LETTERS TO THE EDITORS

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IN THE PRESENT CIRCUMSTANCES, PROOFS OF "LETTERS" WILL NOT BE SUBMITTED TO CORRESPONDENTS OUTSIDE GREAT BRITAIN.

An Enzyme from Bacteria able to Destroy Penicillin

FLEMING[1] noted that the growth of *B. coli* and a number of other bacteria belonging to the coli-typhoid group was not inhibited by penicillin. This observation has been confirmed. Further work has been done to find the cause of the resistance of these organisms to the action of penicillin.

An extract of *B. coli* was made by crushing a suspension of the organisms in the bacterial crushing mill of Booth and Green[2]. This extract was found to contain a substance destroying the growth-inhibiting property of penicillin. The destruction took place on incubating the penicillin preparation with the bacterial extract at 37°, or at room temperature for a longer time. The following is a typical experiment showing the penicillin-destroying effect of *B. coli* extracts. A solution of 1 mgm. penicillin in 0·8 c.c. of water was incubated with 0·2 c.c. of centrifuged and dialysed bacterial extract at 37° for 3 hours, in the presence of ether, and a control solution of penicillin of equal concentration was incubated without enzyme for the same time. (The penicillin used was extracted from cultures of *Penicillium notatum* by a method to be described in detail later. It possessed a degree of purity similar to that of the samples used in the chemotherapeutic experiments recorded in a preliminary report[3].) The growth-inhibiting activity of the solutions was then tested quantitatively on agar plates against *Staphylococcus aureus*. The penicillin solution incubated with the enzyme had entirely lost its growth-inhibiting activity, whereas the control solution had retained its full strength.

The conclusion that the active substance is an enzyme is drawn from the fact that it is destroyed by heating at 90° for 5 minutes and by incubation with papaïn activated with potassium cyanide at *p*H 6, and that it is non-dialysable through 'Cellophane' membranes. It can be precipitated by 2 volumes of alcohol, but much of its activity is lost during this operation. The activity of the enzyme, which we term penicillinase, is slight at *p*H 5, but increases considerably towards the alkaline range of *p*H. It is very active at *p*H 8 and 9. Higher *p*H's could not be tested as penicillin is unstable above *p*H 9.

The mechanism of the enzymatic inactivation of penicillin is being studied. No oxygen uptake occurs during the reaction, and the inactivation proceeds with equal facility under aerobic and anaerobic conditions. No appearance of acid groups could be detected by *p*H measurement with the hydrogen electrode. Extracts of a number of other micro-organisms, made by crushing the bacteria in the bacterial grinding mill, were tested for penicillinase. The enzyme was absent from extracts of the penicillin-sensitive *Staphylococcus aureus*, of yeast and of *Penicillium notatum*. It was present in a Gram-negative rod, insensitive to penicillin, found as a contaminant of some Penicillium cultures. Unlike *B. coli*, it was not necessary to crush the organism in the bacterial mill in order to obtain the enzyme from it; the latter appeared in the culture fluid. The enzyme was also found in *M. lysodeikticus*, an organism sensitive to the action of penicillin, though less so than *Staphylococcus aureus*. Thus, the presence or absence of the enzyme in a bacterium may not be the sole factor determining its insensitivity or sensitivity to penicillin.

The tissue extracts and tissue autolysates that have been tested were found to be without action on the growth-inhibiting power of penicillin. Prof. A. D. Gardner has found staphylococcal pus to be devoid of inhibiting action, but has demonstrated a slight inhibition by the pus from a case of *B. coli* cystitis. The bacteriostatic action of the sulphonamide drugs is known to be inhibited in the presence of tissue constituents and pus.[4] That the anti-bacterial activity of penicillin is not affected under these conditions gives this substance a definite advantage over the sulphonamide drugs from the chemotherapeutic point of view. The fact that a number of bacteria contain an enzyme acting on penicillin points to the possibility that this substance may have a function in their metabolism.

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[1] Fleming, A., *Brit. J. Exp. Path.*, **10**, 226 (1929).
[2] Booth, V. H., and Green, D. E., *Biochem. J.*, **32**, 855 (1938).
[3] Chain, E., Florey, H. W., Gardner, A. D., Heatley, N. G., Jennings, M. A., Orr-Ewing, J., and Sanders, A. G., *Lancet*, 226 (1940).
[4] MacLeod, C., *J. Exp. Med.*, **72**, 217 (1940).

Morphological Effects of Penicillin on Bacteria

WHILE working with Chain, Florey and others on the inhibition of bacterial growth by penicillin[1], I noticed that concentrations of less than full inhibiting power caused a change in the appearance of the growth of *Cl. welchii* in fluid media. The normal uniform turbidity was replaced by a flocculent growth with a heavy deposit. Microscopical examination showed an extreme elongation of the majority of the cells, which took the form of unsegmented filaments ten or more times longer than the average normal cell.

I have now examined a number of bacteria grown in broth or serum broth with penicillin, and I have found similar microscopical changes in all the rod-shaped organisms that have shown any inhibition. These changes may be traceable, in the form of a distinct average lengthening of the cells, to a dilution eight or ten times, and even sometimes thirty times, higher than that which completely inhibits growth.

The Gram-negative rods, which are relatively resistant to penicillin, show the effect very well. Thus