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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'' 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 (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


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

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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 and chocolate agar plates.
On the latter an Oxoid Multidisk 30-9B was placed to enable a tentative sensitivity to be reported.
The Cloxacillin agar 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 pancreatic-
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

<table>
<thead>
<tr>
<th>Mucopurulent</th>
<th>Mucoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatin-trypsin</td>
<td>H. influenzae</td>
</tr>
<tr>
<td>Pneumococci</td>
<td>24 (19%)</td>
</tr>
<tr>
<td></td>
<td>43%</td>
</tr>
<tr>
<td>N. acetyl-l-cysteine</td>
<td>H. influenzae</td>
</tr>
<tr>
<td>Pneumococci</td>
<td>17 (17%)</td>
</tr>
<tr>
<td></td>
<td>34%</td>
</tr>
</tbody>
</table>

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 standard-
ized crystalline trypsin as described batch variations will
not be encountered and consistent results will be obtained.
The material is supplied in sterile containers and pan-
creatin-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 pneum-
ococci 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, how-
ever, reflect the true bacterial flora of the sputa examined
as each specimen was divided into two parts and there-
fore 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.

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