isolated from 21 cases, but culture showed 10 strains to be anaerobic streptococci, 4 to be *Strep. pyogenes*, 2 *Strep. viridans*, and 2 non-haemolytic streptococci. Three strains were seen only in films. Thus, although "streptococci" were common, several species were represented. Fusiform bacilli were found in 10 cases; in 7 of these they were accompanied by streptococci of one species or another, and in one case by both streptococci and staphylococci.

The commonest organism, therefore, was *Staph. aureus*. Fusiform bacilli and anaerobic streptococci came next in order of frequency. *Strep. pneumoniae*, *P. vulgaris*, and *Strep. pyogenes* were also found in several cases.

Discussion

Although staphylococci were present in 15 of the 48 cases in this series and in 14 of 27 cases reported by Alpers (1939), other authors (Hasslauer, 1907; Lund, 1926; Atkinson, 1934) found them in only a few of their cases of brain abscess. It is difficult to account for this discrepancy. The predominance of staphylococci in the present series was not due to the inclusion of cases in infants, in which staphylococci are said to be very common (Sandford, 1928). Several authors have stated that streptococci are the commonest organisms, but the results of culture in this series suggest that several species of streptococci may have been represented. It is unjustifiable to group these species together and misleading to state that streptococci are the commonest organisms in brain abscess. Fusiform bacilli and anaerobic streptococci were often found, and on several occasions pneumococci grew only anaerobically in primary cultures. It is clear that anaerobic culture is an essential part of the examination of pus from brain abscesses.

It appears from Table I that more than one species of organism was present in a large proportion of the abscesses from which anaerobic organisms were isolated. Wámoscher and von Vásárhelyi (1933) showed that the injection of agar furnished a nidus in which tetanus spores could germinate, and it is possible that the use of agar in the inoculum was responsible for the production of abscesses in rabbits' brains with anaerobic streptococci and fusiform bacilli by Falconer *et al.* (1943). It seems therefore quite probable that brain abscesses due to anaerobic organisms originate in foci of lowered O-R potential (Fildes, 1927) produced by a vascular accident or by aerobic organisms which later die out.

In this series clinical evidence of encapsulation was obtained in 5 cases two months after the onset of symptoms, and in 8 cases with three months' history. Histological examination showed well-marked fibrous walls in 2 (pneumococcal) cases with six weeks' history, in 3 cases with two months' history, and in 6 cases with three months' history. However, the clinical observations concerned the superficial part of the abscess wall, where encapsulation is more rapid than in the parts nearer the ventricle (Northfield, 1942; Falconer *et al.*, 1943). Moreover, a deeper unencapsulated loculus was present in several abscesses which had apparently been present for six months or longer. It was therefore impossible to draw any general conclusion about the time relations of capsule formation or to compare the speed of encapsulation of abscesses due to different organisms.

Of the two cases without a capsule, one in which cerebral symptoms had been present for a week was due to anaerobic streptococci, and the other, in which symptoms had been present for a fortnight, was due to *Strep. pyogenes*. It is clear that no deductions as to delay in capsule formation should be drawn from these two presumably recent abscesses. The same argument applies to the three abscesses in which the absence of a capsule was noted at operation. One of these with a fortnight's history was due to *P. vulgaris* and anaerobic streptococci, one with a three-weeks history to fusiform bacilli and anaerobic streptococci, and one with a four-weeks history to *Staph. aureus*.

There are repeated statements in the literature (Neumann, 1909; Alpers, 1939; Grant, 1941) that anaerobic and Gram-negative bacilli inhibit capsule formation. Histological examination demonstrated well-formed collagenous capsules in two cases in this series from which fusiform bacilli were isolated (in one case together with anaerobic streptococci and in the other with streptococci seen only in films). One of these cases has already been cited by Falconer *et al.* (1943), who found that encapsulation occurred in brain abscesses caused by the intracerebral inoculation of fusiform bacilli or anaerobic

streptococci in rabbits. There is therefore both clinical and experimental evidence that encapsulation does occur in the presence of fusiform bacilli or anaerobic streptococci. Similarly the authors just cited showed that encapsulation occurred in experimental abscesses due to *P. vulgaris*, and well-formed capsules were found in two abscesses in this series in which *P. vulgaris* was present. These observations agree with Atkinson's (1934) statement that *P. vulgaris* is apparently not highly virulent in brain abscesses, but do not support the statement, probably derived from Lund's (1926) record of twelve fatal cases due to coliform bacilli, that Gram-negative bacilli inhibit capsule formation. Thorotrast, which favours encapsulation (Falconer *et al.*, 1943), had not been introduced into the abscesses considered here.

Summary

Specimens of pus from 48 brain abscesses were examined. Details of the cases, of the bacteriological technique, and of the organisms isolated are set out. Of the cases 33 yielded a single organism and 14 more than one species of organism. One specimen was sterile.

Staph. aureus was present in 15 cases, fusiform bacilli and anaerobic streptococci each in 10 cases. *Strep. pneumoniae*, *P. vulgaris*, and *Strep. pyogenes* each appeared in several cases. "Streptococci" were found in 21 cases, but several species were represented, and the statement that streptococci are the commonest organisms in brain abscess is misleading.

Anaerobic culture is a necessary part of the examination of pus from brain abscesses both for the isolation of obligate anaerobes and for the isolation of some strains of pneumococci.

Data concerning the encapsulation of the abscesses were not sufficient to permit any general conclusions about the rate at which the capsule is formed or the effect on that rate of the species of the infecting organism. Two abscesses from which fusiform bacilli were isolated were shown histologically to be well encapsulated, as were two abscesses from which *P. vulgaris* was isolated. These cases and some experimental studies (Falconer *et al.*, 1943) are at variance with the statement that anaerobic and Gram-negative bacilli inhibit capsule formation.

I wish to thank Dr. Dorothy S. Russell for allowing me to use her histological findings in the discussion of capsule formation, and the neurosurgeons, particularly Brig. Cairns, without whose help the study could not have been undertaken.

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ON THE HANDLING OF SMALL BIOPSY MATERIAL

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The increasing frequency of diagnostic biopsy, especially of the epithelium of the uterus and the respiratory tract, has prompted this description of the technical procedures used in this laboratory. First, however, it is important to state that biopsy material removed with a blunt scalpel or curette, or subjected to squeezing, tearing, crushing, or deep coagulation during removal will, no matter how good the technique of the pathologist, show nuclear changes that are characteristic of intravital trauma; it should be more widely known that these changes may make the difficulties of histological diagnosis insurmountable.

Many of the methods to be described are of long usage, and are due to predecessors or colleagues; a few are personal innovations, but all have formed an established and satisfactory routine for several years. The importance of scrupulous technique in the handling of such delicate material can scarcely be over-

emphasized, and the description and recommendations are therefore given in a somewhat dogmatic tone.

Fixation

The fixative used exclusively is formal-corrosive. This is prepared by adding 10 parts of commercial formalin (not neutralized) to 90 parts of saturated aqueous solution (5.6%) of mercuric chloride; to this is added enough saturated aqueous magenta acid (acid fuchsin) to give a pale pink colour (depth of 1 in 20,000 KMnO_4). This mixture keeps satisfactorily for months. The addition of the dye has the advantage not only of distinguishing the solution in the laboratory but of imparting to the small fixed fragments a very visible and so helpful red colour. The fixative is supplied in 4-oz. wide-mouthed bottles with bakelite screw caps, about three-quarters full. Metal caps must not be used. The tissues are dropped direct into the fixative, although with endometrial curettings it is of proved value to follow the method employed by Dr. Adam Barr, gynaecologist to this hospital. The curettings are dropped on to an adjacent sterile towel, and the theatre sister, with clean forceps, immediately transfers the fragments of tissue into the fixative. Fixation should be for eight or more hours; in practice this means until the following morning. The tissue is then transferred direct to 95% ethanol (methylated spirit) in a 4-oz. wide-mouthed glass-stoppered specimen jar, where it lies till evening.

Dehydration is completed by transfer to "absolute spirit" (industrial methylated spirit 74 degrees overproof) or absolute ethanol, in which the tissue lies until the morning of the next day (second day after biopsy). **Clearing** is done by benzol (commercial 90%), into which the tissue is now put; two hours is usually enough. Thanks to the fuchsin the cleared tissue is still easily visible—an important point. **Paraffin impregnation** follows, and at 54° C. is adequate in three to six hours (depending on the size and consistency of the tissue). The tissues are thus ready for cutting by the evening of the second day after operation; in practice they are usually cut and stained the following morning.

Notes and Comments

The use of a fixative containing mercuric chloride holds the fragments together by the rapid coagulative effect, and gives a much better fixation of nuclei than fixing for the same period in ordinary formal-saline. The absence of a chrome salt (as in Zenker's solution) removes the need for washing out the fixative, while the presence of formalin in the mixture produces a better fixative for diagnostic purposes than plain aqueous corrosive sublimate (Lendrum, 1941a). It is of course necessary to remove the corrosive by treating the sections with iodine during their hydration before staining.

The fixative thus used is generally quite suitable for filtering and subsequent re-use in fixation of the more gross surgical tissues; the bottles are scrupulously cleaned before refilling with fresh fixative.

In handling small bits of tissue it is of great help to use, as Dr. Janet Niven suggested, dental plugging forceps rather than the usual tissue forceps; they certainly add a noticeable delicacy to the touch.

Denser and tougher tissues (such as biopsies of skin) can be subjected to the same routine if the pieces are small. If, however, a piece is thought to be too large one can cut it with a sharp knife after eight or more hours' fixation, knowing that the face thus cut, thanks to the coagulative power of the corrosive, is not likely to distort. In practice the larger fragment of tissue is left uncut until the next morning; it is then bisected (e.g., through a suspected ulcer), returned to the fixative till midday, then transferred to 95% ethanol, and in the evening to the "absolute spirit" as usual.

If an unsuspected toughness or hardness in a tissue is discovered only when the block is being cut on the microtome, the following procedure is used. The mixture called "mollifex" (B.D.H.; see Baker, 1941) has the power of softening tissue in a paraffin block. In practice if such a troublesome tissue is encountered the paraffin block, still on its wooden chuck (see Carson, 1941), with the tissue already exposed by the attempted cutting, is immersed in mollifex for three or four hours (or till the next day), and it is then found that a satisfactory section can usually be cut. Glycerin has a similar but slower action. If unsuspected calcification is revealed at the stage of cutting, the block and chuck are immersed

in a modified Ebner's decalcifying solution (6% mercuric chloride in 15% sodium chloride, with 1% of hydrochloric acid), and by the following day it is usually possible to obtain a satisfactory section. Slightly longer staining with haematoxylin is necessary because of the acidity of the tissue.

Lymph nodes present a particularly difficult problem, and, since diagnosis of the reticulos is so dependent on perfect histology, they should be treated by a slower method of dehydration (Lendrum, 1941b).

Summary

Satisfactory diagnosis of biopsy material demands good technique on the part of surgeon or gynaecologist and of the pathologist.

The routine as used at the Western Infirmary Laboratory is described in detail, and recommended as being capable of giving consistently good results.

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Medical Memoranda

Congenital Atresia of the Oesophagus

In recording a case of congenital atresia of the oesophagus in the *Journal* (Nov. 12, 1938, p. 983) J. W. D. Bull says: "The following case is recorded not on account of its rarity but because the condition has been so seldom mentioned in the English literature." For the same reason it may be worth while to record another case, in which the fistulous tract opened at the extreme end of the trachea between the bronchi, thus differing from Dr. Bull's case, in which the opening was a little way up on the posterior wall.

CLINICAL HISTORY OF CASE

The mother attended ante-natal clinics and was confined at this hospital. She was a primigravida aged 33. As in Dr. Bull's case, hydramnios had been noted. Apart from premature rupture of the membranes labour was normal. A female child was born on March 17, 1943, at about 35 weeks' gestation. She weighed 4 lb., measured 17½ in., and was apparently healthy. The following night the child vomited thick mucoid material. Cyanosis and rapid shallow respirations were noted. Oxygen, brandy, and glucose were given, but it was found that the child seemed better when left alone. Next day the vomiting continued and fluid was regurgitated through the nose. Cyanosis occurred after each feed and disappeared when the vomiting ceased. Inspection of the palate showed no abnormality, and tracheo-oesophageal fistula was tentatively diagnosed. Feedings were stopped and rectal and subcutaneous glucose-saline substituted. Later the same day frothy white material was ejected from nose and mouth, during which cyanosis persisted. Meconium was passed per rectum. The following day the condition was similar, and the child died early next morning—i.e., after three days.

Post-mortem Examination.—Since the essential points are shown in the photograph, it will suffice to say that no other abnormality was discovered and to describe the procedure. After removal of the thoracic and abdominal viscera in one piece, a metal catheter was passed into the oesophagus from above and was arrested after about 1½ in. The oesophagus was opened on to the catheter and found to end blindly. After opening the stomach the catheter was passed up the oesophagus from below. It passed quite readily and emerged from the larynx. Cutting down on to the catheter showed that the lower part of the oesophagus entered the trachea at its bifurcation. There was no evidence of bronchopneumonia, which is apparently the usual cause of death in such cases.

Photograph of specimen