LETTERS TO THE EDITOR

The Metabolism of 5-Hydroxytryptamine in Cultures of *Claviceps purpurea*

SIR,—The metabolism of 5-hydroxytryptamine (5-HT) has been studied extensively in animal tissues, but knowledge of its fate in micro-organisms and plant tissues is scanty. Jannes, Leppanen and Saris (1962) found an unidentified 5-HT metabolite in cells of *Escherichia coli*. 5-Hydroxy-indole-3-acetic acid has been detected by Tyler and Smith (1960) in several species of fungi. *NN*-dimethyl-5-hydroxytryptamine has been isolated from plant sources such as the seeds of *Piptadenia peregrina* (Stromberg, 1954) and the toxic mushroom *Amanita mappa* (Wieland, Motzel and Merz, 1953).

For this study, claviceps mycelia were grown by the methods of Sim and Youngken (1951) and Taber and Vining (1958) from sclerotia of a Secale strain of *Claviceps purpurea* obtained from the Department of Plant Pathology, University of Minnesota. These cultures failed to produce alkaloids with or without the addition of 5-HT, but significant amounts of the added 5-HT disappeared rapidly during 2-4 hr.

Mycelial pads after 14-67 days growth were separated from substrates, washed twice with 50 ml. of saline, and well drained. 5-HT creatinine sulphate (12 mg.) in 50 ml. saline solution was added to the pads in the culture flasks, which were protected from light and shaken at 105 cycles per min. at 20-25° for 1-4 hr. Aliquots of the medium (3 ml.) were analyzed for 5-HT, (Udenfriend, Weissbach and Clark, 1955), at 1, 2 and 4 hr. intervals. An initial 3 ml. aliquot was analyzed just after adding the 5-HT solution and analyses of subsequent aliquots were calculated as a percentage of this initial value. The results are shown in Table I. A marked loss of 5-HT was noted in the medium of cultures of 21–67 days growth. Aliquots from control flasks without mycelia showed no decrease of 5-HT, nor was it demonstrable in those mycelial cultures to which 5-HT was not added.

TABLE I

5-HT REMAINING IN REPLACEMENT CULTURES OF CLAVICEPS AT SHORT INCUBATION PERIODS

Age of mycelia (days)	pH medium at harvest	pH medium end of incubation	Percent of 5-HT remaining (hr. incubation)		
			1	2	4
14	5·8	5·8	104	99	99
21	7·2	6·7	86	66	30
22	7·4	6·8	85	71	57
30	7·5	6·2	66	54	33
33	7·7	7·0	68	49	22
36	8·5	7·7	76	62	28
41	7·7	6·8	40	26	16
44	7·3	6·1	54	34	20
61	8·0	7·0	59	28	9
67	7·6	7·3	60	21	13

Mycelia of similar age (16, 20, 29, 39 days) were then separated from culture substrates by centrifugation at 17,500 × *g* for 10 min. at 3°. They were frozen overnight and allowed to thaw slowly at room temperature to promote cellular disruption. While cold the tissues were homogenized in glass in 0·1 M phosphate buffer, pH 7·0, and the homogenate quickly transferred to chilled nylon centrifuge tubes and centrifuged for 10 min. (600 × *g*) at 3°. Samples of the supernatant (2 ml.) were incubated with 0·4 ml. of 0·1 M phosphate

buffer, pH 7·0, and 0·6 ml. of 5-HT (final concentration 0·025 M). Control vessels omitting the supernatant were similarly prepared. The mixtures were shaken for 1 hr. at room temperature under air. The pH remained at 7·0–7·1. The reaction mixtures were pooled, concentrated to 1-2 ml. under reduced pressure and analyzed by chromatography by the methods of Matthias (1954), using a descending strip method with butanol: acetic acid: water (4:1:5) as the developing solvent. When dried, the strips were sprayed with Ehrlich's reagent (Jepson, 1955). The mycelial extract gave two intense blue fluorescent spots one of which had the same R_F value (0·46) as the single spot given by the control and by a reference spot of 5-HT creatinine sulphate. The second spot, R_F value 0·33, was not identified. The R_F value did not correspond with those of a number of indole reference compounds. It was concluded that it was not identical with any of the metabolites reported thus far by others for animal or plant tissues. The unidentified spot was present in mycelial growth cultures of all ages and was most prominent in those of 16 and 20 days growth. Sufficient quantities of the unknown compound were not obtained for identification. However, R_F values in different solvent systems, colour reactions and ultra-violet absorption peaks were obtained for it as described for indole compounds by Chadwick and Wilkinson (1960), McIsaac and Page (1959) and Keglevic and others (1959).

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