Pyruvic acid egg medium for tubercle bacilli

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SYNOPSIS Sputum culture for tubercle bacilli on a solid egg medium containing glycerol was compared with culture on a similar medium containing pyruvic acid and no glycerol. Tubercle bacilli from the sputum of five out of 99 patients grew on pyruvic acid medium but not on glycerol medium. The addition of pyruvic acid is therefore essential when an egg medium is used for the cultural diagnosis of tuberculosis. There was no clear relation between catalase activity or isoniazid resistance and preferential growth of tubercle bacilli on pyruvic acid medium.

Marks (1963) has redirected attention to the use of pyruvic acid in solid egg media for the routine primary isolation of tubercle bacilli. This procedure had previously been advocated by Stonebrink (1958) as a result of earlier work by several authors quoted in his paper.

Marks compared a pyruvic acid medium with a similar medium containing glycerol and reported that nearly twice as many cultures grew on pyruvic acid alone as on glycerol alone. This finding showed that pyruvic acid was a good medium, but if the only advantage of its use were to detect the lingering remnants of scanty bacilli in treated patients, small laboratories might be excused the feeling that the addition of yet another medium to their normal routine was not justified by the marginal advantage secured. Cruickshank (1965) recommended the use of pyruvic acid medium 'for the isolation of human strains that are drug-resistant and difficult to grow'. The work reported here was undertaken in order to discover whether the use of a pyruvic acid egg medium ought to be considered mandatory in a laboratory undertaking routine sputum cultures for tubercle bacilli on solid egg media.

METHODS

Sputum specimens were received in 1 oz. Universal screw-capped glass containers. To each specimen was added an equal volume of 4% sodium hydroxide and the Universal containers were then shaken mechanically for 10 minutes and allowed to stand in an incubator at 37°C. for a further 30 minutes. Each alkaline homogenate thus produced was decanted into a fresh Universal container containing 15 ml. of distilled water which was then centrifuged. Most of the supernatant fluid was discarded and the deposit, well mixed with the remaining fluid,

was divided between a pyruvic acid and a glycerol slope by means of a disposable drinking-straw pipette. In alternate weeks either the pyruvic acid or the glycerol slopes were inoculated first. The cultures were inspected after a fortnight and thereafter weekly; negative cultures were discarded after six to seven weeks, save that cultures from microscopically positive specimens were not discarded before 10 weeks.

The medium resembled that of Marks (1963) and is made up as follows:

KH ₂ PO ₄	11.4 g.
Na ₂ HPO ₄ , anhydrous	6.0 g.
Mg ₂ SO ₄ .7H ₂ O	0.3 g.
L-asparagine	3.6 g.
Glycerol	12.0 ml. (in glycerol medium only)
Distilled water	to 600 ml.

Boil to dissolve solids, cool to 60°C., and add 30 g. potato starch.

Heat to suspend starch, cool, and add to the well-mixed contents of 20 aseptically broken large fresh eggs. Mix and add 10 ml. of 2% solution of malachite green in distilled water.

Pyruvic acid (technical grade, British Drug Houses) is neutralized with 2N NaOH and 25 ml. of this mixture is added in the case of pyruvic acid medium from which the glycerol has been omitted.

After filtration through sterile gauze add 100,000 units of benzyl penicillin dissolved in 10 ml. of sterile distilled water. Dispense in 10 ml. amounts in Universal containers, slope, and sterilize in an autoclave at 75°C. for 20 minutes on two successive days.

RESULTS

All sputa received during a period of 14 months were cultured on one slope of pyruvic acid and one slope of glycerol medium.

Excluding cultures in which one or both slopes were contaminated, 14 months' work yielded 365

cultures positive for *Mycobacterium tuberculosis* derived from the sputa of 99 patients.

Two hundred and seventy-four cultures grew on both pyruvic acid and glycerol slopes. Growth was detected on one slope at least two weeks before the other on 14 occasions, on 11 of which the glycerol slope, and on three the pyruvic acid slope, yielded the faster growth.

Sixty-eight cultures grew only on pyruvic acid and 23 grew only on glycerol. When these were more closely analysed it was seen that 33 of the cultures growing only on pyruvic acid were derived from the sputa of five patients. Bacilli from patient A seemed to have become pyruvic-dependent half-way through the study period; patient D yielded a few colonies on pyruvic acid alone on four occasions, whilst on a fifth both slopes were positive but the pyruvic acid grew a fortnight before the glycerol. Tubercle bacilli from the other three patients never grew on glycerol medium.

Apart from these five patients there were 35 positive cultures on pyruvic acid alone and 23 on glycerol alone. Many of these were cultures yielding from one to five colonies only and growth on one medium or the other may often have been due to the chance distribution of the few viable bacilli in the inoculum between one tube and the other, although the balance was still slightly in favour of the pyruvic acid medium. There was no patient whose tubercle bacilli consistently grew on glycerol only.

Preferential growth on pyruvic acid medium was not clearly related to the drug sensitivity of the bacilli. Sensitivities for the five pyruvic-dependent strains are shown in the Table.

TABLE

DRUG SENSITIVITY OF PYRUVIC-DEPENDENT TUBERCLE BACILLI

Patient	Streptomycin	Isoniazid	P.A.S.
A	Resistant	Resistant	Resistant
B	Moderately resistant	Moderately resistant	Moderately resistant
D	Sensitive	Sensitive	Sensitive
N	Sensitive	Resistant	Sensitive
W	Sensitive	Sensitive	Sensitive

Sixteen other patients yielded among them 49 cultures of isoniazid-resistant tubercle bacilli, of which four were positive on pyruvic acid, one was positive on glycerol only, and 44 were positive on both. In seven of these patients glycerol gave slightly quicker (one week) or more luxuriant growth of tubercle bacilli, whereas in three patients there was better or quicker growth on pyruvic acid. Isoniazid-resistant bacilli from the remaining six patients grew equally well on both media.

DISCUSSION

In 94 out of 99 patients the use of a pyruvic acid egg medium was not clearly advantageous in comparison with a glycerol medium, and indeed in some cases glycerol yielded quicker and slightly more luxuriant growth. The significant observation was that tubercle bacilli from five out of 99 patients did not grow at all on glycerol medium in repeated tests spread over considerable periods of time. If only the standard glycerol egg medium had been used these patients would have been diagnosed as arrested, non-infectious, or non-tuberculous as far as bacteriological criteria went. Five per cent of patients is too high a proportion to miss and the results reported here emphasize Marks' dictum (*loc. cit.*). 'If a choice had to be made, pyruvic medium would be recommended in preference to glycerol medium'. A further point is that pyruvic acid medium, as Marks points out, will grow bovine tubercle bacilli more satisfactorily than glycerol. No evidence on this point was collected during the present work, however.

Marks stated that pyruvic acid discourages the growth of certain anonymous mycobacteria, and for this reason suggested that one slope of each medium should be used. Too few anonymous mycobacteria were isolated during this work to test Marks' statement, but three out of the five strains encountered grew on both media and two on pyruvic acid alone. *M. kansasii*, said to dislike pyruvic acid, did not occur. Some strains of *M. tuberculosis* grew more quickly and slightly better on glycerol medium and this in itself may be an argument for retaining one glycerol slope in laboratories where the preparation of two different media is practicable.

Stonebrink (1961) found that all the isoniazid-resistant cultures he tested grew better on pyruvic acid than on glycerol medium. This was not the case in this investigation where some isoniazid-resistant strains seemed to be slightly favoured by glycerol, some to be indifferent, and some to be favoured by pyruvic acid. Isoniazid resistance is not, therefore, the factor responsible for growth enhancement by pyruvic acid.

Three out of five pyruvic-dependent strains and 12 out of 16 pyruvic-indifferent but isoniazid-resistant strains of tubercle bacilli were available for testing for catalase production, for Stonebrink (1958) suggested that neutralization of hydrogen peroxide might be the means by which pyruvic acid enhanced growth. Such neutralization would of course be more beneficial to catalase-negative than to catalase-positive bacteria.

All three pyruvic-dependent strains tested were catalase-negative. Of 12 pyruvic-indifferent, isoniazid-resistant strains, five were catalase-negative.

three weak positive, and four positive. It appears therefore that although pyruvic-dependent strains lack catalase, catalase-negative strains are not necessarily pyruvic-dependent, and thus that catalase deficiency is not the whole explanation of the dependence of some strains of tubercle bacilli on pyruvic acid for growth on solid egg medium.

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