficacy of the new swab culture method is comparable to that of the WHO method under satisfactory conditions. However, the new method offers a number of extra advantages:

(1) The medium (Ogawa or modified Ogawa) that it requires is comparatively easy to prepare and is cheap, since it does not contain l-asparagin—an expensive component that is not available in many countries.

(2) The inoculation procedure is simple because it necessitates no special equipment and the buffering capacity of the medium makes it possible to inoculate the treated specimen without adjusting the pH.

(3) The method is less likely to produce contaminated cultures under unfavourable conditions—e.g., where the technicians who perform it are not well trained or the laboratory is inadequately equipped.

II. STUDIES ON THE PRESERVABILITY OF SPUTUM SPECIMENS AND INOCULATED MEDIA

The only way to ensure that sputum specimens collected in remote places are cultured is to send the specimens themselves or slopes of medium on which they have been inoculated to a well-equipped central laboratory. When specimens are transported over long distances—as is frequently done—care must be taken to prevent the sputum from putrefying, by keeping it cool or adding antiseptic. To transport inoculated media, it is necessary to use a stable technique that can be performed in infavourable conditions. Many such trials have failed on account of a high degree of contamination.

In order to ascertain whether it is preferable to transport sputum specimens or inoculated medium, several experiments were conducted.

EXPERIMENT 1. CULTURE OF SPUTUM STORED AT 37°C

Materials and methods

Sputum specimens were collected over a 12-hour period from 8 inpatients in whom direct microscopy of smears had given positive results. The specimens from each person were pooled, homogenized by mixer, and divided into two parts. One part was mixed with an equal amount of saline solution and the other with an equal amount of 1% boric acid solution, which is recommended by the Japan Public Health Association (3) as an antiseptic for sputum. Both mixtures were kept at 37°C in an incubator and inoculated into media periodically by means of the WHO and new swab culture methods.

Results

Table 5 shows that less contamination occurred among the sputum specimens mixed with boric acid solution than among those mixed with saline solution. The new swab culture method was superior to the WHO method in the same respect. It appears, therefore, that the addition of boric acid solution to sputum and the use of an acid egg medium prevent contamination to some extent. However, the numbers of colonies and of positive results decreased daily even when the growth of saprophytes was inhibited. Thus culture results cannot be relied upon when the specimens have been kept longer than 3 days at a temperature as high as 37°C. The lower the temperature, the longer will be the preservation period.

Table 5. Results of culture with the WHO and new swab culture methods according to length of storage of sputum specimens

Method	Specimen	Days of storage								
		0	3	5	7	10	12	14	17	21
WHO swab culture (Löwenstein–Jensen medium)	sputum with saline	101	37	29	16	4	X	X	×	×
		++++	+++	X	30×	28	×	×	—	—
		46	20	3	4	—	×	×	—	—
		135	19×	30	–×	×	—	—	—	—
		+	98	X	—	—	X	×	×	×
		26	3×	—	—	×	×	—	—	—
		400	X	×	–×	×	—	X	×	×
		3	—	×	–×	×	×	×	×	×
	sputum with 1% boric acid		82	18×	4×	0.5×	0.5×	0.5	—	×
			++++	104	10	1.5×	×	0.5	—	×
			10	5	4	—	—	0.5	—	×
			223	17	6	—	0.5	—	—	—
			++	95	0.5	—	—	—	—	—
			1.5	×	×	—	—	—	—	×
			260	×	×	×	—	—	—	—
			—	×	×	×	×	—	—	—
New swab culture (modified Ogawa medium)	sputum with saline	107	137	130	37	11	13	6	0.5	—
		++++	++++	++	85	1	—	—	—	—
		17	27	11	6	0.5	—	—	—	—
		70	175×	145	14	—	—	—	—	×
		++	++	—	—	—	X	X	X	X
		21	10	3	0.5	—	—	—	—	—
		282	26	—	—	—	—	—	—	—
		2	—	×	×	—	×	×	×	×
	sputum with 1% boric acid		167	42	14	2	1	—	—	—
			+++	300	114	4	—	—	—	—
			9	3	—	0.5	—	—	—	—
			260	137	27	4	—	—	—	—
			++	++	13	—	—	—	—	—
			8	3	0.5	—	—	—	—	—
			250	—	—	—	—	—	—	—
			—	×	—	—	—	—	—	—

— = no growth × = partial contamination X = full contamination.

Table 6. Results of culture (numbers of colonies) after storage of inoculated media for different durations

Temperature and location	Method	Days of storage after inoculation								
		0	1	2	3	4	5	6	9	12
Bangkok 30–23°C	Ogawa medium 0.1 ml pipette	52 45 139	92 37 182	62 30 ++	100 39 ++	40 30 122				
Tokyo 27–12°C	Modified Ogawa 0.05 ml pipette	6 53 20	5 42 18	6 36 10	6 37 17	6 37 11	5 32 11			
Tokyo 20–14°C	Modified Ogawa 0.05 ml swab	2 9.5 172 299			1 5.5 152 240			0 8.5 116 185	1 3.5 99 210	3.5 2 84 186
Bangkok 4–8°C	Ogawa medium 0.1 ml pipette	36 148	44 190	25 200	27 150	20 120				
Tokyo 5–8°C	Modified Ogawa 0.05 ml swab	6 48 19	9 61 50	6 48 18	5 40 19	7 43 28	3 47 18			

EXPERIMENT 2. CULTURE ON MEDIUM KEPT AT ROOM TEMPERATURE AFTER INOCULATION

Materials and methods

Sputum specimens were collected as for Experiment 1, but from 10 inpatients. The specimens were treated with strong alkali without neutralization and were inoculated direct on slopes of medium. The slopes were stored at room temperature or in a refrigerator for periods ranging from 1 to 12 days, after which they were moved into an incubator and kept at 37°C. The same experiment was performed at different times and in different places.

Results

The results for the cultures on which the numbers of colonies could be counted exactly are presented in Table 6. It is concluded from these results that final colony counts are not significantly influenced by keeping medium for a fairly long time in conditions in which growth cannot occur—e.g., if there is an interval of 12 days between inoculation and incubation.

EXPERIMENT 3. COMPARATIVE STUDY OF IMMEDIATE AND OF DELAYED INCUBATION

Materials and methods

Specimens of purulent sputum were selected from among routine specimens at our laboratory. Each specimen was inoculated on four slopes of modified Ogawa medium by means of the new swab culture method. Two of the slopes were incubated immediately after inoculation and the other two were stored at room temperature (17–23°C during this experiment) for 1 week and then placed in the incubator.

Results

As is shown in Table 7, there was no difference at all between the two groups as regards the positivity and contamination rates.

EXPERIMENT 4. SURVIVAL OF BACILLI AT VARIOUS TEMPERATURES

This experiment was made in order to confirm that tubercle bacilli can survive on the surface of egg medium for a long time, even if their growth is arrested.

Materials and methods

A 3-week stock culture of *M. tuberculosis*, strain H37Rv, grown on the surface of Sauton's liquid medium, was ground by the crystal-bead method. Suspensions (10^{-2} and 10^{-3} mg/ml) were made in distilled water. These suspensions were then diluted 1 : 10 with distilled water and with water that had been collected from the surface of modified Ogawa medium (throughout the remainder of the present

Table 7. Comparative study of positivity and contamination rates with incubation immediately and one week after inoculation

Incubation	No. of sputum specimens examined	Positive cultures		Partly contaminated		Fully contaminated [a]	
		No.	%	No	%	No.	%
Immediately	178	35	19.7	14	7.9	2	1.1
After 1 week	178	34	19.1	13	7.3	1	0.6

[a] Fully contaminated cultures were regarded as negative.

paper, this will be referred to as " condensation water ").[a]

Two series each of 63 slopes of modified Ogawa egg medium were inoculated with 0.1 ml of the 10^{-3} and 10^{-4} mg/ml suspensions in distilled water (the inoculum sizes were 10^{-4} and 10^{-5} mg per medium). Three slopes from each series were placed in the incubator at 37°C immediately after inoculation. The remaining slopes were divided into 4 groups of 15, the first group being kept at 5°C, the second at 15°C, the third at 22°C, and the fourth at 30°C. On the 4th day after inoculation, 3 slopes from each group were placed in the incubator at 37°C, this procedure being repeated with the remaining slopes on the 8th, 12th, 16th, and 20th days after inoculation.

The 10^{-3} and 10^{-4} mg/ml suspensions in distilled water were also divided into 5 groups kept at 5°C, 15°C, 22°C, 30°C, and 37°C, respectively. On the 4th, 8th, 12th, 16th, and 20th days, 0.1 ml of each suspension was inoculated into slopes of modified Ogawa medium and these were placed in the incubator at 37°C.

The suspensions in condensation water were kept at 22°C until 0.1 ml of each was inoculated, at the same intervals, into slopes of modified Ogawa medium. These also were placed in the incubator at 37°C.

Results

Fig. 3 shows the decrease in the number of viable units for the inoculum of 10^{-5} mg/ml—the highest at which the number of colonies can be counted exactly. The logarithmic numbers of colonies are shown in the ordinate and the duration of storage (in days) in the abscissa. It is clear that the survival rate of tubercle bacilli suspended in distilled water is reduced daily, the reduction being the more rapid the higher the temperature. On the other hand, tubercle bacilli inoculated on the surface of egg medium survived for a fairly long time, even at a high temperature. For example, at 30°C, the colony counts had dropped to one-hundredth in distilled-water suspensions by the 10th day, whereas, on the egg medium, they had not decreased by the 20th day. The curve showing the decrease in viability of the colonies in the suspensions in condensation water is almost the same as that for the inoculated media.

[a] In this experiment, the amount of monopotassium phosphate in the medium was reduced to 1 g because the inoculum was neutral.

DISCUSSION

Sputum specimens collected in outlying areas without well-equipped laboratories are usually sent to a central laboratory for culture. If the time of transport is long, the results of culture may be less reliable owing to the deterioration of the sputum.

Studies on the preservation of sputum for culture have been reported by Honda (4), Konuma (5), Ito et al. (6), Šula et al. (7), Engbaek & Bentzon (8), and Henkel & Meissner (9), who nearly all agree that contamination increases with the duration of storage and increased temperature.

Several antiseptics (e.g., trisodium phosphate, boric acid, and cetylpyridinium bromide) to prevent sputum from decomposing have been proposed and tested. However, even if contamination could be successfully controlled by adding antiseptics, it would still be difficult to prevent the growth rate of tubercle bacilli from decreasing (see Experiment 1, page 76). The viability of tubercle bacilli is also adversely affected by long storage and high temperatures (see Fig. 3). Therefore, if sputum is to be preserved without a loss of viability of the tubercle bacilli and without an increase in the contamination rate, it would have to be kept at a low temperature—e.g., 4°C, as proposed by Engbaek & Bentzon (8). How-

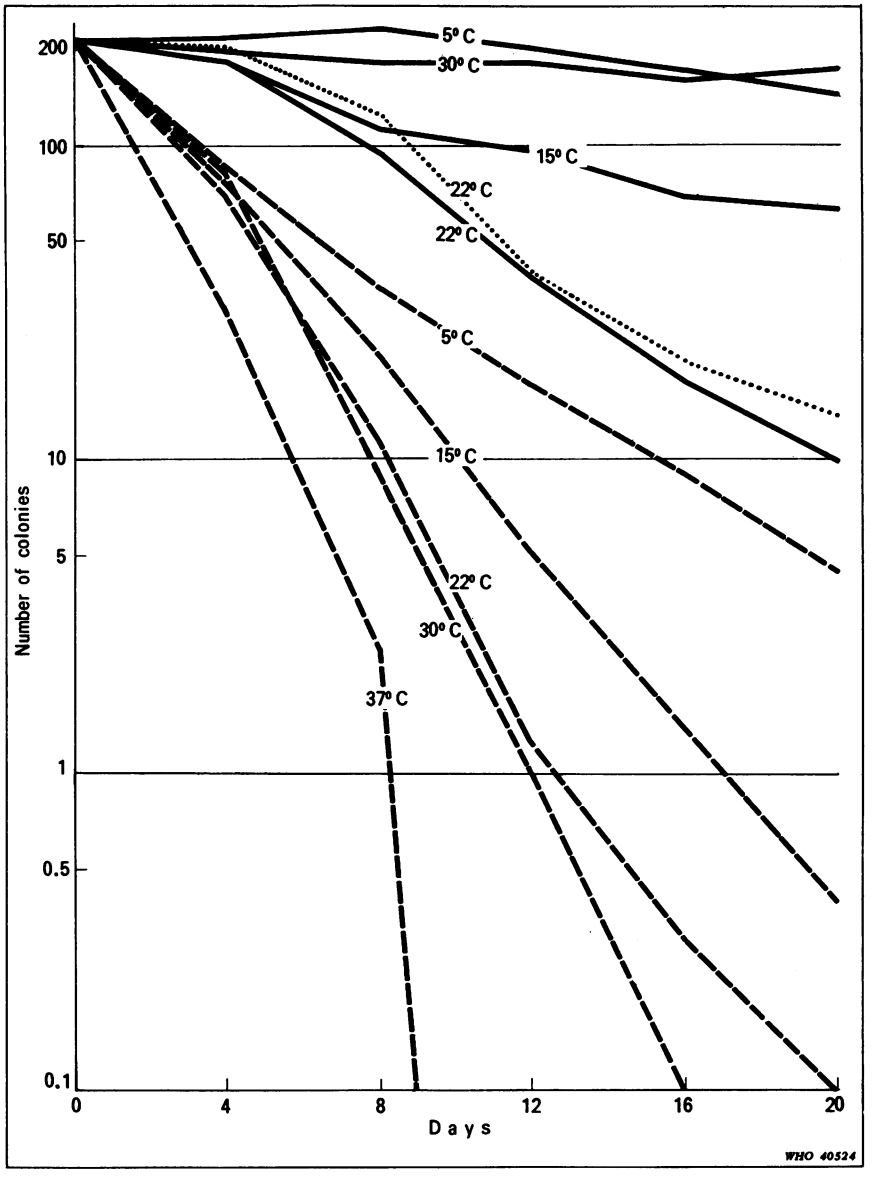

Fig. 3. Survival of tubercle bacilli inoculated on the surface of egg medium (continuous line) and suspended in distilled water (broken line) and in condensation water (dotted line), at various temperatures.

ever, it is often difficult—especially in rural areas in the tropics—to keep sputum cool.

We therefore studied the feasibility of storing medium on which sputum had already been inoculated at the place where the specimens were collected. The failure of similar experiments carried out in the past seems to have been due to extensive contamination of the cultures. This difficulty can be overcome by the use of the new swab culture technique described in part I of this paper.

It is evident from the results of Experiments 2, 3, and 4 (part II, pages 78-79) that tubercle bacilli inoculated on the surface of egg medium can survive for a fairly long time without growing. This phenomenon is attributed to the rich amino acid contained in the medium (the work of Cho & Obayashi (10) on thermostable BCG vaccine is relevant in this connexion).

On the basis of the results obtained in our studies, we would emphasize the need for providing peripheral health institutions with ready-made culture medium, so that sputum specimens can be inoculated on the spot by the means available (e.g., coconut fibre for making swab sticks). A simple decontamination method can be applied without jeopardizing the culture results, and the inoculated slopes of medium can then be sent to a well-equipped laboratory for incubation and reading of the results.

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Annex 1

NEW SWAB CULTURE METHOD

(1) Test tubes (100 mm in length and 25 mm in diameter, if possible) are wrapped in paper 10 at a time and sterilized by oven or autoclave. (As many test tubes as sputum specimens are required.)

(2) Swab sticks 20 cm long are made from coconut leaves or bamboo fibre. A small amount (0.05–0.1 g, dry weight) of absorbent cotton wool is firmly attached to the end of each swab stick with a cotton thread. The swab sticks are then wrapped in paper, 10–50 at a time, and the packages are sterilized in an oven or autoclave.

(3) Sputum specimens are collected in waxed carton cups or any other type of sputum container.

(4) Slopes (two per specimen) of Ogawa's medium or modified Ogawa medium are prepared by coagulating the medium in test tubes with cotton wool plugs. The tubes are then placed in racks, usually 20 to a rack. McCartney bottles or Universal containers with screw caps may be substituted for the test tubes.

(5) A 300-ml plastic fountain bottle is disinfected with an ether–ethanol mixture and a sterile solution made up of 4 g of sodium hydroxide and 100 ml of distilled water is placed in it. One bottle of the solution suffices for 100 sputum specimens. Two-millilitre volumes of the solution are then filled into test tubes. These test tubes also are placed in racks.

(6) One speck of purulent sputum is smeared on to each swab (it is not necessary to wet the cotton wool beforehand) and the swab is immersed in the sodium hydroxide solution in a test tube.

(7) After thorough stirring with the swab stick for 1–2 min, taking care that the sputum does not stick to the inner wall of the tube, the specimen is inoculated on the medium by smearing and squeezing the swab over its surface. (The inoculum size should not be greater than 0.1 ml per slope.)

(8) The inoculated slopes are kept moist by placing them in a plastic bag or screw-capped jar.

(9) After use, the swabs, swab sticks, and sputum containers (if these are made of waxed carton or other combustible material) are incinerated on the spot.

RÉSUMÉ

UNE TECHNIQUE SIMPLE DE CULTURE DES BACILLES TUBERCULEUX

Les échantillons de crachats recueillis dans des régions rurales démunies de laboratoires bien équipés sont ordinairement expédiés à un laboratoire central où l'on procède à la culture. Si la durée du transport est trop longue, cette culture risque de donner des résultats douteux ou nuls par suite de la décomposition ou de la contamination du matériel.

Pour pallier ces inconvénients, les auteurs proposent une technique simple. Les instruments et réactifs nécessaires sont préparés au laboratoire central puis expédiés au laboratoire périphérique. C'est dans ce dernier que les échantillons sont inoculés au milieu de culture (milieu à l'œuf d'Ogawa, modifié) selon un procédé décrit dans le présent article qui limite le danger de contamination. Les milieux inoculés sont ensuite envoyés au laboratoire central pour mise en incubation et lecture ultérieure des résultats.

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