investigations to date are now recorded. A more detailed account will be given elsewhere.

Of the twenty strains of *B. subtilis* examined, the strain *A.T.C.C.* 6633 displayed greatest antibacterial activity, the metabolism solutions being adjusted to *p*H 2·5 and autoclaved at 114° C. for ten minutes. The active concentrate subtilin had been isolated from surface cultures of this strain by Jansen and Hirschmann[1]. We used quick-freeze dried cells grown in submerged culture with aerating agitation on a medium of corn-steep liquor and sucrose. It was found, after several trials with different solvents, dilute acids, and inorganic salts, that the active principle was most advantageously separated when the cells were disintegrated in alcoholic suspension with glass beads or autoclaved with 80 per cent aqueous alcohol. By the use of the latter procedure, followed by dilution of the alcoholic extract with adjustment of the *p*H to 2·3, an active concentrate, which we will term subtilin *C*, was obtained as an amorphous powder.

Subtilin *C* in high concentration (1/2,000) was inactive against the Gram-negative bacteria tested, but Gram-positive organisms were generally susceptible to high dilutions; acid-fast bacteria showed a range of sensitivity. The substance had no lytic effect on a variety of organisms. It diffused only with great difficulty.

Antibacterial activity was determined by serial dilution of solutions or fine suspensions of subtilin *C* which had been adjusted to *p*H 2·5 and autoclaved at 114° C. for 10 minutes.

	Highest dilution of subtilin *C* giving 50% inhibition of growth after 24 hr. at 37 C. in glucose broth
Sarcina lutea	1/10,000,000
Staphylococcus aureus (6 strains)	1/80,000–1/8,000,000
Corynebacterium xerosis	1/5,000,000
Lactobacillus helveticus	1/2,400,000
Micrococcus conglomeratus	1/600,000
Mycobacterium from butter	1/300,000
Mycobacterium phlei	1/60,000

Complete inhibition of growth was given by two to four times the amounts of subtilin *C* required for 50 per cent inhibition.

Subtilin *C* is stable under certain conditions. A preparation stored for five months at room temperature in a desiccator in the dark showed no loss of activity. In solution in glucose broth, *p*-aminobenzoic acid (200 μgm./ml.), nicotinic acid, pantothenic acid and riboflavin (5 μgm./ml.), aneurin and pyridoxin (2 μgm./ml.), biotin (0·1 μgm./ml.), folic acid (0·01 μgm./ml.), 2 per cent casein hydrolysate, and asparagine and tryptophane (0·1 per cent), exerted no antagonistic effect in the concentrations given. The same was true of solutions of sodium chloride (2 per cent), glucose (2 per cent) and cysteine (0·04 per cent).

Under some conditions, the antibacterial properties of subtilin *C* were impaired. Exposure to sunlight for two days reduced the activity to 10 per cent of darkened controls at various *p*H values. With serum the results were not clearly defined. Using *Staph. aureus* as test organism, no significant loss in activity was observed over short periods (24 hr.), but contact for three days reduced the activity to 25 per cent of the original. Seitz filtration of a solution of the antibiotic resulted in loss of activity. Studies on the stability of solutions at various *p*H values showed that subtilin *C* was most stable at *p*H 2·5. It is rapidly destroyed in alkaline solution.

The properties of subtilin *C* would support the view that the active principle is a polypeptide. Hydrolysis by trypsin and pepsin resulted in loss of activity. It gave a strong ninhydrin reaction for α-amino-acids and positive tests with Ehrlich's reagent for indole derivatives and the Folin–Denis reagent for phenols. No coloration developed on treatment of an alcoholic solution with ferric chloride; this differs from the results obtained by the American workers.

I am indebted to Dr. F. Bergel and Prof. A. R. Todd for helpful suggestions and a continued interest in this work; also the assistance of Dr. Klein, Dr. Moss and Miss Ward in the microbiological work is gratefully acknowledged.

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Nov. 4.

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Serological Position of *Streptococcus bovis*

Streptococcus bovis, a conspicuous streptococcus of cow dung and also occurring in human fæces, has proved difficult to classify. Biochemically it is a well-defined species (Orla Jensen), and although it has some of the characteristics of Lancefield's Group *D* streptococci, its serological identity has long been obscure because of its failure to yield a group serum. While many strains of *Str. bovis* reacted with Group *D* sera, a not inconsiderable proportion gave negative or equivocal results[1,2,3], and until group sera for *Str. bovis* could be prepared its serological placing was necessarily left in abeyance.

Using an improved method of extraction, it has now been shown that virtually all strains are precipitated with a Group *D* serum. Moreover, by further improvement in the method of preparation of suspensions for inoculation, it has been possible to produce group specific sera from *Str. bovis* itself. Reciprocal absorptions with these sera and with sera prepared from other authentic Group *D* strains confirm that *Str. bovis* now takes its place with the enterococci as a member of Group *D*.

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Nov. 12.

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Intravenous Methylene Blue: a New Method for Studying the Nervous System

In 1886, Ehrlich[1] obtained striking results by the use of methylene blue as a stain for the nervous system. Of the many methods since developed for utilizing the staining properties of methylene blue perhaps the simplest and most reliable is the local injection technique of Weddell *et al.*[2]. The two main disadvantages of this method are the inevitable distortion of the tissue by the direct injection of the

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