LETTERS TO THE EDITORS

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An Antibiotic from *Aspergillus parasiticus*

RECENTLY we had occasion to examine the possible production of antibacterial materials by about twenty strains of *Aspergillus flavus*, *A. oryzæ*, *A. tamarii* and *A. parasiticus*. The culture fluids showed no significant antibacterial titres when a medium of the Czapek type was used; the test organism was *Staphylococcus aureus* and both the serial dilution method and the plate test[1] were used. When the medium contained bactotryptone or 7–8 per cent of corn-steep liquor together with 4 per cent of glucose and inorganic salts, several of the strains of *A. flavus* and *A. tamarii* produced culture fluids which were weakly antibacterial, being completely inhibitory at dilutions of 1 : 30–80 after 5–14 days incubation at 22–24°. Far superior were the filtrates obtained from four strains of *A. parasiticus*, which gave titres of complete inhibition of 1 : 200–600 after 5–12 days at 24°. A concentrate of the product of one of these four strains was obtained by absorbing the antibacterial principle on charcoal and eluting with aqueous acetone, 50 per cent of the activity being so recovered.

It was later found that in presence of corn-steep liquor additional inorganic salts were unnecessary for the production of antibacterial activity, though copper (1 : 100,000) had a very marked effect on the growth of *A. parasiticus*; with added copper the mould grew luxuriantly with rapid formation of olive-green spores; in its absence, growth was less vigorous and the mycelium remained lemon-yellow for the whole period of growth (up to 14 days). Even added carbohydrate was unnecessary, for the full antibacterial titre developed in 7·5 per cent corn-steep liquor adjusted to *p*H 7 (the sample of liquor contained a small amount of fermentable carbohydrate). Experiments on the length of incubation using varying amounts of glucose in the culture medium threw some light on the probable reason for the apparent superiority of *A. parasiticus* over *A. flavus*. The former grown in 7·5 per cent corn-steep liquor with 0·5 per cent additional glucose produced maximum antibiotic activity in 4–5 days; the antibacterial titre decreased after 7–8 days though appreciable activity was still present after incubation for one month. With two strains of *A. flavus*, however, activity was markedly more transient, and in one case had disappeared on the ninth day of incubation. The glucose content of the medium had a pronounced effect on the *rate* of production of activity by both species. In cultures of *A. parasiticus*, addition of 2 per cent of glucose (compared with 0·5 per cent of glucose) caused a delay of 48 hours in the production of similar activity, the maximum being reached only on the eleventh day. *A. flavus* cultures showed a similar time-lag, and in addition the maximum activity attained was considerably less in 2 per cent or 5 per cent of glucose than in 0·5 per cent of glucose; indeed, with one strain of *A. flavus* appearance of activity was completely suppressed when 5 per cent of glucose was used.

The products from all the strains of *A. parasiticus* grown with or without additional glucose or salts lost all activity on standing at *p*H 2 for 30 min. or at *p*H 11 for 30 min. The active material was extracted by ether, chloroform, or amyl acetate from aqueous solution at *p*H 2–3, and was recovered in aqueous solution by shaking the extract with a suspension of barium carbonate. The antibiotic was approximately as active against *B. fascians* (a Gram-positive plant pathogen) as against *Staph. aureus*, but was inactive against *B. coli*, *B. pyocyaneus*, *B. prodigeosus* and several other Gram-negative bacterial species.

In both chemical and antibacterial properties the new antibiotic resembles penicillin. Antibiotics of similar character have also been obtained from strains of *A. flavus* in surface[2] and submerged[3] culture and from *A. giganteus*[4], so that the production of such materials is evidently more generally possible than has been supposed. The antibacterial titres obtained from at least one strain of *A. parasiticus* are sufficiently high to make its culture of possible practical value. There is insufficient evidence to decide the identity of penicillin or other Aspergillus products with that from *A. parasiticus*, and it is provisionally proposed to designate the new product 'parasiticin'.

We thank Prof. I. M. Heilbron for his encouragement and the Rockefeller Foundation for financial assistance.

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March 15.

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Trypan Blue and Growth of the Adrenal Cortex in Mice

ACCORDING to a recent communication, Calma and Foster[1] have been unable to demonstrate centripetal cell migration in the adrenal gland of the rat by the use of trypan blue. Salmon and Zwemer[2], using the same vital stain, had previously reported inward cell movement. These last-mentioned workers injected the dye subcutaneously and found it taken up first by the cells in the capsule and after varying intervals by cells of the glomerulosa, fasciculata, and reticulosa successively, while the outer layers became dye-free.

More than two years ago, while I was working on the *X*-zone of the mouse, adrenal experiments similar to those of Salmon and Zwemer were started but discontinued with the publication of their report. The appearance of Calma and Foster's letter prompted a re-examination of old slides and a study of more mice. In one experiment five animals received ¼ c.c. of 1 per cent trypan blue for two days, and then left and right adrenals were examined separately after intervals of approximately six days; thus each animal served for two observations. The last adrenal was removed sixty days after the termination of injections.

Inspection of histological preparations of these glands supports the findings of Calma and Foster, namely, that this method yields no evidence of inward cell migration in the adrenal gland. Dye was present in the capsule of every gland, and in greatest amount (to judge by granular size) in the region of the glomerulosa and outer fasciculata.