approximately 80% of the isolates grew well in semi-solid media. Growth was evident after 2-4 days and reached a maximum in 5-8 days. On the plates and slopes at 25°C about 60% of the isolates produced visible colonies; these were evident in 3-4 days and reached a maximum in 5-8 days. With some of the isolates, the colonies grew to a size comparable to that attained at the higher temperature. With others smaller ones only were produced. Slopes and semi-solid media showed no growth at 23°C within 20 days of incubation. Plates were not tested at this temperature.

As yet no attempt has been made to culture gonococci at temperatures below 36°C direct from clinical material. It is apparent however, that once isolated a large proportion of strains with little or no "training" will grow well, albeit slowly, at a temperature as low as 25°C. Information in current texts on this characteristic of the species is therefore inaccurate and misleading.

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Growth of *Neisseria gonorrhoeae* in brain heart infusion

Although relatively complex liquid media are available for growing *Neisseria gonorrhoeae*^{1,2} a simply prepared, heat stable liquid medium would be welcomed by venereologists for a variety of general purposes. Attention was recently drawn to a simple semi-solid medium consisting of Brain Heart Infusion, agar and starch which supported growth of all 65 isolates of gonococci tested.³ Although this medium was very useful, its agar content was a disadvantage for some procedures such as those requiring centrifuged deposits of cells. Further work showed what gonococci grew well in unmodified Brain Heart Infusion (BHI) (Difco) but

that the physical conditions under which the organisms were grown greatly influenced the yield. Growth was remarkably increased by growing liquid cultures in shallow layers. While this was in part, undoubtedly due to the aeration provided it was also attributable to the heavy growth which occurred at the medium-vessel interface, an observation which does not appear to have been made previously.

Fifty isolates of gonococci, some freshly isolated and others from desiccates, were tested and found to grow well in BHI. Many of the isolates were tested in several batches of the same medium and all gave similar and satisfactory results. Inocula were usually taken from a semi-solid culture and the final concentration of organisms in the broth before incubation was approximately 10⁵ colony forming units (CFU)/ml.

In conventional tube cultures at 36°C, growth gradually occurred at the surface of the medium as it did in semi-solid media. On prolonged incubation and if left undisturbed, growth gradually progressed down the interface of medium and tube.

Shallow layer cultures were carried out in 200 ml flat-sided glass bottles. The depth of the medium was approximately 2-3 mm. In such cultures turbidity developed both in the free medium and at the interface of the medium and glass surface. During the early stages of incubation faint patches of turbidity developed at the interface before any clouding of the medium itself was obvious. As incubation continued the interface layer became quite dense and the medium above it turbid. Gentle shaking of the vessel at this stage released the loosely adherent interface layer and yielded a dense homogeneous suspension. As would be expected there was considerable variation between strains but even the lightest growth was reasonably turbid. Several cultures yielded counts of approximately 10⁸ CFU/ml within 24-48 h of incubation.

As would be expected from established work in this field, aeration of the cultures by shaking also resulted in copious growth. No additional CO₂ was supplied in these experiments as it was obvious that heavy growth was attainable without such a measure. Also relatively heavy inocula were used and no attempt was made to determine minimal inocula required to initiate growth.

It was somewhat paradoxical that in deep unagitated culture, the organisms had a strong predilection for growth at the

medium-air interface, whereas in shallow layers the preference was for growth at the medium-vessel interface at the bottom of the culture. Numerous factors including CO₂:O₂ ratios were probably important factors in determining growth responses. However, it appeared that the solid surface *per se* provided a stimulus for the proliferation of the organism.

Many formulations of gonococcal media include proteose peptone which is a basic constituent of BHI. Although the latter medium is recommended for growing "fastidious" organisms, it does not appear to have been used for growing gonococci except in blood culture media where its properties are unavoidably modified by addition of the blood inoculum itself. While it is probable that BHI could be improved by various measures such as addition of starch, haemin and yeast extract, the results here demonstrate that the unmodified medium is adequate for growing gonococci and should be useful for at least some purposes. Auxotrophic mutants would of course pose their own specific nutritional requirements.

If the full potential of BHI for work with gonococci is to be realised, manufacturers must ensure that the medium is consistently suitable for the purpose. It may well be rewarding to investigate other types of unmodified dehydrated media currently available for their ability to support the growth of gonococci. Whatever the medium used it would seem that the propensity of the gonococcus to flourish at the vessel-medium interface is a characteristic worth exploiting.

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