able conditions for the inquiry. Subsequently it was found that the laboratory at No. 4 Canadian General Hospital was not prepared to undertake the investigation, this hospital having only recently been transferred from Salonika, and the laboratory was not as yet in full working order.

From the office of the D.M.S. directions were forwarded to each participating laboratory detailing the scope and plan of the investigation. Those directions were full and precise. Their main features may here be referred to in abstract. The attempt was made to reduce to a minimum the procedure for differentiating between the different members of the diphtheria group, and to provide methods which had been tested and proved reliable. (1) Sterilized swabs, labelled, were to be provided for use in the wards. (2) Fresh smears were to be examined by either Loeffler's or Neisser's method, and attention was called to the fact that the majority of wound diphtheroids are indistinguishable from the Klebs-Loeffler bacillus under the microscope. (3) Blood serum plates were recommended in place of slanted test-tubes for the first cultures. (4) Pure cultures of the diphtheroids recognized were to be obtained on blood serum slants (*B. diphtheriæ* colonies remain white, wound diphtheroids often, as they become older, acquire a pinkish or yellowish tinge). (5) For the sugar tests of these pure cultures Hiss's serum water medium was recommended as base, plus 1 per cent. of the sugar, together with azolitmin or litmus as indicator.* Four sugars only were to be used: dextrose, lactose, saccharose, and dextrine. These four saccharides were chosen in the first place to ensure the recognition of *B. diphtheriæ*. Dextrose (glucose) is a typical monosaccharide, lactose a useful disaccharide. Neither saccharose (sucrose) nor dextrine are for general bacteriological purposes regarded as of first importance, i.e., with other bacterial species. The latter, it is true, is a representative polysaccharide, yet care is requisite in the preparation of dextrine broth, lest it be overheated and undergo hydrolysis with production of dextrose and levulose (this was noted in the directions given). But with a striking constancy the Klebs-Loeffler bacillus ferments dextrine with production of acid, and fails to ferment saccharose. Saccharose and lactose are also useful in differentiating between the other members of the group. As regards the other saccharides, levulose (as between the main members of the group) reacts in the same way as does dextrose, and might therefore be excluded. The same is largely true of maltose, and where it is not, the confusion that has been introduced by some members of what in other respects is the same sub-group fermenting this sugar, and others failing to ferment it, renders its employment inadvisable. Mannite could be neglected, since it is not fermented by any members of the group. Other glucosides are both difficult to procure in a state of purity, and when procured do not afford constant results; their use, that is to say, is corroborative rather than of fundamental importance. We admit that glycerine might have been added to the series. Mellon has found this representative of the alcohols of distinct service in differentiating between the diphtheroids. Our object, however, was not so much to make a full differentiation between the many members of the group, as with the greatest economy of material and labour to recognize the main types, and above all to distinguish clearly between *B. diphtheriæ* and the other members. All forms which produced acid with dextrose and dextrine, and were negative with saccharose, were to be regarded provisionally as the Klebs-Loeffler bacillus; all others as diphtheroids. The following formulæ were afforded:—

| | Dextrose | Lactose | Saccharose | Dextrine |
|---|---|---|---|---|
| *B. diphtheriæ* ... ... | + | + | − | + |
| *B. hofmanni* ... ... | − | − | − | − |
| *B. xerosis* .. ... ... | + | − | + | − |
| Wound diphtheroids ... | + | + | + | ± |

(6) *Toxin Formation.*—The determining test as between the diphtheria bacillus and diphtheroids is toxin formation. The investigation was planned to discover in the first place whether virulent micro-organisms are to be encountered in open wounds, and only in the second place how frequently other members of the group gain entrance to wounds. What determines whether a given organism is the true Klebs-Loeffler bacillus, and therefore of serious import, is not its morphology or staining reactions, or cultural characteristics, but its capacity to produce ectotoxins, and thus to affect the health of the patient. Detailed directions were therefore given as to the preparation of a broth, according to Dean's method, found to be most favourable for the rapid production of toxins. Having made twenty-four hours' growths in Erlenmeyer flasks of the bacilli to be tested, a guinea-pig was to be inoculated subcutaneously with 1·0 c.c. of the same. If toxic effects were produced, then four guinea-pigs were to be taken; two of them were to be given each 300 units of anti-diphtheria serum, and now one normal and one immunized guinea-pig were to be inoculated with 0·5 c.c. of the twenty-four hours' broth culture, and the other pair with 1·0 c.c. If the growth was one of true virulent diphtheria bacilli, the larger dose should cause death of the untreated guinea-pig within twenty-four hours.

A separate report was asked for each case affording members of the group, both true Klebs-Loeffler bacilli and diphtheroids.

It is generally agreed by those engaged in this investigation that no dependence can be placed upon smears from wounds as discovering the presence or absence of bacilli of the diphtheria group. This fact, in itself, is evidence of the absence of widespread diphtherial wound infection. Where there is widespread infection with membrane formation it should not be difficult to encounter the characteristic bacilli in relative abundance. Presence or absence of diphtheroids was determined by smears from the first, mixed, cultures on blood serum plates. This has introduced a difficulty in affording exact statistics, e.g., at Folkestone bacilli resembling *B. diphtheriæ* were detected forty-one times, and that from forty cases, but from only twenty-four out of these forty cases were pure cultures isolated and studied,* one wound affording two separate diphtheroid organisms. Thus our first table giving the number of cases affording bacilli belonging to this group does not tally with the second table giving the relative frequency of the different members of the group isolated.

### RESULTS.

The results obtained by this combined investigation may here be succinctly tabulated:—

| Laboratory | Observer | Number of cases examined | Number affording true *B. diphtheriæ* | Number affording diphtheroids |
|---|---|---|---|---|
| No. 1 Canadian General Laboratory, Folkestone | Captain F. Adams ... ... ... .. | 121 | 1 | 40 |
| No. 16 Canadian General Hospital, Orpington ... | Captain C. Imrie ... ... ... ... | 60 | 1 | 7 |
| No. 15 Canadian General Hospital, Taplow ... | (1) Captains A. G. Fleming and R. M. Janes | 100 | 0 | 0 |
| | (2) Captain C. D. Farquharson ... ... | 25 | 0 | 9 |
| | | 306 | 2 | 56 |

The following additional data deserve note:—

(1) *Folkestone.*

Two cases afforded growths which culturally, tinctorially, and by the fermentation tests had the properties of the Klebs-Loeffler bacillus. One of these (No. 69, a sloughing stump of the thigh) afforded two separate strains of diphtheria-like bacilli. Of these one gave the sugar reactions of a wound diphtheroid, the other of the Klebs-Loeffler

* Mellon objects that litmus is not a sufficiently delicate indicator, and recommends Andrade's medium, or, failing this, neutral red. Azolitmin was recommended after a careful test. Major Bowman and Captain Adams found that constant results were obtained by eight days' and even by four days' growth in the Hiss's medium.

* That any of the seventeen remaining cases were those of true diphtherial infection is improbable from the fact that the method of isolation employed was that which has proved itself the optimum for isolation of *B. diphtheriæ*.