recently tested in the laboratories of the Royal Army Medical College. It is considered that these values more nearly represent the present fuel value of war-time food-stuffs than earlier figures obtained from the calorification of pre-war foods.

A FURTHER REPORT UPON DIPHTHEROID INFECTION OF WOUNDS.

With a Note upon the Frequency of Diphtheroid Bacilli in Cases of Urethritis and Prostatitis.

By R. M. JANES, M.B.Tor., Captain, C.A.M.C.,

N. O. THOMAS, B.A., M.B.Tor., Captain, C.A.M.C. Pathologists to Granville Canadian Special Hospital, Buxton, Derbyshire.

ABOUT four months ago a routine investigation of wounds infected with diphtheroid organisms was begun here, with a view to corroborating, or otherwise, the combined report of Canadian pathologists recently published, in June, 1918, in the Bulletin of the Canadian Army Medical Corps, and then undergoing publication. This report was compiled as a result of an article which appeared in the Journal of the American Medical Association for September of 1917 by Majors J. G. Fitzgerald and D. E. Robertson, of Toronto, Canada. In view of the fact that practically all of the cases sent to this hospital showing open wounds had some bone injury, and had, in many cases, undergone prolonged treatment in primary hospitals before being sent here, it seemed that we were dealing with a type of cases not available for investigation in most military hospitals. It is also of interest that these are the cases which are being returned to Canada while their wounds are continuing to discharge, and that they are, therefore, much the same type of case as those reported on by the Toronto investigators, and perhaps differed somewhat from the cases examined by the investigators of the combined report.

The following technique was employed : A sterile cotton swab was rubbed well over the surface of the wound and sent to the laboratory by the medical officer in charge of the case. This swab was planted on a tube of Loeffler's blood serum, and the inoculated tube incubated for a period of eighteen hours. Smears were then made from the growth obtained and stained with methylene blue, as well as by Gram's and Neisser's methods. This was carefully searched under the microscope for Gram-positive bacilli showing diphtheroid arrangement. If diphtheroid organisms were found, a loopful of growth from the original culture at a place where the majority of the colonies were of the character of those produced by diphtheroids was emulsified by thorough shaking in a tube containing about 4 c.c. of bouillon. A loopful of this broth was then smeared on a plate of neutral nutrient agar, which was incubated for twenty-four hours, when suspicious colonies were fished. If no likely colonies appeared the process was repeated, and this time a loopful was also smeared on a plate of Loeffler's blood serum, as B. diphtheriæ are occasionally hard to grow on agar. The agar was used as routine because, being a transparent medium, it is easier to fish the colonies, and, moreover, the diphtheroids grow quite well on it. Any suspicious colony was inoculated on a tube of Loeffler's blood serum and the tube incubated. This growth was next day examined for purity, and, if pure, the sugar tubes inoculated from it. Sugar reactions were always carried out on Hiss's serum water, to which 1 per cent. of the desired sugar was added. Before the sugar reactions were finally read each tube was planted again on agar, in order to ensure that latent contamination had not occurred. Sugars were incubated for four days before final readings were taken. It was found by experience that this time was quite sufficient.

CHARACTERS OF GROWTHS.

On plain agar diphtheroids produce in twenty-four hours small pin-head colonies, translucent and lenticular on nakedeye examination, under the low power of the microscope appearing finely granular, with a slightly irregular margin. On Loeffler's blood serum colonies have the same general

characteristics, but grow somewhat larger. Colonies always tend to remain discrete.

Pure cultures of diphtheroids on serum slants are more profuse than the growths ever obtained with true Klebs-Loeffler. Cultures kept at room temperature develop the creamy appearance noted by Captain Adams in the combined report. The growth is moister than that of Klebs-Loeffler, and in some cases, where heavy, becoming almost slimy. Cultures of *B. diphtheriæ* kept under similar conditions do not lose their characteristic appearance. Similar to Klebs-Loeffler cultures, those of diphtheroids can be kept for at least four months, and at the end of that time good growths are obtained on subculture.

We have been unable to detect any consistent difference between the morphology and staining of diphtheroids and true Klebs-Loeffler bacilli. All strains of the former encountered by us stain well by Neisser's method, although usually the granules are larger. Generally speaking, diphtheroids are shorter than *B. diphtheriæ*. Grown on different media, they show the pleomorphism so characteristic of Klebs-Loeffler.

All the diphtheroids which we have isolated from wounds have produced acid from dextrose, lactose, saccharose, maltose, lævulose, galactose, and have failed to ferment dextrin and mannite. Acid production in lactose is somewhat slower than in the other positive sugars. They may all be considered as falling under the B. hoagi type (Morse, 1912). One diphtheroid isolated from an acute otitis media complicating a case of influenza during the recent epidemic showed the sugar reactions of true B. diphtheriæ. On animal inoculation it proved avirulent, and belongs, therefore, to the class of B. quasi-diphthericus (combined investigation). Sugar reactions have been controlled on each batch of sugar media prepared by cultures of Klebs-Loeffler isolated from the throats of clinical cases of diphtheria. These have always fermented all the above sugars with the exception of saccharose and mannite.

For animal inoculation a twenty-four hour neutral broth culture has been employed. Guinea-pig No. 1 was inoculated in the subcutaneous tissue of the abdomen with 2 c.c. of the culture. Guinea-pig No. 2 was similarly inoculated with 2 c.c. of culture, which, however, had remained in contact with 1,500 units of diphtheria antitoxin at room temperature for a period of one hour. In the case of wound diphtheroids of a few pigs, No. 1 showed slight ordema after twenty-four hours, amounting to a tumour as large as a bean. But no general reaction was observed in any case. At the end of three days this local reaction had always subsided. In the case of true B. diphtheriæ, death of pig No. 1 occurred in from twenty-four to seventy-two hours. Pig No. 2 showed no local or general reaction. Autopsy findings were in all cases typical. Virulent Klebs-Loeffler was in one case isolated from the pleural fluid of a pig which had received B. diphtheriæ isolated from a wound. (It may be noted that the pleural effusion consisted of clear, straw-coloured fluid, not bloody fluid, as reported by some observers.)

In all we have examined swabs from one hundred and twenty-nine cases, eighty-two of which, or $63^{\circ}5$ per cent., showed diphtheroid organisms. Of these thirty were isolated in pure culture at intervals during a period of four months. Three of these proved to be true Klebs-Loeffler, and twentyseven wound diphtheroids—that is, 10 per cent. of the isolated organisms were *B. diphtheriæ*, and assuming that this is a fair proportion, 6^o4 per cent. of the wounds were infected with *B. diphtheriæ*.

We give below brief clinical notes on the three cases infected with B. diphtheriæ, and on three infected with wound diphtheroids.

Case I.-No. 1005736 Pte. T. Wounded, Passchendaele, November 11, 1917. Shrapnel wound, outer surface of left arm. Wound excised and dressed at C.C.S. the same day. Although the original wound was small, it continued to discharge until March, 1918, when it healed, leaving the arm apparently well in every way. About the first week in May, 1918, wounded area became swollen, red, painful, and tender. Swelling incised, much pus freed, and a piece of shrapnel came away in the dressing. The wound continued to discharge, and considerable sloughing took place, leaving a dirty ulcer. A swab taken May 20, 1918, shows the Klebs-Loeffler bacillus. The following note was made on his Medical