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Haemophilus ducreyi infections – time for reappraisal

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SUMMARY

As the literature on *Haemophilus ducreyi* and clinical chancroid is reviewed, it becomes obvious that many significant findings have been forgotten over the years. As a result, from the time of Ducrey's original description of the organism in 1890 until about 1977, both clinical and laboratory experts in the United Kingdom believed that *H. ducreyi* infections were rare, generally acquired abroad, and almost impossible to confirm in the routine laboratory! In consequence it was a common view that it was not worth looking for *H. ducreyi* until all other possible causes of genital ulceration had been excluded. Moreover, the search for such an infection stopped as soon as any other cause for the patient's lesions had been found.

A decision to ignore this 'rule' in Sheffield led to our looking for *H. ducreyi* in specimens from an unselected series of patients with genital ulceration including a number with herpes genitalis infections. The surprise finding of *H. ducreyi* in circumstances suggesting that it was a secondary invader made us re-examine the whole question of *H. ducreyi* infections and chancroid and wonder if the same organism can act as a primary pathogen and as a secondary invader. An account of the media and methods we used and of the characteristics of the organism is presented. In an attempt to find out more about the characteristic coherent colonies of *H. ducreyi* we studied them with the scanning electron microscope. It is clear that the whole subject of *H. ducreyi* infections has been neglected in the United Kingdom, but we believe that interest has now been aroused and progress will surely follow. Some areas for further investigation are suggested.

INTRODUCTION

In 1947 the combined effect of the end of the war and the use of penicillin brought about a dramatic fall in the incidence of what were then called venereal diseases. For a short time it seemed possible that they would vanish as a serious clinical problem. Unfortunately, this did not happen and after a few years at a low level the incidence of sexually transmitted diseases increased dramatically. Since then the number of new cases seen in special clinics has risen steadily and the range of infections has also increased greatly. Sexually transmitted genito-urinary diseases due to a greater diversity of organisms are now diagnosed more than ever before, and we have also become aware of the importance of mixed infections in such patients. It is interesting to note that during the past 4 years

new gonococcal infections in Sheffield have remained steady at an average incidence of about 925 per year, but as a result of the steep increase in other infections they now make up no more than 10% of the work of the clinic. The problems today are herpes genitalis, chlamydial infections, non-specific urethritis. trichomoniasis, and gardnerella infections – and also non-syphilitic genital ulcerations. It was the rising incidence of the latter that made us decide that despite the textbook view of the relative rarity of *Haemophilus ducreyi* as a cause we should look carefully for the organism in a sample group of patients. We did so and found an unexpectedly high incidence of positives (Hafiz, Kinghorn & McEntegart, 1981). This sent us back to search the literature and, as can be seen from the following review, it is hard to understand why, with all the information about *H. ducreyi* which is available, the infection failed to make more impression. It is startling to find methods still being employed to confirm the diagnosis of chancroid in 1979 that were familiar to Ducrey in 1890!

The literature on *H. ducreyi* and chancroid falls roughly into three periods. The early period from the late 1890s until the end of the century, the second from about 1915 for another 40 years and, finally, the period of renewed interest dating from the work of Kilian in 1976 until the present day.

Early period. Although the flora of soft sores had been studied by other workers, the first clear account of a characteristic organism was given by Ducrey (1890) when he recorded the typical appearance of bacilli in stained smears made from such sores. Although he did not succeed in culturing the organism on laboratory media, he was able to produce skin lesions on the forearm of his patients by intradermal auto-inoculation of material from their lesions. Stained films of these skin lesions showed bacilli with the same characteristic appearance as those seen in the genital lesions. Serial passage by auto-inoculation was demonstrated, and three strains were transmitted for 15 passages. Auto-inoculation was shown to be a useful method for getting rid of any contaminants present in the original specimen. It is doubtful if any ethical committee today would sanction such methods!

Ducrey's observations were confirmed by other workers, but it was not until 1900 that the organism was obtained in pure culture by Bezançon, Griffon and Le Sourd using nutrient agar plus 20% whole fresh rabbit blood. From this earliest account of the growth of *H. ducreyi* on solid medium we have a description of a characteristic colony which is unlike that given by any other organism (save possibly gravis diphtheria). The colonies have a curious coherence which enables them to be pushed about the surface of the medium like lentils. These curious colonies are a recurring theme in the accounts which have accumulated over the years. The first observation on animal pathogenicity is that of Nimmel in 1901 who grew the organism and demonstrated its pathogenicity in rabbit and guinea-pig skin. Stein in 1908 reporting the growth on a medium similar to that of Bezançon, Griffon and Le Sourd, also comments on the peculiar colonies.

Second period. Soon after 'soft sore' became clinically recognized it was noticed that there was a marked male bias in the incidence of cases. From this it was concluded that asymptomatic infection in women must occur. The first confirmation of this view was provided by Bruck (1915) when he recorded tracing the symptomless female contact of two soldiers with soft sore.

It is interesting to record that Teague and Deibert, as early as 1920, reported

favourably on the use of culture in the diagnosis of chancroid, despite which diagnosis continued and indeed may still continue to be based on the 'typical appearance' of the organism in gram-stained smears. Often the smears were prepared from 24–48 h cultures of material from suspected lesions grown in media such as defibrinated whole rabbit blood.

Brams (1924) also suggested the occurrence of symptomless infections when he reported the isolation of a gram-negative streptobacillus from the smegma of 6 out of 30 normal men and added 'we have recorded a similar organism from the cervix and clitoris of women having no genital sores'. He therefore concluded that persons 'apparently normal may be carriers of the Ducrey bacillus'.

Saelhof (1924) presents an account of 'work undertaken to make bacteriological diagnosis of chancroid accurate and fairly rapid'. Primary inoculation was made by dropping the 2-in inoculating loop used to sample the lesions into a slope of clotted rabbit blood and subculturing film positive tubes onto a phosphate ascitic agar containing 5% sheep corpuscles. The author suggested that failures were due to incorrect media, inconstant temperature, or insufficient moisture which was most important. His success rate was 65% from clinical chancroid. It is also very interesting to note that he prepared smooth suspensions by putting washings from 6–8 plates in 'the globe of a ball mill which was run continuously for 48 h after which the emulsion was centrifuged slowly to remove clumps'.

The state of knowledge up to 1929 was summed up by Hewlett in an article on the subject which he contributed to the M.R.C. System of Bacteriology. This compilation is a mine of information in which bacteriologists may still rediscover forgotten facts about their subject. After the introduction of the sulphonamides in the latter part of this middle period there was some consolidation of understanding and a growing interest in the use of antimicrobials in the treatment of chancroid.

The successful use of sulphanilamide and sulphathiazole in the treatment of chancroid was recorded in 1941 by Kornblith, Jacoby and Chargin following a study based upon 175 successive ambulant patients. Greenwald (1943) writing about the diagnosis and treatment of chancroid comments that it is a relatively common venereal lesion. A further reference is made to carriers by Satulsky (1945) who comments 'there is evidence that the Ducrey bacillus may exist in the female genitalia as a saprophyte and when transplanted to other tissues may become a pathogen'.

Heyman, Beeson & Sheldon (1945), using whole defibrinated rabbit blood, reported successful isolations in 50 of 60 cases of chancroid they had studied. They tested the pathogenicity of the strains isolated by inoculation into rabbit skin.

In the same year Strakosch *et al.* (1945) studied 370 cases in which the diagnosis was based on the history and clinical evidence of infection. Positive cultures were obtained from 59% of these compared with 43% in which positive smears were obtained.

The following year (1946) Beeson added further to our knowledge of the growth requirements and antibiotic sensitivity of the organism. In particular he showed that both whole serum and washed erythrocytes would support growth when added to an agar base but reported that whole blood was best. He also emphasized the need for high humidity and said that cultures will not grow in a normal incubator.

In 1956 Deacon and his co-workers grew *H. ducreyi* in a clot medium with

subsequent transfer to solid medium and reported once more that 'all colonies when touched tend to remain intact'. Kaplan *et al.* (1956) in the same issue of the journal recorded the results of intradermal inoculation in rabbits and showed that the strains tested consistently produced lesions when inoculated intradermally, but failed to do so when simply rubbed into the scarified skin.

Also in 1956 Ajello *et al.* made a much-needed attempt to define the nutritional requirements for the culture of a virulent strain of *H. ducreyi*. In some measure their precise definition of the amino acids in the base medium was invalidated by the use of a serum overlay in all cultures. One significant comment is borne out by our own findings and that is 'the nature of *H. ducreyi* to grow colonially or in chains even in liquid medium made any precise quantitative estimate of growth impossible'.

In a study of penile lesions in U.S. armed forces in Japan, Barile and his colleagues (1962) set out to make a comprehensive microbiological investigation of the lesions in 32 patients. The group is a small one, but the emphasis is on the thoroughness of the laboratory investigations. The study group was matched by 47 control patients from the same units. The most interesting finding was that herpes virus was isolated from just over 31 % of lesions and *H. ducreyi* from almost 23 % and from two patients both organisms were isolated. Once more, comment is made on the tendency of the colonies to remain intact.

Kerber, Rowe and Gilbert (1969) give an account of the experience of the Army Medical Center in Saigon where specimens from chancroid were second only to gonococcal specimens in frequency. Although sent in transport medium, culture was unsuccessful and was discontinued after the first 40 failures. This may well have been due to a lack of humidity when attempting to culture the organism.

After these papers, which show a growing awareness of the value of culture, it comes as a surprise to find Borchardt and Hoke in 1970 recommending, as a simplified technique, the inoculation of serous fluid from the lesions into 10 ml of the patient's blood, inactivated for 30 min at 56 °C. Nevertheless, 21 of 24 patients with a presumptive diagnosis of chancroid were confirmed by this method of culture and smear. The appearance of the films is shown in a series of colour illustrations, and they are obviously characteristic as the following names suggest: 'tangled chains', 'parallel rows', 'railroad tracks'. The look is familiar and rather reminiscent of the 'cording' seen in the growth of tubercule bacilli.

So we come to the end of this middle period with little fundamental change in the thinking about chancroid. Standard textbooks on venereology still repeat the old concepts of the disease as rare and generally acquired abroad. The organism is considered to be difficult to isolate and indeed few laboratories, even when providing the diagnostic service for the special clinic, considered the organism either as a serious clinical possibility or as an attainable isolate bacteriologically.

The modern period. Little comment seems to have been made on the taxonomic position of *H. ducreyi* before Kilian in 1976 presented a study of 426 strains of the genus Haemophilus. In the course of this he examined nine stock strains of *H. ducreyi*, all originally from the cases of chancroid, and commented on their differences. He divided them into two which he felt were acceptable as members of the Haemophilus genus and seven that were unacceptable. However, as he was unable to judge which of the two groups agreed with Ducrey's original description he recorded the whole nine as *H. ducreyi*!

The following year Tan *et al.* (1977) gave an account of 500 cases of chancroid, which had been diagnosed on clinical evidence in one year in Singapore. Fifty six per cent of these cases were confirmed bacteriologically. The confirmation, however, was not based on colonial morphology but simply on the older method of inoculating swabs from lesions into 10 ml of the patient's own serum inactivated at 56 °C for 30 min. These cultures were incubated for 48 h at 37 °C and positive results recorded when gram-stained films showed typical G-bacilli. Attempts to grow the organisms on solid media were unsuccessful. A control group of 100 asymptomatic patients tested in the same way gave uniformly negative results.

In 1977 Khoo, Sng and Goh applied the same methods to a group of 200 asymptomatic prostitutes and found 29 positives, none of whom showed any genital tract lesions.

Chapel *et al.* (1978) in the course of a study of the microbiological flora of penile ulcerations in 100 consecutive patients found a polybacterial flora in 97. Herpes simplex was the most prevalent pathogen with syphilis next. *H. ducreyi* was isolated from two patients only, in each case in the presence of herpetic infection. It is interesting to note the results of their detailed study of the anaerobic bacteria and to see that *Bacteroides fragilis* was isolated from 17 patients and *B. melaninogenicus* from 18. The low recovery of *H. ducreyi* may, in part, have been due to the method employed, whereby samples taken from the ulcers with a wire loop were inoculated into the serum overlying freshly clotted human blood. Presumptive isolates were identified by the typical appearance in gram-stained smears. Suspected strains were sent to a reference expert for confirmation by immunofluorescence. In 1977 there was an epidemic of chancroid in Greenland which was described by Lykke-Olesen *et al.* (1979). In this outbreak, which spread along the west coast of Greenland, there were 975 cases in a population of 32000. The male/female ratio was 1·6/1·0. Symptomless cases were not considered to be important, and with the rapid response to sulphonamides, which were reported to effect a cure in about 1 week, it was anticipated that the outbreak would die out. Strains were recognized by direct microscopy of exudate taken from the ulcers or from auto-inoculation sites. In general the group's attempts to grow the organism met with very little success but the few organisms which were isolated corresponded with the earlier descriptions of *H. ducreyi.*

This outbreak demonstrates the ability of chancroid, as a new infection, to spread rapidly amongst a susceptible population. It is surprising that as late as 1977 auto-inoculation was considered to be justifiable when simpler and safer cultural methods for identification were available. Indeed, it illustrates the remarkably slow progress of ideas about *H. ducreyi*. The outbreak started just too soon to benefit from the medium described by Hammond *et al.* (1978*a*) and used by them in their study on 16 patients with chancroidal ulcers. This very successful medium consisted of chocolate blood agar plus 1% Isovitalex plus 3 μg/ml of vancomycin. With this medium the authors claim that the clinical suspicion of chancroid can now be supported by the laboratory isolation of *H. ducreyi* from the lesions in a large number of cases. Their hope that better culture methods would not only be valuable for epidemiological and patient management purposes but would also lead to further studies of the organisms was soon realized. Hammond's group in Manitoba reported their findings on the antimicrobial susceptibility of strains of *H. ducreyi* in which 19 isolates were tested *in vitro*

against 13 antimicrobials (Hammond *et al.* 1978*b*). This study showed that the strains which were resistant to penicillin and ampicillin were β-lactamase producers.

The following year (1979) Brunton, Maclean & Albritton gave an account of further study of the three β-lactamase-producing strains which showed the presence of a $6{\cdot}0 \times 10^6$ dalton plasmid. This plasmid could not be transferred to *H. influenzae*. Transfer experiments demonstrated that a strain of *H. ducreyi* was able to receive and donate an ampicillin resistance plasmid from *H. influenzae*. This suggests that multiple antibiotic resistant variants might arise as a plasmid-mediated effect. Further work by the same group (McLean, Bowden & Albritton, 1980) showed that the β-lactamase produced by their three strains was similar to the TEM-1 type originally from the ampicillin transposon T A^2. The source of the plasmid is yet to be established.

Further observations on the plasmids of *H. ducreyi* are given by Deneer *et al.* (1982) reporting that a clinical isolate of the organism they studied had three plasmids, a 23·5 megadalton cryptic plasmid, a cryptic 7·0 megadalton ampicillin resistance plasmid and a 4·9 megadalton sulphonamide resistance one. The resistance plasmids could be transfered to *H. influenzae* providing the receptor strain possessed the 23·5 megadalton plasmid which appeared to mediate transfer.

An interesting account of the change in the incidence of chancroid in Rotterdam after 1977 is given by Nayyar, Stolz & Michel (1979). In a retrospective study they report that prior to 1977 there were fewer than five cases per year. The incidence increased fivefold during 1977–8. In this period the clinic saw 673 men and 23 women with genital ulcers. Chancroid was diagnosed on the basis of a positive smear or a positive culture (on 25 % fresh rabbit blood agar) in 50 men and 3 women. Had smears alone been used as a basis for diagnosis eight cases would have been missed (18 %). Symptoms were generally mild and treatment with co-trimoxazole was highly successful in achieving clinical cure. There is no comment on coincident herpes genitalis infections.

Sottnek *et al.* (1980) studied 17 strains of *H. ducreyi* isolated in the CDC Atlanta and one reference strain originally derived from the Pasteur Institute. The effectiveness of five isolation media was compared, and human and five animal sera considered for their value as supplements. Of these only foetal bovine serum was consistently seen to improve growth. Of the five media used for primary isolation three containing vancomycin (3 μg/ml) were found to be better. All the Atlanta strains were α-haemolytic on rabbit blood and were oxidase-positive and also weakly urease-positive. All except the reference strain were β-lactamase producers. The authors confirmed the view expressed by other workers that a moist atmosphere enhances the growth of *H. ducreyi*.

In 1981 Hafiz, Kinghorn and McEntegart in Sheffield reported the isolation of *H. ducreyi* from 22 patients seen in the Sheffield Special Clinic with genital ulcers. This unexpected finding stimulated further observations, including attempted isolation from asymptomatic contacts.

In 1981 Handsfield and his colleagues in Seattle gave an account of seven cases of clinical chancroid in the United States, four being imported and three others acquired from one of the imported cases. All these strains were β-lactamase producers, and the plasmids identified were indicative of the epidemiological source

of the infections. All strains were grown on a modified medium prepared by adding 5% of heated sheep blood to gonococcal base agar (BBL) and adding 1% of Isovitalex (BBL) and 3 μg/ml of vanocomycin. Later, 10% of fetal calf serum was added, as this enhanced growth. Incubation was at 35 °C in candle extinction jars without further enhanced humidity. Typical colonies were seen within 4 days. A careful bacteriological assessment of these strains showed that they were typical gram-negative bacilli with a requirement for haemin but not for V factor. All were catalase-negative but weakly oxidase-positive. The spread of the infection was clear from the clinical histories. There were no asymptomatic infections identified.

The authors conclude that in view of the conflicting accounts of the role of the asymptomatic carrier in *H. ducreyi* infections, a re-evaluation should now be undertaken using the more successful media available for diagnosis.

Our own observations suggest a pool of infection amongst patients in Sheffield, the great majority of whom were not infected abroad (Hafiz, Kinghorn & McEntegart, 1981).

MATERIALS AND METHODS

Media. The development of the original culture medium has been described (Hafiz, Kinghorn & McEntegart, 1981). However, we later beame convinced that the addition of 0·4% wet weight of gelatin enhanced the growth of *H. ducreyi* so that it was usually possible to identify typical colonies after only 48 h incubation rather than 4 days (Kinghorn, Hafiz & McEntegart, 1982*a*). The final composition of the modified medium was as follows.

Part I consists of proteose peptone no. 3 (Difco), 30 g; corn starch, 1 g; potassium phosphate (dibasic), 4 g; potassium phosphate (monobasic), 1 g; sodium chloride, 5 g; gelatin (Oxoid), 4 g; Bacto agar, 10 g. The constituents are soaked in distilled water (1 l) for 30 min, mixed and the pH adjusted to 7·2–7·4. The medium is sterilized by autoclaving at 115 °C (10 lb/sq. in) for 15 min and cooled to 56 °C in a water bath.

Part II, the stable defined supplement (Kellogg *et al.* 1963), consists of gelatin, 1 g; glutamine, 1 g; glucose, 40 g; ferric nitrate, $Fe(NO_3)_3 . 9H_2O$, 0·05 g. The gelatin and glutamine are dissolved in 90 ml distilled water at 100 °C, the glucose added to the gelatin solution and the mixture cooled to approximately 10 °C. The ferric nitrate is dissolved in 10 ml distilled water without heating and the solution is added to the other constituents and mixed by vigorous stirring. The medium is distributed in 10 ml amounts and sterilized by autoclaving at 115 °C (10 lb/sq. in) for 15 min and stored at room temperature in the dark. The supplement is used when required, 10 ml being added to each 1000 ml of the medium. (N.B. Isovitalex (BBL) can be used in place of defined supplement.)

Part III is made by dissolving 250 mg of Type III Equine haemin crystalline (Sigma) in 1 ml of 0·2 M potassium hydroxide in 47·5% ethanol and adding 4 ml distilled water. This component is sterilized by filtration.

The basic medium consists of 1 l of Part I, 100 ml Part II and 5 ml Part III. The options for the final medium are (*a*) to add 3 μg/ml of vancomycin for the isolation of *H ducreyi* from clinical specimens especially from multiple lesions of long standing, (*b*) to add 5–7% horse blood to the medium in order to assess the haemolytic effect of isolates or (*c*) to add both vancomycin and horse blood.

Source of clinical material and cultivation

Exudate from genital ulcers was taken on cotton swabs and inoculated directly onto two plates of *H. ducreyi* media, one without blood and one with 5% horse blood. The cultures were then returned to the laboratory within 2 h and incubated in a CO_2 incubator (5% CO_2) at a temperature of 33 °C for up to a week before being discarded. Typical colonies could usually be recognized after 48–72 h incubation. The addition of 5% horse blood appears to result in a slightly softer colony and also allowed us to assess the haemolysis the organism produced.

On this media we have not only improved our isolation rate from genital ulcers (from 11% to 28%) but we have also isolated typical *H. ducreyi* from asymptomatic patients presenting for other reasons or as consorts of established cases.

All of the sixteen strains so far tested have on intradermal inoculation of rabbit skin shown the typical response of pathogenic *H. ducreyi*.

Preparation of H. ducreyi *colonies for examination under the scanning electron microscope*

An agar block about 1 cm in diameter containing colonies was cut and suspended in 3% gluteraldehyde for 2–3 days. It was then rinsed in distilled water and dehydrated through ascending grades of acetone ranging from 30–100% (10 min in each grade) and subsequently transferred for drying to a Polarou 'E3000' critical point drying (C.P.D.) apparatus. The block was flushed with liquid CO_2 to remove traces of acetone, and left soaking in CO_2 for 1 h in the C.P.D. chamber at 35 °C. At this temperature all the liquid CO_2 changes through its critical point to the gaseous state. The CO_2 gas was then vented off very slowly and the agar block mounted on a stub with Dag 915 (electrical conductivity silver point) and coated with gold in an Edwards S-150 sputter in an atmosphere of argon using two 2 min periods at 45 mA. The specimens were viewed in a Phillips S.E.M. 500 at 15 kV using viewing angles of 30° and 45°.

RESULTS

Isolation of H. ducreyi *from genital ulcers*

In our most recent study we have isolated *H. ducreyi* from 46 of 161 patients with genital ulceration (80 males and 81 females) (Kinghorn, Hafiz & McEntegart, 1982*b*). These strains all gave rise to typical entire, brownish, lenticulate colonies with the characteristic coherence which made it easy to push them about but difficult to film them or to prepare any smooth suspension for pathogenicity testing. The general features of our strains corresponded with those studied in Seattle and Manitoba. By the kind co-operation of the directors of these laboratories we have been able to exchange strains and they have confirmed that the organisms we have isolated are indistinguishable from those isolated by them or sent to them for identification. Within the limits of preparing suitable standardized suspensions, to ensure comparable doses, we have established the pathogenicity of our strains for rabbit skin. With the addition of strains isolated previously, the total number now studied in Sheffield is 72, of which only three were β-lactamase producers, two being imported and the third a contact of one of those cases.

Our attempts to grow the organisms in liquid medium have so far failed, our most successful attempt resulting in 'stalactites' of growth adhering to the upper part of the broth tube. We have been unable to produce a uniform turbidity in broth. It will remain very difficult to study dose effects in tests for pathogenicity until we are able to quantitate inocula.

Colonial form

We have referred to the recurring remarks in the literature on the characteristic colony of *H. ducreyi*. In an attempt to explain this we studied colonies under the scanning electron microscope and the results are shown in Plate 1. We have included for comparison a gonococcal colony of about the same age. The gonococci in the colony look somewhat like heaps of dried peas (Plate 1*a*, *b*). By contrast the *H. ducreyi* colony at a similar magnification shows a most remarkable smooth surface (Plate 1*c*). Higher magnification (Plate 1 *d*, *e*) confirms this impression and leaves one feeling that there is some interstitial matrix bonding the organisms together and giving rise to the coherent colony. So far none of our attempts to produce a smooth suspension by breaking up colonies with enzymes such as trypsin, papain or cellulase have been successful. Suspension in 20% H_2SO_4 or 20% KOH did not even disperse or indeed make any impression on the colonies.

A chance observation on cultures of *H. ducreyi* growing on a clear, starch-containing medium led to a useful though not specific test for identification. After 48–72 h we noticed that colonies were surrounded by an opalescent halo, which when treated with diluted Grams iodine (1 in 3) stained a typical blue-black. It appears possible that a change in the particle charges around the colonies may result in aggregation of colloidal starch which then stains blue-black with iodine.

DISCUSSION

In little more than 2 years our concept of *H. ducreyi* has changed from that of a rare organism which was so difficult to isolate as to be beyond the skill of all but the most specialized laboratories, to that of an organism which, with care, can be grown and identified in any good routine laboratory.

We have had reports from colleagues in various parts of the country of their success in isolating the organism. We have also had reports of the loss on passage of stock strains that we have sent for testing media. It appears that the organism survives for long periods of passage on optimum media. However, although it will also grow on quite simple media, such as chocolate blood agar, it dies out within two or three passages on this medium. It may be that the organism can carry over with it sufficient quantities of essential nutrients to ensure growth for one or two passages, but that if these are not replaced the strain will die out. There may, of course, be some simpler cause such as a failure to provide an adequate humidity for growth. Now that we have a small range of reliable media and a better understanding of the organism it should be possible to re-examine the precise nutritional needs of the organism and try to establish its essential requirements.

We have indicated some of the peculiarities in the growth of *H. ducreyi*. The absence of dispersed growth in liquid medium is a major handicap in any experiments requiring accurate quantitation as, for example, comparing the

pathogenicity of strains from different sources. It may be that investigations directed towards explaining the extraordinary colony form might help to indicate ways in which dispersed growth in liquid medium could be obtained.

Finally, despite our interest in the fascinating bacteriology of the organisms, we must not lose sight of the remaining clinical and epidemiological problems. Are we looking at geographically varied local pools of infection? Has the organism persisted in and around Sheffield following introduction at some earlier date? This seems possible, especially as our colleagues in Seattle assure us it does not exist in their population apart from rare and clearly marked importations. It may well be that early and energetic treatment prevented the persistence of *H. ducreyi* following the outbreak in Greenland in 1977. In this explosive outbreak symptomless carriage was not considered to be important.

At present there are no useful epidemiological markers such as phage or bacteriocine to help in tracing the spread of the organism in a community like Sheffield.

Although there is no laboratory evidence it is still possible to explain these apparent contradictions by suggesting that *H. ducreyi* varies in virulence and that the Greenland strain produced clinical disease in all persons infected, thus ensuring treatment and prompt eradication. Other strains are less pathogenic and so give rise to asymptomatic infections which may only be diagnosed when they subsequently infect already damaged tissue as secondary invaders. Once established in damaged tissue the organisms contribute to the persistence of lesions until specific treatment is undertaken.

What then is the way ahead. First, we need much more information about the incidence of the organism not only in clinic patients, consorts, etc. but also in other groups such as patients from family planning clinics who are not only asymptomatic but not, so far as anyone can judge, high risk patients. Secondly, we must try to persuade bacteriology laboratories servicing genito-urinary medicine clinics in the U.K. that the organism is worth looking for. If we can do this we should soon see how far our experience in Sheffield can be repeated elsewhere. We also need some epidemiological markers to help us to follow the spread of the disease. Happily chancroid and *H. ducreyi* infections are amenable to treatment and unless this situation changes it should be possible to act effectively through the special clinics once the true size of the problem due to this long-neglected organism has been defined.

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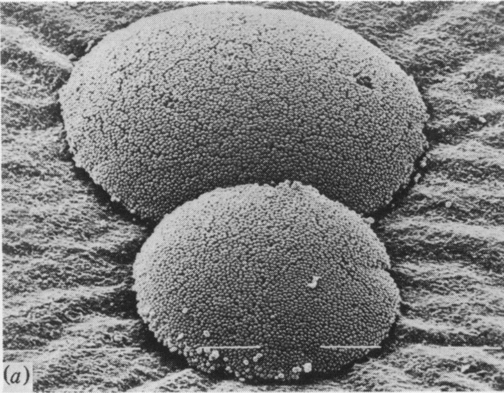
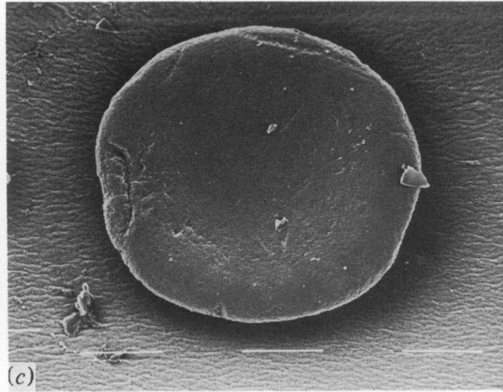
EXPLANATION OF PLATE

A comparison of colonies of *Neisseria gonorrhoea* and *H. ducreyi* by scanning electron microscopy. Plates 1*a* and *b* are *N. gonorrhoea* ×2500 and ×10000, respectively. Plates 1*c–e* are *H. ducreyi* ×320, ×5000 and ×10000, respectively.


(*a*)

(*b*)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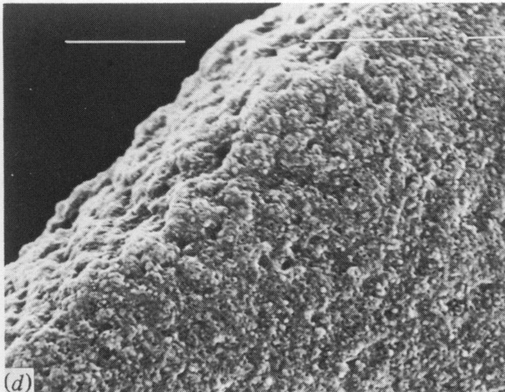
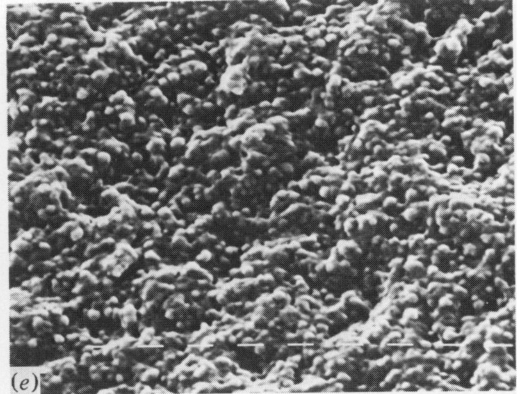

(*d*)


(*e*)