

The titre of the anti-D was again measured by the albumin replacement method using CcDE phenotyped cells. No loss of avidity or decrease in solubility or titre was detected after testing at intervals throughout 10 years.

The activity of the papain-cysteine content of the mixture was investigated by using the mixture with an incomplete anti-c serum and testing against R'r, R''r, and rr phenotyped cells suspended in saline. All proved negative when equal volumes of the mixture and anti-c serum were used but with two drops of the papain-cysteine anti-D mixture (\equiv one drop of Löw's solution) and one drop of anti-c serum, complete agglutination of the red cells resulted within one hour at 37°C. The anti-c serum was checked to ensure absence of any saline agglutinating anti-c.

After testing, the reconstituted material was stored frozen at -20°C except during periods of re-tests. Each ampoule was re-tested three to seven times during the following four weeks. No loss of avidity, titre, or specificity was detected.

The pH of the reconstituted material was 6.1 but that of the original mixture had not been determined. However, a similar mixture, prepared later, had a pH of 6.3.

REFERENCES

- Löw, B. (1955). *Vox Sang.*, 5, 94.
 Gilbey, B. E., and Lindars, J. M. (1962). *J. clin. Path.*, 15, 47.

Use of a pancreatin-trypsin solution for the liquefaction of sputa for routine bacteriological examination

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The irregular distribution of pathogens within a single specimen of sputum from patients with chronic bronchitis was first demonstrated by May (1953), who found that a true assessment of the bacterial flora was obtained only after multiple cultures. The liquefaction of the sputum using a 1% pancreatin solution in saline buffered at pH 7.6 and at 37°C was introduced by Rawlins (1953) to overcome the need for multiple cultures, but this technique suffered from the disadvantage that some sticky specimens were slow to liquefy. Other workers (Mead and Woodhams, 1964; Woodhams and Mead, 1965) used N-acetyl-L-cysteine (N.A.C.) as a liquefying agent which they claimed gave a higher rate of isolation of *H. influenzae* and was more rapid in its action than pancreatin.

Recently it has been found in this laboratory that the addition of trypsin to pancreatin considerably reduces the time required for liquefaction, and in the present report the efficiency of the pancreatin-trypsin mixture is compared with that of N-acetyl-L-cysteine. Since from time to time it is necessary to examine sputa for the presence of eosinophils, which may also be unevenly distributed within the specimen, the effect on the detection of these cells of adding trypsin to the pancreatin has been included in the assessment.

MATERIALS AND METHODS

Five-hundred and fifty sputa were examined. The liquefying agents were each used on alternate days in order to rotate their use for the various outpatient clinics. In the analysis of the results no account was taken of the clinical diagnosis or treatment since the details supplied were often inadequate for the classification of the specimens.

Pancreatin-trypsin solutions were prepared by dissolving two tablets of Oxoid buffered pancreatin tablets in 100 ml of sterile distilled water and adding 25 mg of pure crystalline trypsin in 5 ml of sterile diluent. The trypsin used was Novo Crystalline Trypsin (Duncan Flockhart & Evans, Ltd.), in vials of 50 mg amounts, together with a vial of sterile diluent. Preliminary trials of trypsin concentration and alternative sources had shown that this product and concentration were satisfactory. N-acetyl-L-cysteine solutions were prepared as described by Woodhams and Mead (1965).

Sputa were liquefied in sterile 2 oz containers by adding

an equal volume of the liquefying agent and shaking for 15 to 20 minutes at room temperature. It was found that almost all sputa were liquefied in that time, but those specimens which had not converted into a homogeneous fluid were shaken additionally as necessary.

Cultures were made on blood agar and chocolate agar plates. On the latter an Oxoid Multodisk 30-9B was placed to enable a tentative sensitivity to be reported. The Cloxacillin included on this disc assisted the isolation of *H. influenzae* by inhibition of most other bacteria. Both plates were incubated overnight at 37°C.

To establish that prolonged exposure to pancreatin-trypsin did not impair bacterial viability, six sputa known to contain *H. influenzae* were liquefied and kept at 37°C for four hours, cultures being made at hourly intervals.

Dried films of the liquefied materials were stained by Leishman stain and the morphology of the eosinophils was assessed.

RESULTS

In the analysis of the results particular attention is paid to the incidence of *H. influenzae* and pneumococci, which have been shown in bronchitis to be significantly related to the presence of pus in the sputum (May, 1953).

It can be seen from the Table that 29 (24%) of the 123 mucopurulent sputa liquefied by the pancreatin-trypsin solution yielded a growth of *H. influenzae* compared with 17 (17%) of the 102 liquefied by N-acetyl-l-cysteine. Mucoid sputa contained *H. influenzae* in about the same proportion whichever liquefying agent was used, but the pneumococcus was isolated more frequently using a pancreatin-trypsin solution. When mucopurulent sputa are considered alone, 53 out of 123 (43%) of those liquefied by pancreatin-trypsin were found to contain either *H. influenzae* or pneumococci, whereas only 34 out of 102 (34%) sputa liquefied by N-acetyl-l-cysteine showed these organisms.

TABLE
INCIDENCE OF *H. INFLUENZAE* AND
PNEUMOCOCCI IN 550 SPUTA LIQUEFIED BY
PANCREATIN-TRYPSIN OR N-ACETYL-L-CYSTEINE

Mucopurulent	Mucoid
<i>Pancreatin-trypsin</i>	
<i>H. influenzae</i> 29 (24%)	10 (7%)
Pneumococci 24 (19%) } 43%	17 (11%) } 18%
<i>N. acetyl-l-cysteine</i>	
<i>H. influenzae</i> 17 (17%)	14 (8%)
Pneumococci 17 (17%) } 34%	10 (6%) } 14%

Prolonged exposure to either liquefying agent did not appear to affect the results as cultures made at hourly intervals showed no qualitative change in flora, only minor quantitative variations being exhibited.

Eosinophils could be estimated in sputa liquefied by either method, but the effect of the pancreatin-trypsin solution appeared on average to be less detrimental to cell morphology than N-acetyl-l-cysteine.

COMMENT

The addition of crystalline trypsin to a 1% buffered

pancreatin solution reduces the time taken to liquefy sputa. The concentration of trypsin used does not appear to be critical, but if the concentration is too low the time taken for the sputum to liquefy will be increased and the value of the trypsin will be lost. The addition of 12 mg of trypsin to each 100 ml of pancreatin solution is the lowest practicable concentration and at this level only those sputa containing fairly viscous mucus will take longer than 20 minutes to liquefy. By using the standardized crystalline trypsin as described batch variations will not be encountered and consistent results will be obtained. The material is supplied in sterile containers and pancreatin-trypsin solutions, made up in a sterile diluent, remain sterile and stable for at least one week when stored at 4°C. Trypsin used out of the bottle (Trypsin 1:250, Difco) is less active and the solutions made from it tend to become contaminated.

Any method of culturing sputa from patients with bronchitis and other respiratory disorders should be directed towards the isolation of *H. influenzae* and pneumococci as these two organisms are generally regarded as being of the greatest significance. *H. influenzae* and pneumococci were isolated from 43% of mucopurulent sputa when a pancreatin-trypsin solution was used to liquefy the specimen. This compares favourably with isolations using N-acetyl-l-cysteine where only 34% of the mucopurulent specimens revealed these pathogens. Woodhams and Mead (1965) found *Haemophilus* spp. (non-haemolytic) and pneumococci in 33% of sputa liquefied by pancreatin alone and in 34% of those liquefied by N-acetyl-l-cysteine. These results may not, however, reflect the true bacterial flora of the sputa examined as each specimen was divided into two parts and therefore the irregular distribution of organisms within a single specimen was not taken into account.

Comparing the results obtained in this laboratory with the two liquefying agents, it appears that a pancreatin-trypsin solution is more effective in liquefying cellular or 'infected' sputum than N-acetyl-l-cysteine, possibly because the latter is primarily a mucolytic agent, whereas a pancreatin-trypsin solution breaks up cellular debris releasing *H. influenzae* more readily from the cellular material.

SUMMARY

The examination of 550 sputum specimens either using a pancreatin-trypsin solution or N-acetyl-l-cysteine as a liquefying agent has shown that the former is more efficient for the isolation of *H. influenzae* or pneumococci. Liquefaction is carried out in a shaker and at room temperature. Such specimens may also be examined for eosinophils.

I wish to thank Dr W. R. G. Thomas for his advice and helpful criticism in the preparation of this paper.

REFERENCES

- May, J. R. (1953). *Lancet*, 2, 534.
Mead, G. R., and Woodhams, A. W. (1964). *Tubercle (Edinb.)*, 45, 370.
Rawlins, G. A. (1953) *Lancet*, 2, 538.
Woodhams, A. W., and Mead, G. R. (1965). *Tubercle (Edinb.)*, 46, 224.