LETTERS TO THE EDITORS

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Production of Penicillin

PENICILLIN, the metabolic product of *Penicillium notatum*, discovered by Fleming in 1929, has been shown to be a chemotherapeutic agent of great importance. The exceedingly high bacteriostatic potency of this substance, together with its low toxicity to tissue cells, are such that penicillin has been described as "the most powerful antibacterial agent with predominantly bacteriostatic action so far known"[1], and its value in human therapy has already been demonstrated[2].

It is, therefore, unfortunate that the production of crude penicillin is a somewhat lengthy process in which the yield of this valuable but unstable product is small[1,2]. Further, the process involves the culturing of the mould in large volumes of fluid medium in culture vessels in which the depth of medium is small. The incubation space therefore required is so great that the facilities of even the larger laboratories are inadequate for the production of penicillin in quantity sufficient for therapeutic use, or for the further work required in the elucidation of its chemical structure. By the modified method of production outlined below, the bacteriostatic metabolic product of *P. notatum* is produced in 5–6 days in yield comparable to that obtained in 10–11 days by the method as previously described[1,2]. This means that for a given incubation space the *output* of the crude active substance may be considerably increased (for example, doubled) and, in addition, the modified method has other advantages.

Attempts were made to increase the yield of penicillin by adding to the medium (as used by Florey *et al.*[2]) substances which might serve as growth factors. In this connexion it was thought that the culture fluid itself might contain such substances. Therefore, small quantities (0·1–10 c.c.) of fluid from an 11-day culture of a previous batch were added to culture vessels containing 200 c.c. of medium, after which the bottles were seeded with *P. notatum* and then incubated at 24° C. along with cultures to which no culture fluid was added. These experiments were strikingly successful and the course of the metabolism was profoundly altered as judged by the following criteria: growth and sporulation of the mould, pigmentation of the culture fluid, *p*H changes and appearance of coloured droplets on the surface of the mycelium were all considerably accelerated as compared with untreated cultures (controls), and the rate of production of the antibacterial substance after 4–5 days' incubation, as judged by the ring assay test described by Florey *et al.*[2], was increased considerably. However, these results were not maintained during further incubation, the assay tests then indicating a considerable diminution in the amount of antibacterial substance present in the culture fluid. Observations of the changes in the *p*H of the culture fluid suggested that the antibacterial substance was being destroyed by the excessive amount of acids produced under the changed experimental conditions.

Accordingly, similar experiments were carried out in a medium adequately buffered by the addition of a strong solution of mixed phosphate buffers, the proportion of the mono- and dihydrogen phosphates being determined after consideration of the *p*H ranges most favourable for the stability and production of penicillin. The results of these experiments were entirely satisfactory, and the amount of antibacterial substance produced in 5–6 days (so far as could be judged by ring assay tests on the *diluted* culture fluid, and by the serial dilution method as used by Fleming[3]) was at least equal to that produced in the control experiments in 10–11 days, that is, in cultures seeded from the same suspension of the mould and incubated along with the test cultures. Assay tests on the culture fluid after several days' more incubation (in addition to the 5 days) showed no diminution in the amount of active substance present, and work is in progress in order to ascertain if the yield of the active substance *per given volume of culture fluid* may be increased, that is, as compared with the yields obtained in 5–6 days by the modified method or in 10–11 days by the older method.

Pigment production during the shorter incubation period of the modified method was less than in the 10–11 day period of the unmodified method, and this is an advantage of importance, since the isolation and purification of the active substance will therefore be facilitated.

In preliminary experiments, buffering of the medium as in the successful experiments outlined above, but without the addition of the culture fluid, appeared to alter the metabolism of the mould as in the experiments in which both culture fluid *and* buffer were added. Further work is in progress in order to ascertain whether the addition of culture fluid is necessary.

At present only small quantities of the crude antibacterial substance have been prepared by the modified method. The substance, however, was found to be extractable at *p*H 2 by amyl acetate and from the amyl acetate solution the active substance was re-extracted at *p*H 6·4 with aqueous barium hydroxide. There is therefore no reason to doubt that the bacteriostatic metabolic product of *P. notatum* produced was penicillin, although rigid proof cannot be brought forward until the active substance has been isolated in a more highly purified form.

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[1] Abraham and Chain, *Brit. J. Exp. Path.*, **23**, 103 (1942).
[2] Abraham, Chain, Fletcher, Florey, Gardner, Heatley and Jennings, *Lancet*, **241**, 177 (1941).
[3] Fleming, A., *Brit. J. Exp. Path.*, **10**, 226 (1929).

Experimental Production of a Pouch in the Male of *Trichosurus vulpecula*

THE female of *Trichosurus vulpecula*, the common Australian phalanger or possum, has a well-developed pouch, while the male possesses in the corresponding region a large pendulous scrotum. On injecting large doses of œstrogen into males of varying ages there appeared at the sides of the scrotum skin folds which strongly suggested the formation of a rudimentary pouch, particularly in immature animals[1].

To obtain more definite information as regards the true nature of these folds the small scrotal sac of three pouch young of three to four months of age was amputated with or without the testes.

The best result was obtained from a pouch young of three months of age where the testes occupied a