

blackish colonies also." Anderson, Happold, McLeod, and Thomson (1931) also describe dark colonies due to *B. influenzae*, some streptococci, and certain proteolytic aerobes as occurring on their tellurite medium. Sutherland and Iredale (1937) describe a dark colony on Clauberg's medium due to a Gram-positive bacillus.

In the nine false positive results in this series staphylococci were recovered from the throat in five cases and diphtheroids in two; in the other two cases no investigation was made apart from the exclusion of the diphtheria bacillus.

In the course of the investigation it was found that if a growth of the Klebs-Loeffler bacillus on Loeffler's medium was subjected to the application of the tellurite solution used for the throat experiments and retained at body temperature in the incubator for about twenty minutes definite darkening of the colonies would occur. Acting on the assumption that this reaction *in vitro* was comparable to that obtained *in vivo* in the throat, the following experiment was undertaken.

EXPERIMENT TO DEMONSTRATE THE PREVALENCE OF NON-DIPHTHERITIC THROAT FLORA THAT MIGHT GIVE RISE TO DARKENING

A batch of forty-four swabs sent in from the city in the usual way were plated out on Loeffler's medium. The majority of these came from the presumably healthy throats of school children swabbed in an attempt to discover a diphtheria carrier. After eighteen hours' incubation the growths were examined for the Klebs-Loeffler bacillus. In no case was the diphtheria bacillus found. The tellurite solution was then applied to the cultures, and they were returned to the incubator for half an hour. At the end of this time, on examination thirty-six out of the forty-four cultures were seen to show definite darkening. The prevalence of non-diphtheritic organisms which showed darkening in this experiment confirmed the impression that the percentage of potential false positive results in this tellurite test must be very high.

The difference between the figures obtained in this series and those of Dr. Manzullo can possibly be explained by the assumption that epidemicity occurs in all those organisms which give darkening, whether pathogenic or not, and that they may be present in exudates in sufficient numbers to give darkening with the test even though they are not necessarily the causative organism. It is possible that there may have been in this series an epidemic increase in some such organism.

### Summary

The technique of this suggested clinical test for diphtheria is simple.

A negative result is of value in that it supposes with great accuracy (100 per cent. in this series, 92 per cent. in that of Dr. Manzullo) that the disease is not diphtheria.

It is unlikely that any case of diphtheria would be missed through reliance being placed on the test, if the technique were not at fault.

Such a high percentage of false positive results may be obtained that no definite diagnosis of diphtheria should be made on a positive result.

It is possible theoretically, and in view of the discrepancy between these results and those of Manzullo, that the proportion of false positive results will vary from place to place and from time to time.

This test can in no way take the place of the clinical and bacteriological methods of diagnosis already in use.

I should like to thank Dr. Mackenzie, medical superintendent of the hospital, for permission to undertake and publish this work and for the help he has given throughout its course. I should also like to thank Dr. Ward, city pathologist, and his staff for help on the bacteriological side.

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## THE IMMEDIATE TELLURITE TEST IN DIPHTHERIA

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Since Conradi and Troch (1912) suggested the addition of calcium tellurate to a medium for the isolation of *Corynebacterium diphtheriae* the value of this method of distinguishing its colonies has been generally recognized. Later workers who devised modifications of the earlier media recommended the use of potassium tellurite instead of calcium tellurate. Owing to the power of *C. diphtheriae* or its products to reduce these salts of tellurium, colonies of *C. diphtheriae* have a distinctive appearance, being black or greyish-black in colour. Reducing activity towards tellurite is not shown only by *C. diphtheriae*, and a variety of organisms commonly present in throat swabs exert a similar action, which causes colour changes in their colonies. *C. hoffmanni* and other organisms of the diphtheroid group, streptococci, and staphylococci form colonies of a greyish-black colour, while *B. proteus* and some Gram-positive spore-bearing bacilli grow as brownish colonies. A fuller account of these appearances is given by Allison and Ayling (1929), and they also note that a characteristic somewhat pungent odour is emitted from cultures of *C. diphtheriae* on their improved medium.

In a recent communication Manzullo (1938) of the Bacteriological Institute of the National Department of Hygiene at Buenos Aires describes a method of rapid diagnosis in cases of diphtheria. A 2 per cent. solution of potassium tellurite is prepared, the salt being dissolved in distilled water at a temperature not above 40° C. This solution should be renewed at intervals of thirty days. A swab dipped into the solution is applied to the membrane or exudate on the patient's throat. If the infection is caused by *C. diphtheriae* the area that has been in contact with the swab is said to show obvious blackening within five to ten minutes. If not, there should be no change in colour. The tongue should not be touched, since its surface is the only part of the buccal mucous membrane which possesses the power under normal conditions of blackening potassium tellurite. The throat should not have been recently treated with hydrogen peroxide, tannic acid, or methylene-blue. Manzullo states that in sixty-nine out of seventy-five patients with pharyngeal exudate the immediate tellurite test, the provisional clinical diagnosis, and the result of bacteriological culture corresponded. In three of the remaining six cases the tellurite test and culture were in complete agreement. While the culture was positive in three cases with a negative test, in no other case was the test positive with

a negative culture. A high degree of agreement was thus shown to exist between the test and the recognized methods of diagnosis in diphtheria.

### Present Investigation

In view of the knowledge that such a variety of bacteria commonly present in throat infections exhibit reducing activity towards tellurite in artificial media, it was thought that confirmation of the specificity of the test was required. We decided, therefore, to apply the method in a series of 200 unselected patients with pharyngeal exudate, the diagnosis being established by the clinical appearances and bacteriological culture. For the most part, cultures on Loeffler's medium were examined on three successive days before a case was pronounced negative for *C. diphtheriae*. In approximately fifty cases two cultures were examined, and the presence of *C. diphtheriae* was confirmed by isolation of the organism, virulence tests, and typing into gravis, mitis, and intermediate strains. In assessing the clinical diagnosis we considered the results of bacteriological examination before admission to hospital as well as the local appearances in the fauces.

After a slight experience of the test it was found that some cases of diphtheria required more than one application of the tellurite solution before a positive result could be obtained. This was attributed to the mechanical difficulty of application, especially in a young patient, and 40 per cent. glycerin in distilled water was substituted as the solvent for the potassium tellurite. This fluid adheres more effectively to the swab and to the fauces, and does not in any way affect the reading of the test. Approximately two-thirds of the cases were investigated with this solution. If the exudate showed no blackening at the end of ten minutes the test was recorded as negative, and throughout our series we saw no results which suggested the advisability of lengthening this period.

In about one hour after performing the test a pungent garlic odour is detectable in the patient's breath. This has been described by Allison and Ayling (1929) as characteristic of tellurite plates containing a growth of *C. diphtheriae*; in our cases, however, the odour has not been entirely limited to diphtheritic infections. The smell is persistent, often remaining for several weeks after the faucial condition has cleared up; but, while unpleasant to the attendants, it is apparently not objectionable to the patient.

### Analysis of Results

The results of investigating the tellurite contact test, bacteriological culture, and the final clinical diagnosis in 200 patients with pharyngeal exudate or pseudomembrane are analysed and classified in Tables I, II, and III.

TABLE I.—Tellurite Contact Test and Bacteriological Culture

Tellurite Positive — Culture Positive	....	108 cases
.. Negative — .. Negative	....	27 ..
.. Positive — .. Negative	....	43 ..
.. Negative — .. Positive	....	22 ..

We have compared in Table I the tellurite contact test with bacteriological examinations for *C. diphtheriae* after admission to hospital. In 151 (75.5 per cent.) of the 200 cases a positive immediate tellurite reaction was obtained, while *C. diphtheriae* was grown on culture in 130 cases (65 per cent.). The findings on culture and on application of the tellurite test were in agreement—that is to say, either both positive or both negative—in 135 cases (67.5 per cent.). Dissimilar results were found in sixty-five cases (32.5 per cent.); in twenty-two cases negative to the tellurite test culture was positive, and in forty-three cases a positive test was not confirmed by the bacteriological

examinations. Of the 130 cases from which *C. diphtheriae* was grown on culture, 108 (83.1 per cent.) gave a positive tellurite reaction. In forty-three (61.4 per cent.) of the seventy cases in which *C. diphtheriae* was not isolated a positive reaction to the tellurite solution was observed.

TABLE II.—Tellurite Contact Test and Clinical Diagnosis

Tellurite Positive : Diphtheria	..	129 cases
.. Negative : Not Diphtheria	..	25 ..
.. Positive : ..	..	22 ..
.. Negative : Diphtheria	..	24 ..

In Table II we have analysed the results of the tellurite test in comparison with the final clinical diagnosis. The clinical diagnosis was assessed in the manner previously indicated. In all, twenty-three cases have been transferred from the non-diphtheritic to the diphtheritic group; bacteria morphologically resembling *C. diphtheriae* had been found in throat cultures of twenty-two patients before admission to hospital, and one patient showed the characteristic clinical features of diphtheria, though unconfirmed by culture. Of the 200 cases 153 were diagnosed as diphtheria. A positive tellurite reaction was found in 129 (84.3 per cent.) of the cases of diphtheria, while twenty-four cases (15.7 per cent.) gave a negative result. In 154 cases (77 per cent.) the findings of the test and the clinical diagnosis agreed in being positive or negative. Of the forty-seven cases in the non-diphtheritic group twenty-two (46.8 per cent.) showed a positive reaction on applying the tellurite solution.

The value of the immediate tellurite test as an aid to the diagnosis of diphtheria would depend on its yielding a high proportion of positive results in diphtheria and negative findings in other pharyngeal infections. To summarize, we may state that the tellurite test was positive in 83.1 per cent. of cases where we found *C. diphtheriae* on culture and in 84.3 per cent. of cases finally diagnosed as diphtheria. A positive tellurite test was noted in 61.4 per cent. of cases in which *C. diphtheriae* was not found after admission to hospital, and in 46.8 per cent. of cases in which the diagnosis of diphtheria was satisfactorily excluded.

The exclusive use of the classical Loeffler method of culture has been criticized as unsatisfactory in view of the aberrant morphology of certain strains of *C. diphtheriae gravis*. Brahy and his colleagues (1934) were able to furnish accurate reports in 83 per cent. of cases of clinical diphtheria within eighteen hours by the Loeffler slope method. We would claim greater accuracy, since in most of our cases examinations were done on three successive days before recording a negative bacteriological finding. Where the appearances on staining were suspicious, culture was continued up to twenty-four to thirty-six hours. This opinion is borne out by our figures, which show that *C. diphtheriae* was found in 130, or 86.3 per cent., of the 153 cases finally diagnosed as diphtheria. We consider, therefore, that a stringent standard was used in requiring negative findings on three successive days for the exclusion of diphtheria.

The apparent failures of the test have been submitted to a detailed examination. Of the twenty-four cases of diphtheria with negative reactions to the tellurite test eight showed scanty patches of membrane, and the absence of blackening might be explained on this ground. Six patients with severe tonsillar or nasopharyngeal diphtheria gave a negative tellurite reaction, and in three of these cases repeated applications failed to produce any darkening of the membrane. Individual differences in reducing activity towards tellurite have been noted by Stuart (1938) in some starch-fermenting strains of *C. diph-*

*theriae*, and a lowered reducing power *in vivo* might explain the absence of reaction in some cases. A slightly higher proportion of negative reactions was found in cases from which a gravis strain was obtained, but the thirty typed cases in our series are too few to give conclusive evidence on this point.

TABLE III.—*Non-diphtheritic Cases (47)*

Diagnosis	No. of Cases	Tellurite Contact Test	
		Positive	Negative
Tonsillitis .. .. .	34	16	18
Scarlet fever .. .. .	9	3	6
Vincent's angina .. .. .	4	3	1

In Table III we have analysed the forty-seven cases of non-diphtheritic infections. Sixteen of the thirty-four cases of tonsillitis, three out of the nine cases of scarlet fever with tonsillar exudate, and three out of the four cases of Vincent's angina without associated diphtheria were positive on testing by the tellurite method. As controls these cases are open to the objection that in some instances their allocation to the non-diphtheritic group depended on excluding diphtheria by bacteriological methods. Reasons which support our opinion that a high degree of accuracy in this respect has been achieved are stated in an earlier section of this paper. In ten of the twenty-two patients positive to the tellurite test the clinical features suggested a diagnosis of streptococcal tonsillitis, scarlet fever, or Vincent's angina, and were not compatible with diphtheria.

### Discussion

As has often been emphasized before, the death rate in cases of diphtheria increases with delay in the administration of antitoxin. The only safe course open to the practitioner is to base his judgment on the clinical appearances and give antitoxin immediately if a provisional diagnosis of diphtheria is made. Since the significance of the findings is often doubtful in cases which show exudate on the throat, any bedside procedure offering immediate and reliable confirmation of a diagnosis of diphtheria would be of obvious value. The immediate tellurite test raised hopes of a high degree of usefulness in practice, if the apparent specificity of the reaction was confirmed on extended trial by other workers.

Some technical limitations of the test have been mentioned above—namely, the necessity for frequent renewal of the solution and the difficulty of applying it to a resistive patient. Without assurance of adequate application a negative result is unreliable. The reading of the test may be subject to confusion in the presence of natural greyness of the diphtheritic pseudomembrane.

Positive results have been obtained in a considerable proportion of patients with other pharyngeal infections such as tonsillitis, scarlet fever, and Vincent's angina. A small percentage of cases of diphtheria show no reaction to the test while recognizable by clinical appearances or routine bacteriological methods. Mild cases of diphtheria may fail to give a positive reaction owing to scantiness of the patches of membrane. We have not found the tellurite test helpful in the nasopharyngeal type of diphtheria, where early diagnosis is of great importance. No blackening occurs in this form when the local lesion is in the early phase of oedematous swelling, with an ill-defined pellicle of membrane. Some cases of nasopharyngeal diphtheria with widespread membrane gave a negative result even after repeated applications.

In view of these serious objections reliance cannot be placed on the immediate tellurite test in confirming or excluding a clinical diagnosis of diphtheria, and it cannot replace bacteriological methods at present in use. Speed and reliability in the morphological diagnosis of diphtheria have been improved by the use of specially prepared swabs, as described by Solé (1934) and Brahdny and others (1934). In special circumstances when bacteriological confirmation of the clinical diagnosis was desired urgently we have been impressed with the value of the method of rapid culture by the Folger-Solé swab. In the main the clinical appearances must continue to be the primary and decisive means of diagnosis in diphtheria.

### Summary and Conclusions

Two hundred cases of pharyngeal infection with exudate have been examined by the immediate tellurite test. Correspondence with bacteriological diagnosis was obtained in 67.5 per cent., and with clinical diagnosis in 77 per cent. of cases.

Definite blackening of the membrane occurred in a high proportion (84.3 per cent.) of cases of diphtheria.

Similar blackening occurred in a proportion (46.8 per cent.) of faucial lesions due to other organisms.

No blackening occurred in a small number of cases of diphtheria, often of a severe type.

With careful application, therefore, a negative finding affords presumptive evidence against diphtheria, but a positive finding does not establish the diagnosis. Though the test possesses a certain value, in view of the anomalous results in some cases of non-diphtheritic infections and severe diphtheria it cannot replace clinical diagnosis, either alone or supplemented by cultural methods.

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A new link in a chain of special institutes established in connexion with the University of Stockholm, an institute for biological research, was recently opened by the Crown Prince of Sweden. The new institute has been made possible largely through a generous grant from a fund established by the Swedish industrial magnate Axel Wenner-Gren, whose name it bears, and through other donors, including the Rockefeller Foundation and the late B. A. Hjort, the city of Stockholm contributing the site. The purpose of the institute is to facilitate research into the basic features of the physiology of the cells and tissues, with the double object of increasing knowledge and creating a broader basis for medicine. It comprises five departments: for cellular physiology, evolutionary physiology and heredity, physiological chemistry, metabolic research, and biophysics, respectively. The department for metabolic research is the link between theoretical research and practical clinical work, and the principal metabolic derangement which is there to be studied is diabetes. The new institute, with which several of Sweden's foremost scientists are associated, will maintain constant contact with Swedish medical institutions as well as industrial establishments, in order to contribute more directly to improvement of practical working conditions.