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EDITORIAL

For some a Conference may be but an enjoyable social event, but for those whose interest it is designed to serve, the benefits are more real and lasting.

Probably the greatest of these is the personal gain in meeting members with special interests, discussing their subjects with them, and being able to question authors of papers on aspects which need fuller explanation. Scientific publications, which should always be models of clarity, often omit the precise details of greatest interest or refer to other publications impossible to obtain easily; personal contact bridges this gap.

Then, too, there is the encouragement in meeting others working in the same field, and we would do well, in this relatively small community of scientific workers, to remember that any honest attempt to take a problem and explore it to the best of our ability, is worthy of every support; the final result may not necessarily be a whit less important to scientific work in general than work done with the aid of all facilities overseas.

An Association is, of necessity, composed of people with differing views, so that it is essential for periodical meetings to be held to hammer out a broad policy. In the case of our own Association, still in its infancy, there is much to do, and a letter from our President, published in this issue, touches on some of the problems. Other problems are the general staffing of laboratories, and in particular the smaller laboratories under Social Security regulations, and the policy of this Journal.

It is therefore urged that all members who can possibly do so will attend the Conference to be held in Palmerston North on August 3rd, and thereby assist the continued progress of our Association.

THE ISOLATION OF CL. TETANI FROM TALCUM POWDER

(L. G. ECCERSALL)

(From the Pathological Dept., Public Hospital, Auckland.)

The recent occurrence of several fatal cases of tetanus in infants has focussed attention on the isolation of *Cl. tetani* from infected material. In this paper the isolation of *Cl. tetani* from suspected talcum powder is discussed, the basis of isolation being the formation by *Cl. tetani* of a spreading outgrowth on solid media which will outgrow other anaerobes, and from which pure cultures may be obtained.

Media

(1) ROBERTSON'S COOKED MEAT MEDIUM. (Lepper and Martin's modification. 1929.) Details of the preparation may be found on reference to Mackie and McCartney (1945), or any other book dealing with laboratory technique. The medium is prepared in accordance with the original instructions, but sterilised on three consecutive days by autoclaving at 10 lbs. pressure for thirty minutes, a layer of approximately one-eighth of an inch of paraffin oil being added prior to the final autoclaving. The paraffin not only gives a more satisfactory anaerobiasis, but also prevents evaporation of the medium.

(2) 2% NUTRIENT AGAR.

(3) FILDES' PEPTIC DIGEST OF BLOOD. This is prepared in the same manner as described in Mackie and McCartney (1945). The pH, however, was adjusted in accordance with Fildes' original figures, (Fildes, 1920), the Beckman pH meter being used as the indicating device instead of indicators.

(4) PEPTIC-DIGEST-AGAR SLOPES. These are prepared by the addition of 5% of peptic digest of blood to the 2% nutrient agar.

(5) PEPTONE-WATER SUGARS. These are enriched by the addition of 1% of peptic digest of blood, phenol red being used as

indicator. Glucose, lactose, and saccharose were considered sufficient.

Technique in General

For the purpose of anaerobic incubation a McIntosh and Fildes' jar was used. The hydrogen, from a cylinder of compressed gas fed through a reducing valve, was introduced into the jar by means of a football bladder. Two bladders of hydrogen were forced through the jar by firm pressure with the hands, the outlet valve was closed, and a third bladder of hydrogen was left attached to the inlet valve for thirty minutes, while combination of the hydrogen and the remaining oxygen was taking place under the influence of the electrically heated element. A description is given in Mackie and McCartney (1945).

In this investigation the material under examination was talcum powder which had been used on the umbilical stumps of two newly-born infants, who subsequently died of clinical tetanus. The two tins of powder used on the respective infants, talc supplied from the same source, and a crude talc used in its manufacture were all examined. A suspension of the talc was made by placing a sample in a sterile 6in. x $\frac{3}{4}$in. tube and adding an equal volume of sterile water. This was well shaken and approximately 1 cc. inoculated with a Pasteur pipette into the bottom of a tube of cooked meat medium. Care was taken to avoid the introduction of air bubbles into the medium. This was incubated for three days. One to two ccs. of the culture were then pipetted off into a smaller sterile tube and utilised for the following investigations:—

(a) Smears were made and examined for the presence of morphological *Cl. tetani*.

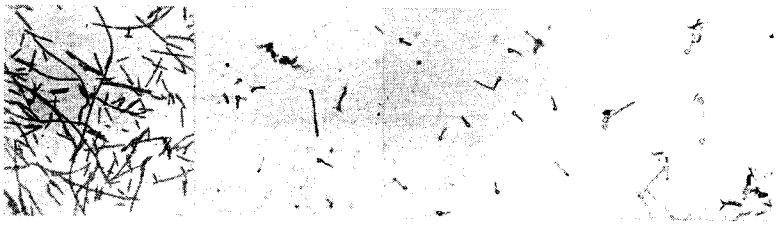
(b) If morphological *Cl. tetani* were present, animal inoculations were carried out as described hereunder.

Irrespective of whether or not morphological *Cl. tetani* were present, the cultures were heated for 30 minutes at 80° C in a water bath, the temperature being checked by a thermometer, and inoculations made into the water of condensation of a peptic-digest agar slope. These were incubated anaerobically in a McIntosh and Fildes jar and examined after 15 and 36 hours for outgrowths of *Cl tetani*, from which, if present, subcultures were made into cooked meat medium, or on further peptic-digest-agar slopes, for

obtaining the organism in pure culture. From such pure cultures the organism could be investigated for its biochemical reactions, further animals could be inoculated if deemed necessary, and any other required investigations carried out.

Morphology

From the primary culture in cooked meat medium all the tales proving positive showed typical organisms. The staining was invariably Gram-negative, "drum sticks" were frequent and there were a small number of "dumb bell" forms. The bacilli appeared as slender, straight and curved rods and some filamentous forms were seen. The spores showed as spherical rims, being two to three times the diameter of the bacillus. Fildes (1925a) used peptic-digest broth for primary cultures and states that spores are not readily formed in this medium. He therefore subcultured from all primary cultures to peptic-digest-agar without examination of the primary cultures. As a routine in this investigation, subcultures were made after heating for 30 minutes at 80° C. It is interesting to note that only those cooked meat cultures in which morphological *Cl. tetani* were demonstrated proved positive.



1 2 3 4

Fig. 1. Outgrowth of *Cl. tetani* showing filaments, on peptic digest agar. × 470.

Fig. 2. Primary culture in cooked meat medium showing early spore formation. × 600.

Fig. 3. Pure culture in cooked meat medium, 4 days. × 600.

Fig. 4. Primary culture in cooked meat medium showing "drum stick" and "dumb bell" forms. × 600.

In direct contrast to the typical appearance of the organism in primary culture, young cultures on solid media frequently consisted of filaments, many of which extended across the 1/12in. field. No spores could be found, and the tendency towards this filamentous formation was even more marked if the surface of the medium was at all moist. Some cultures consisted entirely

of long filaments, and when dealing with *Cl. tetani* it is important to bear this fact in mind, as no particular stress is laid on this point in the literature. After further incubation the organisms appeared more typical and many spores could be found. These spores stained solidly and tended to retain Gram's stain, many being only slightly larger than the diameter of the bacillus. Spherical spores were always present in mature cultures, although oval spores were frequent, especially in the early stages of growth. Occasionally a bacillus containing a central spore was found.

Growth on Solid Medium

When inoculated into the water of condensation of a peptic-digest agar slope, the organism grew as a spreading film on the surface of the medium and extended to the uppermost limits of the agar in a very short time. Fildes states that the rate of growth is approximately 1 cm. per hour. *Cl. tetani* will grow well on plain agar, but the growth on peptic-digest agar proved to be more rapid and slightly more luxuriant. A meticulous examination of the agar was necessary to detect the outgrowth of the organism. Good lighting, and an eyeglass or hand lens were essential, and a dark matt background was of assistance. Fildes' peptic-digest agar is well suited for the cultivation and examination of *Cl. tetani* in that it is particularly clear and assists the detection of a delicate film of growth as is found with this organism. At first examination the surface of the medium appeared to be without growth, but on closer examination a very fine film of growth could be detected. At the apex of the agar an extremely fine filamentous growth was found which could be aptly described as wandering, tangled, threads of silk. The splendid photographs of outgrowths of *Cl. tetani* appearing in Fildes' (1925a) paper are worthy of study. This typical appearance is best obtained on dry agar, and it has been recommended that the slopes be allowed to dry out for one or two days before inoculation. If the surface of the agar is moist a more confluent but still delicate growth will be obtained. Such outgrowths were found to consist entirely of *Cl. tetani,* and were used for subculturing to obtain a pure culture. The purpose of heating the mixed culture to 80° C. for thirty minutes is to kill other spreading organisms such as *B. proteus.*

Growth in Other Media

In cooked meat medium a good growth was obtained after two to three days' incubation. No marked characteristics were

observed. The meat was slightly discoloured and after several days very slightly digested. A few bubbles of gas were produced and the odour was sour. No change was observed in peptone water sugars enriched with 1% of peptic digest of blood. Glucose, lactose, and saccharose were inoculated and neither acid nor gas were produced after seven days' incubation. Only in cases where a non-toxic strain of *Cl. tetani* is found (as referred to hereunder) would sugar reactions seem necessary to exclude *Cl. tetanomorphum* and *Cl. sphenoides.*

The recognition of colonial appearance proved difficult owing to the spreading nature of the organism and discrete colonies could not be obtained. Very slight haemolysis was obtained on human blood agar. The difficulties of obtaining supplies of sterile horse blood made the use of horse blood agar plates impossible.

Pathogenicity for Guinea Pigs

The fact that the toxins of *Cl. tetani* are highly pathogenic to laboratory animals is well known, and constitutes the basis of proof of the presence of *Cl. tetani* in suspected material and in cultures. Fildes (1925b) has stated, however, that some strains, although highly pathogenic to man, are not pathogenic to guinea pigs, and in other strains spontaneous loss of toxicity may occur if a culture is kept for some days before inoculation into the animal. The positive cultures in this investigation were all pathogenic to guinea pigs. 0.25 ccs from the cooked meat culture were inoculated intramuscularly into the inner aspect of the right hind leg of each of two guinea pigs. One animal, the control, was protected two to four hours previously with 10,000 units of tetanus antitoxin, injected intraperitoneally. By the following morning, the unprotected animal was either dead or had local tetanus. Postmortem examinations showed little change, a slight bloody exudate from the affected muscle area being the only obvious feature. No attempt was made to recover the organism from the infected animal. In cases where the animal showed local tetanus, the condition was striking. The leg became rigidly fixed in extension, first at the knee joint, and later, as higher groups of muscles became involved, at the hip joint also. On palpation the affected muscles were very firm. Before death, which invariably took place within the next twenty-four hours, spasms were present in the muscles of the back. In the beginning of the investigation, when a smaller amount of antitoxin was used, the control animals developed local tetanus after a period of some

days, but the injection of 10,000 units of antitoxin proved adequate for complete protection.

Fig. 5. Typical local tetanus in right hind leg of guinea pig.

Discussion

In this investigation we were fortunate enough to encounter organisms which showed typical morphology, would spread to the top of an agar slope, and would produce typical tetanus in guinea pigs, whether injected in mixed or in pure culture. However, as strains may be encountered which present great difficulties in both isolation and identification, it is considered justifiable to mention some of the points which may not be generally appreciated after reference to available textbooks of Bacteriology.

(a) MORPHOLOGY. In typical cultures the shape of the spore depends on the stage of development. It begins as an oval enlargement at the end of the bacillus which later becomes a typical spherical terminal spore in a large proportion of the organisms. However strains are encountered in which all the mature spores retain their early oval shape. The position is summed up by Fildes (1929) who states: "The familiar description of the sporing bacillus with spherical, terminal spores is undoubtedly, to some extent, idealistic."

(b) ISOLATION. It is an essential part of the technique to heat the original mixed culture, not only to inhibit spreading aerobes and facultative anaerobes, but also to kill contaminants, which in some cases appear to inhibit the growth of *Cl. tetani.* Rarely strains are encountered which will not spread up an agar slope and these must be isolated by other standard methods.

(c) TOXICITY. The demonstration of toxicity by the injection of the culture into laboratory animals is not an absolutely constant feature of *Cl. tetani.* Although negative toxicity tests are usually associated with old cultures, it may also be found after recent isolation, as was demonstrated by Fildes with a culture from a fatal case of human tetanus which was found to be lethal to a mouse but completely harmless to a guinea pig.

A further point to note is that in mixed cultures the contaminants are frequently capable of destroying tetanus toxin, and thus inoculations with the original mixed culture or the original infected material are very frequently innocuous. This last point is worthy of special note since it is recognised that contaminating organisms in a culture may aid the growth of *Cl. tetani.* In this case the growth of the contaminants reduces the EH of the medium, thus producing more favourable conditions for anaerobic growth. This set of conditions is in no way parallel to the demonstration of toxicity by animal inoculation.

From the foregoing it is seen that individual strains may present difficulties in both isolation and identification and in the absence of toxicity with an otherwise suspicious pure culture, the naming of the organism as *Cl. tetani* may only be made with certainty by agglutination by one of the seven specific type sera of this group.

Summary

A method which proved satisfactory for the isolation of *Cl tetani* from talcum powder is described, and difficulties in the isolation and identification of the organism discussed.

ACKNOWLEDGEMENTS.

The writer expresses his appreciation to Dr. W. Gilmour, Director of Pathology, Auckland Public Hospital, for permission to publish this paper, to Dr. S. Hills, Assistant-pathologist, for much assistance and advice, and to Mr. R. W. Litherland, Clinical photographer, for the photomicrographs.

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AMOEBIASIS

(D. H. ADAMSON)

(*From the Pathological Dept., Public Hospital, Christchurch.*)

It is not wished to over-emphasise the subject of amoebiasis, as an article by the present writer appeared but recently (Adamson, 1945), but it has been found since then in this Hospital that several modifications of a Middle East technique described therein are indicated in laboratory diagnosis here.

1. In all cases a thorough purge, yielding at least six fluid specimens in one day, is necessary to exclude amoebiasis.

Calomel grs. 2-3 should be given at 4.0 a.m., followed by Mag. Sulph. crystals ozs. ½-1 in a little water at 7.30 a.m., and repeated at 8.30 a.m. and 9.30 a.m. with breakfast following. It is in only a few cases that either more or less purgative than this is necessary.

2. The first specimen sometimes contains unidentifiable motile amoebae (perhaps minute ones the size of a lymphocyte) and none can be found in later specimens. A second purge in a few days' time will sometimes show *E. histolytica*.

3. Again, amoebae found may exhibit extremely little motility even in the sixth or ninth stool, or "Structures resembling dead amoebae" only may be seen. In these cases, the typical, actively-motile trophozoites of *E. histolytica* with ingested red cells can often be found in the second or third specimen the next morning after a dose of ½-1 ounce of salts.

4. It is again emphasized that ingested red blood cells are often very difficult to recognise when they become small and pale. If amoebae are unidentified it is advisable to describe their appearance, for future reference, if for no other reason.

5. The T-shaped improvised warm stage, described by Mackie and McCartney (1945) has been found very satisfactory.

It is of interest that some of the cases diagnosed each month have been of soldiers from the Middle East or the Pacific, who have returned over a year previously. So far, their symptoms have subsided after specific treatment.

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TO THE EDITOR

Dear Sir,—

On behalf of the Council and Members of the Association I extend congratulations on your good effort in the production of the first issue of the *Journal*. I wish to endorse the remarks contained in your first editorial, and would urge all members, senior and junior, to give their active support to the Journal.

Our second Conference will be held in the near future and we look for the keenness and co-operation that characterised the first Conference. We are now an established body with a membership of 75. We are also an Incorporated Society, but I feel that we must once again discuss the question of registration in the light of knowledge and experience gained since the deputation approached the Director-General of Health. Following representations from our Association, the Director-General of Health has advised us that "the person qualified and holding the Department's Certificate was to be known as a 'Hospital Bacteriologist'," but I notice that in the Social Security (Laboratory Diagnostic Services) Regulations 1946, para. 5 (b) such persons are still referred to as "bacteriological assistants."

I still seek support to the idea of a preliminary examination to provide for recognition of experience gained in laboratories at present not under a Pathologist.

I strongly urge that CONFERENCE 1946 will have strong representation from all laboratories.

Yours sincerely,

E. L. F. BUXTON, PRESIDENT.

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