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## EDITORIAL

In one's school days, one common type of essay set was that in which one wrote the occurrences during a day in the life of some inanimate object. Supposing you were set to write down the feelings of your laboratory instruments with complete honesty, what story would they have to tell? Would they complain of being left dirty, unoiled, ungreased, overloaded and, in general, in no fit condition to carry out the work expected of them, with their work growing progressively harder, until they were finally forced to give up the unequal struggle, or would they tell of careful, skilful handling, with thought for their wellbeing and a knowledge of their limitations, so that they would be able to live to a ripe old age still giving faithful service?

Good care comes with knowledge of the instrument upon which the maker has lavished all the resources of his workshop and upon which he stakes his reputation. His staff, from an intimate knowledge of the construction, and being guided by the troubles experienced in faulty machines, has compiled handbooks in which is given in clear and concise language complete instructions for the use and care of the instrument. How many people discard the guide book with the wrapping, and then complain that the instrument is no good! It is said that a poor workman blames his tools: Does a good one understand them?

## THE USE OF CLAUBERG'S MEDIUM IN THE ROUTINE DIAGNOSIS OF C. DIPHTHERIAE

A. M. MURPHY

*(From the Pathological Laboratory, Auckland Hospital)*

Clauberg's medium has been used for a number of years in various laboratories throughout Great Britain and Germany and recently has been tried out in this Laboratory with very encouraging results.

Numerous media containing tellurite have been devised for the isolation of C. diphtheriae. Clauberg's medium contains, in

addition to the tellurite, two dyes (methyl blue and metachrome yellow), which give it further useful properties, namely, that C. diphtheriae grows as a blue-black colony surrounded by a blue zone in the medium, while diphtheroids appear as small, pale yellow colonies. The growth of the majority of other organisms is inhibited.

**Materials Required**

Although the medium contains a large number of reagents, once these are made up in bulk, a batch of medium can be poured in about half an hour.

(1) Fresh, sterile, defibrinated ox-blood, not more than three days old. This is best collected in a sterile wide-mouthed bottle, with glass stopper and containing a number of glass beads. The blood is vigorously shaken while in the process of clotting. (About ten minutes.)

(2) Glycerinated blood. To one part of pure glycerol which has been steam sterilised is added two parts of sterile defibrinated ox blood. This mixture improves with age and may be kept in the refrigerator indefinitely. It must, however, be allowed to ripen in the refrigerator for at least six weeks before use.

(3) Dye solutions.

(a) Methyl blue (B.D.H.), 2% in sterile distilled water in which the dye is readily soluble.

(b) Metachrome yellow. (Chrome fast yellow G—Clayton Aniline Co.); 2 grams of the dye is added to 100 c.c.'s of sterile distilled water and allowed to stand for two days with frequent shaking. The remaining precipitate is then filtered out.

There is no need to sterilise either dye solution.

(4) 20% sodium acetate solution (A.R.). Sterilised, 5lbs./30mins.

(5) 50% glucose solution (A.R.). Sterilised, 5lbs./30mins.

(6) Cysteine solution. 1 gram of pure anhydrous sodium carbonate is dissolved in 10 c.c.'s of boiling distilled water, 1 gram of cysteine hydrochloride is added, the whole boiled again, the volume made up to 100 c.c.'s with sterile distilled water, and the whole bottled in a sterile bottle.

(7) Potassium tellurite. 1 gram of fresh potassium tellurite, $K_2TeO_3$, is dissolved in 100 c.c.'s of distilled water by constant shaking. The solution can be sterilised by passing through a Seitz filter after moistening the pad with a little N/10 caustic

soda solution to prevent re-precipitation of the tellurite in the body of the filter.

(8) Buffered nutrient agar. This is made up in a similar manner to ordinary nutrient agar. To four litres of water are added three minced human placentas, 40 grams of peptone, 12 grams of sodium chloride and 8 grams of crystalline disodium hydrogen phosphate. The whole is heated to boiling and allowed to simmer for thirty minutes. It is then filtered through gauze, the remainder of the fluid pressed out, and the pH adjusted to 7.2. To this is added 160 grams of agar and the whole steamed for 15 minutes. The pH is then checked, adjusted to 7.2 if necessary and the medium bottled in amounts of 220 c.c.'s. It is finally sterilised by steaming for 20 minutes on three successive days. If human placentas are unobtainable, 3lbs. of veal free from fat may be used instead.

**Method of Preparation of One Batch of Medium**

*Agar.*—One bottle (220 c.c.'s) is melted in the steamer or by placing in boiling water, cooled to 48 degrees and maintained at this temperature in a water bath until required.

*Blood-tellurite Mixture.*—In a sterile flask of about 1000 c.c's capacity are placed 165 c.c.'s of sterile distilled water, 82.5 c.c.'s of sterile defibrinated ox blood. When lysis is complete, 11 c.c.'s of glycerinated blood and 18 c.c.'s of potassium tellurite solution are added. This mixture is then placed in the water bath at 48° C.

*Indicator Solution.*—In a sterile 100 c.c. flask are placed 30 c.c.'s of methyl blue solution, 10 c.c.'s of metachrome yellow solution, 3.5 c.c.'s of 20% sodium acetate, 15 c.c.'s of 50% glucose solution and 5 c.c.'s of cysteine solution. The mixture is then warmed to 48 degrees C.

When all three constituents have reached the temperature of 48 degrees C., the indicator solution is poured into the flask containing the blood tellurite mixture, and finally the agar is added. It is essential that the mixture of tellurite and indicator solution should not be maintained at 48 degrees C. longer than is absolutely necessary, as the tellurite undergoes a change when heated in the presence of glucose. In pouring the plates it has been found advantageous for one assistant to pour the medium into the plates, while another rotates them rapidly, in order to obtain an evenly-spread very thin layer of medium, which should be quite transparent and of a bright claret colour. It is possible to obtain from 100 to 120 plates 4½in. in diameter from each batch of medium. The plates can be used as soon as they have solidified and if stored in a cool place will remain in good condition for about a week. Those showing a roughness of the surface after

storage should be discarded.

**Inoculation of Plates**

The plate is divided into quarters and on to each a swab is rubbed. There is no need for thin spreading and, in fact, this is a disadvantage, as the blue colour, which is so characteristic of the diphtheria bacillus on this medium, develops more rapidly when the colonies are crowded.

**Time of Incubation**

Cultures made up to 5 p.m. each day are incubated overnight and examined about 9 a.m. the next morning. It is advisable, however, to return the negative plates to the incubator and to make a final examination late in the afternoon. When the growth is very scanty, the blue colour may not develop by 9 a.m., but becomes quite distinct later. An incubation period of 24 hours ensures that these scanty growths are not missed.

**Appearance of Colonies**

A profuse growth of C. diphtheriae consists of fairly large colonies which, on examination by transmitted light, are of dark blue colour. Extending from the margin of the mass of colonies there is a diffuse Prussian blue colouration of the medium usually about 2 m.m. in width. This blue zone surrounding the colonies forms a striking contrast with the red colour of the medium, an effect enhanced by the use of a very thin layer of medium. It is thus of the greatest importance not to pour the plates too thick.

The colonies are soft, moist and easily removed with a platinum loop. A diagnosis of C. diphtheriae should only be made when there is an obvious blue colour in the medium, easily seen in a good light. The use of a hand lens is unnecessary. Diphtheroids grow well on this medium and are pale yellow in colour at the end of 16 hours' incubation, but on further incubation slowly turn black. On two occasions, however, we found that C. xerosis produced a typical blue colouration of the medium. The only other organisms which may be confused with C. diphtheriae are gram-positive, sporing bacilli which appear as minute, pin-point colonies, black in colour and difficult to remove with a platinum loop. The colour produced is more of a greenish hue than that produced by C. diphtheriae and after a little experience these two colours cannot be confused. The majority of other organisms, including Staphylococci and Streptococci, are completely inhibited.

If it is desired to carry out sugar reactions or virulence tests, the colonies should be picked off as soon as possible, as the organism is likely to be killed by prolonged exposure to the tellurite.

**Comparison of Results Obtained with Clauberg's and Loeffler's Media**

In all 2023 swabs were inoculated on to both media. From these 49 positive results were obtained. In every case in which a positive result was obtained on either medium an attempt was made to isolate the organism in pure culture, and to prove the presence of C. diphtheriae by carrying out sugar reactions.

There were 48 positive results on Clauberg's medium, two of which were proved to be C. xerosis; 12 of these positives were negative on Loeffler's medium, although, on making further films and after careful searching, two were found to be positive. A further five were classified as suspicious on Loeffler.

There were 31 positives on Loeffler and a further 10 suspicious, making a total of 41 in all. Of these 6 were negative on Clauberg's medium, but in only one case was the organism proved to be C. diphtheriae. In two cases it was proved to be C. hofmanni and in the three remaining cases the organism was not able to be isolated.

Thus, the proven positives were:—

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**Conclusions**

(1) Clauberg's medium is relatively easy to prepare, but considerable work and care are required in the preparation and maintenance of the stock materials, and for this reason bacteriologists in small laboratories, where but a few swabs are received each day, may not consider the time and energy involved to be justified.

(2) On this medium the colonies of C. diphtheriae are easily recognised by the naked eye after 16-24 hours' incubation, which results in a great saving of time and labour when swabs are examined in large numbers. Up to 100 swabs can be examined in less than ten minutes.

(3) The medium is superior to Loeffler's medium, both as regards the number of positive results obtained and in precluding the risk of diagnosing other diphtheroids as C. diphtheriae.

(4) The medium also facilitates the isolation in pure culture and proof of existence of C. diphtheriae. Under favourable conditions it is possible to have the result of sugar reactions in 24 hours from the time of inoculation of the medium, an operation which may take 72 hours or longer using Loeffler's medium or other tellurite media. This point should not be overlooked, since the time factor is all-important in the diagnosis of diphtheria.

# A MODIFIED METHOD FOR THE MICRO-ESTIMATION OF BLOOD SUGAR BY THE TECHNIQUE OF FOLIN AND WU

J. B. BROWN

*(From the Pathological Laboratory, Auckland Hospital)*

Recent work in this Laboratory on the insulin sensitivity test (1) using the micro blood sugar technique of Haslewood and Strookman (2) showed anomalous behaviour at low sugar values. A spectrophotometric study of both the Haslewood and the micro Folin and Wu (3) methods showed that increasing errors arise, especially in the former method, as sugar values fall below normal limits. When the very small amount of sugar being detected in these methods is considered, this inaccuracy is not surprising. With modern colorimeters requiring but 5 c.c. of fluid, such dilution of the optimum concentrations which are used in the macro Folin and Wu method is unnecessary. The following simple modification of the macro method of Folin and Wu is now in routine use in this Laboratory for all cases where we have to collect the blood samples ourselves. Over 1 c.c. of supernatant fluid is obtained, so that results can be readily checked. Spectrophotometric examination has shown that a "200 mgm" standard is actually more accurate in the normal range for this method than is a "100 mgm" standard.

## Reagents

Reagents as for macro-method, see Harrison (4). Standard is 0.01% glucose in saturated benzoic acid.

## Method

1. Into 1.7 ml. of distilled water (Note I.) pipette 0.1 ml. of blood, rinsing the pipette thoroughly with the distilled water.
2. Add 0.1 ml. of 10% sodium tungstate, mix, add 0.1 ml. of two-thirds normal sulphuric acid, mix, stand three minutes and centrifuge.
3. To a special micro blood sugar tube (Note II.) add 0.5 ml. of clear supernatant fluid and 0.5 ml. of alkaline copper

solution, mix well.

4. To another similar tube add 0.5 ml. of standard sugar solution and 0.5 ml. of alkaline copper solution. Mix well. This is a 200 mgm. standard.
5. Place in a boiling water bath for six minutes.
6. Remove without agitation and cool rapidly.
7. Add 0.5 ml. of molybdic acid reagent to each, mix thoroughly by shaking. Stand ½-minute.
8. Dilute to the mark (6.25 ml.) with distilled water, mix by inverting twice.
9. Match in the colorimeter immediately, setting the unknown at 20 m.m. Then the reading of the standard in millimetres multiplied by 10 gives the result in mgms. per 100 c.c. With results over 800 mgms. per 100 c.c. repeat, using 0.25 ml. of supernatant fluid plus 0.25 ml. of water and multiply the answer by 2.

**Note I.**

Blood sugar values are stable for about six hours in distilled water. For periods longer than this the water should contain as preservative 100 mgms. of sodium fluoride per 100 c.c. of water.

**Note II.**

The tubes can be readily constructed from 6 x ⅝in test tubes by heating near the closed end and pushing out the end with a blunt metal rod to form a constriction about ¼in. diameter at the softened part. The bulb can then be softened and blown to size to contain 1 c.c. fluid with its meniscus in the constriction. A calibration mark at 6.25 c.c. is made with a wet file.

**Summary**

A modified Folin and Wu micro blood sugar technique is described. The method utilises the reagents for the macro method and possesses the same ease of manipulation. By working at a greater glucose concentration than the published micro colorimetric method greater accuracy and ease of colour matching is attained.

**References**

(1) Fraser & Smith—Quart. J. of Med. 1941 10 297.
Fraser, Albright & Smith—J. Clin. End. 1941 1 297.
(2) Haslewood & Strookman—Biochem. J. 1939 33 920.
(3) Harrison—Chem. Meth. in Clin. Med. 2nd Edn. P144.
(4) Harrison—ibid. P142.

# AN IMPROVED PROCEDURE FOR THE DIGESTION OF SPUTA IN THE CONCENTRATION OF TUBERCLE BACILLI

P. H. CURTIS

*(From the Pathological Laboratory, Auckland Hospital)*

An article by Corper, H. J., and Stoner, R. E. (J. Lab. Clin. Med., 1946, 31, 1364), on the use of trisodium phosphate for the preparation of material to be cultured for T.B. suggested a possible improvement in the concentration procedure for tubercle bacilli in use in this Laboratory, and, after some preliminary experiments it has been used exclusively since January, 1947, for this purpose. The salt used is the commercial hydrated trisodium phosphate, as used for the cleaning of glassware, in 23 per cent. strength.

## Procedure

On the afternoon of the day on which a specimen of sputum is received, it is reduced where necessary to a quantity not exceeding 5 c.c., and an equal amount of 23 per cent. trisodium phosphate is added, the specimen is well shaken and incubated at 37° C. overnight. The following morning, the specimen is removed from the incubator, shaken again to complete disintegration, and 8 to 10 drops of indicator (0.2% Pot. alum, 0.002% Brom. Thymol Blue and 0.5% Sodium hydroxide in aqua dest.) is added. The point of neutralisation is then reached by adding in small quantities a 25 per cent. solution of hydrochloric acid until the indicator just changes from blue to a yellowish green and the alum precipitate starts to appear, the specimen being gently swirled during this operation. The specimen is placed in a corked 25 c.c. tube and centrifuged for 5 minutes; the supernatant fluid is then decanted and a film prepared from the deposit and stained by the Ziehl-Neelsen method.

It should be noted that materials other than sputum may be concentrated as above with equal success.

## Digesting Properties

Digestion with trisodium phosphate as the reagent is considerably slower than with sodium hydroxide, but this has proved to be a definite advantage in the saving of actual working time. Whereas sodium hydroxide requires an hour for digestion, trisodium phosphate requires a minimum of 18 hours at 37° C. As will be seen, this enables digestion to take place overnight.

If the minimum period is allowed it has been found that incubation at 37° C. is necessary to assist digestion, but specimens may be left at room temperature (20-25° C.) for 36 hours or more with quite satisfactory results.

**Neutralisation and Precipitation**

Trisodium phosphate is a buffer salt. When neutralising by the addition of 25 per cent hydrochloric acid, the potassium alum contained in the indicator is thrown down as a precipitate more slowly at the point of neutralisation (pH 6.6-6.8) than when using unbuffered sodium hydroxide. This point cannot be stressed too strongly as the entire success of concentration depends on there being a minimum of precipitate present. Thus, this reagent is of the greatest assistance to the technician and ensures a higher degree of accuracy.

**Contaminants**

Corper and Stoner claim that contaminants including saprophytes and most moulds are destroyed after 24 hours' exposure to a 23 per cent. solution of trisodium phosphate at 37° C. By growing a pure culture of B. subtilis in sputum and then adding an equal quantity of 23 per cent solution of trisodium phosphate, it has been found here that this contaminant will withstand exposure to this reagent at 37° C. up to 42 hours, but is destroyed after this time.

They also claim that tubercle bacilli will remain viable up to seven days in this solution, the only effect after this period being a slight retarding of growth in the early stages of culture. Thus, the preparation of cultures is not attended by the haste necessitated by the destructive action of sodium hydroxide. To date this statement has not been verified in this Laboratory, but four successful guinea pig inoculations have been carried out after 48 hours' exposure to this reagent.

Up to the present time, specimen jars have not been issued containing a 23 per cent. solution of trisodium phosphate, but this could obviously be done in order to prevent further contamination, especially in cases where a specimen is required to travel long distances when the process of decontamination and digestion could be taking place.

**Summary**

1. A new reagent for the digestion and decontamination of sputum, trisodium phosphate has been described and compared with sodium hydroxide.
2. Precipitation of the potassium alum at the point of neutralisation in the concentration of sputum is slowed down considerably by the buffer effect of trisodium phosphate. This point is of the greatest importance (vide supra).
3. As trisodium phosphate digests sputum more slowly than sodium hydroxide, this process can take place overnight and thus save valuable working time.

**Acknowledgments**

I wish to thank Mr. D. Whillans, B.Sc., for his very valuable assistance, both in the writing of this article and in tests carried out on the above reagent.

# HERE AND THERE

## Auckland

On Monday, 23rd of June, 65 members and ex-members of the Auckland Hospital Board's Laboratories met at the Mt. Eden Tea Kiosk at 5 p.m. to farewell the Director, Dr. W. Gilmour, whose resignation was effective the following day.

Dr. Gilmour and Mrs. Gilmour were greeted by a burst of applause on entering, and Mrs. Gilmour was presented with a shoulder spray. The guests of honour spent the following half-hour in chatting to those present, and, on the power being turned on again, Mr. G. A. Haylock, Dr. Gilmour's first assistant, now retired, presented Dr. Gilmour with a crystal decanter and sherry glasses on behalf of the staff. In doing so, he recalled some of the small beginnings of the Laboratory, and assured Dr. Gilmour that he took with him the good wishes of his staff, both past and present, into private practice.

In replying, Dr. Gilmour thanked the staff for the presentation and for the farewell party. He said that the good wishes of the staff meant a lot to him and that he hoped to keep in touch with all his former staff. He also recalled the beginnings of the Laboratory, on the top floor of the present Manager's Office, in August, 1920, and mentioned by name many of the former members of staff. He said that he was particularly pleased to have present Mr. E. L. F. Buxton, at present in Auckland on a refresher course.

After the presentation the members of staff took leave of Dr. and Mrs. Gilmour, and the guests of honour left shortly after 6 p.m., carrying with them the good wishes of those present.

On Thursday, 29th May, the members of the Auckland Hospital Board's Laboratories held a dance in honour of the several members who were about to leave the staff, at the Cornwall Hospital Nurses' Recreation Hall. A very enjoyable evening was spent by all. Miss W. C. Corsbie has resigned to be Bacteriologist to J Force in Japan, Miss J. Byers has leave of absence for a year to study in England, and will first go to the Radcliffe Infirmary at Oxford to do Haematology, and Mr. K. B. Ronald has resigned to be Bacteriologist to the Oamaru Hospital Board. Mr. A. M. Murphy has taken over Miss Byers' position as Bacteriologist at Green Lane Hospital, and we have welcomed four new Cadets, Miss J. E. Caughey, B.Sc., Miss M. E. Armstrong, Mr. J. T. Connolly and Mr. M. McL. Donnell.

## Waipukurau

Engagement: Isobel Catherine Munro to Roderick Turner Chisholm.

Resignation: Mr. H. Hutchings to proceed to Palmerston North to complete his training.

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