A SIMPLE BLOOD TELLURITE MEDIUM FOR THE ISOLATION OF *C. DIPHTHERIAE.*

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In a recent important paper by Anderson, Happold, McLeod and Thomson (1931) on the existence of two types of *C. diphtheriae*, a new chocolate tellurite medium has been described for the easy isolation and differentiation of this organism. The authors appear to have shown conclusively the superiority of their medium over the usual serum-tellurite media, and they claim that it permitted the "determination of the presence or absence of *C. diphtheriae* at sight in 90 per cent. of cultures from throat swabs after 18 to 24 hours incubation." The above medium would appear to show a slight superiority over the somewhat similar blood tellurite medium of Clauberg (1931).

In our laboratories extended trials have been made of various serum tellurite media, especially that of Alison and Ayling (1929), but the results have not been sufficiently satisfactory to justify its adoption as a routine medium, one of the disadvantages being its inhibition of the growth of occasional strains of *C. diphtheriae*. O'Meara (1931) recently has found similar difficulties with this medium.

For the detailed preparation of the medium employed by Anderson *et al.* (1931) the original paper should be consulted, but the essential feature of the medium is a broth basis sterilised by filtration (not by heating) to which agar and fresh rabbits' blood 7–10 per cent. is added and completed by the addition of 0·04 per cent. pot. tellurite. The mixture is heated to 75° C. for 10–15 min. before being poured as plates. In this, as in Clauberg's later medium, the superior results obtained would appear to be correlated with the presence of the red blood corpuscles, the type of blood used being apparently of little importance, as Clauberg used sheep or ox.

Taking the results of both media into consideration, it appeared to us that greater simplicity might be obtained by the use of a simple tellurite blood-agar mixture without heating or other treatment. As difficulties of filtration in the Sudan are notorious, we used nutrient agar sterilised by heat in the usual way, to which was added blood 7–10 per cent. and 0·04 per cent. pot. tellurite.

Type of blood. Human, rabbit, ox, horse were all tried, and although there was little difference in their results, ox or horse appeared, on the whole, to

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give more profuse growths and abundant supplies of either were available, a further advantage for a routine medium.

The blood (ox or horse) was drawn from the jugular vein into sterile sod. citrate sufficient to give a final dilution of about 1 per cent. The citrated blood was poured into small rubber-capped bottles (100 c.c.) which were stored in a refrigerator (about 8° C.). In a temperate country where the risks of contamination are much less than in the Sudan, the blood could be taken directly at the slaughter-house into a sterile flask containing citrate solution.

We also investigated the relative values of whole blood, ox or horse, as compared with the washed corpuscles; there appeared to be no difference in the results.

Blood tellurite-mixture. During the course of the work it was found that the addition of the pot. tellurite in the required amount to the fresh blood and storing the mixture in the refrigerator for 2 or 3 days (8° C.) gave even more favourable results than adding the solution separately at the time of pouring the plates. An added advantage, especially in the tropics, is that the tellurite prevents any contamination of the blood. This blood-tellurite mixture gave excellent results up to 21 days, although, at this period, the corpuscles were entirely haemolysed. As after 30 days the results were definitely inferior, 20 or 21 days should be adopted as the maximum limit for storage.

On the above medium *C. diphtheriae* and the diphtheroids grew readily, but many other organisms, especially streptococci and yeasts present in throat or nose swabs, also appeared after 24 hours, and, in the endeavour to render the medium more selective, varying percentages of tellurite were tried.

The results with freshly isolated diphtheria strains were as follows:

	24 hours pot. tell. per cent.					48 hours pot. tell. per cent.				
Strain	0·04	0·11	0·16	0·18	0·22	0·04	0·11	0·16	0·18	0·22
A	+	+	+	±	−	+ +	+ +	+ +	+	+
B	+	+	+	+	+	+ +	+ +	+ +	+ +	+
C	+	+	+	±	−	+ +	+ +	+ +	+	±
D	+	+	+	±	±	+ +	+ +	+ +	±	±

+ + = heavy growth.
+ = good growth.
± = a few scattered colonies.
− = no growth.

From the above figures it would appear that pot. tellurite at about 0·16 per cent. is the maximum concentration that can be used, without definitely inhibiting growth (of some strains) after 24 hours.

After 48 hours with this amount heavy growth was obtained with all strains while three strains showed good growth at 0·18 per cent. but strain D was definitely inhibited. It is interesting to compare this concentration (0·16 per cent.) with the standard concentration of 0·04 per cent. which has been used by most previous workers, but so far we have encountered no strain of diphtheria which is in any way inhibited by this percentage and hence have judged it a safe concentration for routine purposes.

Inhibitory effects of 0·16 per cent. tellurite.

The effects of 0·16 per cent. pot. tellurite on other throat or nose organisms are interesting. Swabs plated directly on to the medium with this concentration fail to show any growth other than *C. diphtheriae* and the diphtheroids in about 90 per cent. of cases; in the remaining plates variable numbers of organisms such as streptococci grow as minute colonies in 36–48 hours.

The following was a more severe test. Loeffler cultures, 24 hours old, from throat swabs selected at random were emulsified to give a strength of 1000 million per c.c. and five loops (3 mm. diameter) of each emulsion were spread on blood tellurite (0·16 per cent.) plates and examined after 24 and 48 hours.

Culture	24 hours	48 hours
(1)	–	A few colonies of *C. hofmanni*
(2)	–	–
(3)	–	–
(4)	–	–
(5)	–	–

– = sterile.

These results demonstrate in a striking way the inhibitory or bactericidal effect of the tellurite.

A second experiment, similar to the above, was tried using a much denser emulsion 5000 million per c.c. and plating as before five loopfuls from each emulsion.

Culture	24 hours	48 hours
(1)	*C. hofmanni*	*C. hofmanni* + Streps
(2)	*C. hofmanni*	*C. hofmanni* + Streps
(3)	*C. hofmanni*	*C. hofmanni* + Streps
(4)	–	*C. hofmanni*
(5)	–	–

The cultures were from nose swabs, which accounts for the frequent presence of *C. hofmanni*. Although not entirely inhibiting the growth of streptococci owing to the massive inocula employed, the results demonstrate the total inhibition of organisms other than the diphtheroids in 24 hours.

Medium.

The medium, as now used by us for routine purposes, is as follows:

(1) Any ordinary nutrient agar 2 per cent. at *p*H 7·6. The broth basis as used in these laboratories consists of meat extract 1000 c.c., peptone (Parke Davis) 10 gm., NaCl 5 gm.

(2) Ox blood.

(3) Pot. tellurite (B.D.H.) 2 per cent. in distilled water.

To tubes of agar (15 c.c.) melted and brought to 50° C. add 1·5 c.c. of both (2) and (3); mix and pour as plates. After pouring tilt the covers of the Petri dishes to dry off water of condensation. There is no risk of contamination. Alternatively, as mentioned above, the blood and tellurite can be previously

mixed in equal parts and stored in a refrigerator; 3 c.c. of the mixture is added to the melted agar.

Anderson and his co-workers have shown that on the basis of morphology, appearance of colonies, fermentation reaction and virulence tests two clear-cut types of *C. diphtheriae* exist and the names *gravis* and *mitis* have been assigned to these types.

In the present investigation all strains were examined and conformed sharply to one or other of these types, and in consequence the authors have adopted this simple, very convenient classification.

APPEARANCE OF COLONIES.

The following are the points which we have found of most assistance in the identification of colonies:

Diphtheria mitis.

24 hours	48 hours
Small, greyish, flat discs (1–2 mm. diameter), rather translucent.	Translucent discs (2–4 mm. diameter), black by reflected light, narrow greyish margin by transmitted light.

Diphtheria gravis.

Small grey discs (1 mm. diameter), with black granular centre, slightly opaque.	Grey discs (1–2 mm. diameter), with black granular centre, greyish margin much wider than *mitis* when examined by transmitted light.

C. hofmanni.

(*a*) Common type—Dull pearly grey, umbonate 2–3 mm. diameter in 48 hours, common in nose.
(*b*) Rarer type—Jet black, translucent, slightly convex discs, 1–2 mm. diameter in 48 hours. It is frequently impossible to differentiate this type from *mitis* on the medium.

Both types of *C. hofmanni* are exceedingly minute after 24 hours, giving the appearance of frosted glass to the naked eye or tiny pearls when examined by a watchmaker's lens.

C. xerosis.

Colonies so similar to type (*b*) *C. hofmanni* that we found it impossible to differentiate them on plate cultures.

In our experience *C. xerosis* is a very rare organism.

ODOUR OF CULTURES.

The unpleasant garlic-like odour of *C. diphtheriae* on tellurite media is well known, and all our strains had this characteristic odour. An observation which was made independently by the present authors was that cultures of *gravis* were much more pungent smelling than *mitis*, but there were insufficient strains of the former in the series to confirm this observation.

The pungent odour is definitely given by some strains of *C. hofmanni* (type (*b*)), and hence although of great help in the differentiation of *C. diphtheriae* cultures, it is not a specific diagnostic point.

Microscopic morphology of *C. diphtheriae* on blood-tellurite media.

This has been discussed in detail in the above-mentioned paper by Anderson *et al.* (1931). In brief, *mitis* type has the characteristic text-book morphology although the granules are not well marked. In our experience, it is difficult, if not impossible, to identify *gravis* type from some strains of *C. hofmanni*, as both show short non-granular bacilli.

Colonies, when picked off and subcultured on Leoffler's slopes, always show the characteristic morphology after 18 to 24 hours.

Types found in clinical conditions.

In a subsequent paper will be shown the value of the medium for the isolation of carriers amongst school children.

In addition, the following is a brief summary of all strains of diphtheria which have been isolated from routine swabs sent in for examination for the first three months of 1932.

The swabs were first rubbed on Loeffler's slopes and then on tellurite plates, and in all suspicious cases colonies were isolated from the latter.

In all: 19 cases were positive by both methods; 22 cases were positive on tellurite plates; 3 cases (carriers) were negative on Loeffler but positive on tellurite plates. No case so far has been encountered which was positive on Loeffler but negative on the tellurite medium.

Appended below is a list of the strains.

Clinical condition	Type	Virulence (intracutaneous method) Mair (1930)
Carriers (4)	*mitis*	Positive
Carriers (9)	*mitis*	Negative
Mild diphtheria (throat) (3)	*mitis*	Positive
Mild diphtheria (nose) (1)	*mitis*	Positive
Convalescent diphtheria (1)	*mitis*	Positive
Fatal case	*gravis*	Positive (marked reaction)
Contact of previous case (no symptoms)	*gravis*	Positive
Carrier	*gravis*	Positive
Carrier*	*gravis*	Negative

* Gave characteristic appearance and fermentation reactions of *gravis*; non-haemolytic.

Summary.

1. A simple blood-tellurite medium has been described which is absolutely selective for *C. diphtheriae* and the diphtheroids. In many cases diagnosis at sight of the colonies is possible after 24 hours' incubation. The medium is especially useful for the isolation of carriers when diphtheria bacilli are present in very scanty numbers.

2. The existence of two main types of *C. diphtheriae* has been confirmed.

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