Pharmacological and Biochemical Study of a Carcinoid Tumor.* (23078)

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The high concentration of serotonin (5-hydroxytryptamine, 5-HT) in malignant carcinoids accounts, at least in part, for the striking symptoms often experienced by patients with this disease(1,2,3). Serotonin is formed by ring-oxidation and subsequent decarboxylation of tryptophane, a pathway that consumes as much as 60% of dietary tryptophane in carcinoid patients compared with 1% in normal individuals(4,5). Another pharmacologically active compound, chromatographically distinct from serotonin, but as yet unidentified, has been obtained from malignant carcinoids(6,7,8). In this work, we have measured both the serotonin content and the activity of 5-hydroxytryptophane decarboxylase in a benign appendiceal carcinoid, and at the same time searched for other pharmacologically active compounds in this tumor.

Methods. The appendix was removed routinely during a pelvic laparotomy on a 48-year-old white housewife. At tip of the appendix, the mucosa and sub-mucosa were replaced by firm, grayish yellow tissue which was identified microscopically as an argentaffin-staining carcinoid tumor. The fibromuscular coat of the appendix was dissected free from the carcinoid. The tan mucosa of a grossly and microscopically normal appendix was dissected from the underlying muscular coat and used as control material. 5-hydroxytryptophane decarboxylase activity in a portion (150 mg) of both appendiceal tissues was estimated by a method reported earlier (9). The remainder of the carcinoid (210 mg) was extracted with 95% acetone and petroleum ether and taken to dryness *in vacuo* (10). Both the extract and residue were kept at −15°C until analyzed. Similarly, blood drawn from the antecubital vein of the patient after removal of the appendix was extracted with acetone and dried. The residue remaining after acetone extraction of the carcinoid was extracted with 0.2 *N* HCl(7) and taken to dryness. Hereafter in the text, this is referred to as the "acid extract." Bioassays for serotonin were carried out on the heart of *Venus mercenaria*,‡ on the rat's uterus, in estrus, and on the ileum of the guinea pig. Each biological indicator was suspended in a 4 ml overflow bath with a constant flow of air. The heart of *Venus mercenaria*(11) was bathed in a nutrient medium previously described(12), to which was added benzoquinonium chloride (6 mg/l) to block the inhibitory effect of esters of choline. Uteri from rats treated with stilbestrol (100 μg/100 g) on the preceding day were placed in de Jalon's solution with atropine sulfate (1 mg/l). Substance P, kindly presented by Dr. T. B. B. Crawford of Edinburgh, was used as a reference substance in tests on the ileum, suspended in Tyrode's solution containing 0.1 mg of atropine sulfate and 1 mg of mepyramine maleate per liter. Ascending paper chromatography in isopropanol and 0.1 *N* HCl (7:3), was carried out on acetone soluble extracts in 8% aqueous NaCl, and in the upper layer of a system containing *n*-butanol, glacial acetic acid, and water (4:1:5). A solution of the acetone insoluble material in 0.1 *N* HCl was chromatographed by using the first mentioned solvent mixture. After drying, the papers were cut into pieces, each corresponding to 1/30th of the whole migration length, eluted with NaCl, and the eluates were tested on Venus hearts. In addition, guinea pig ileum was employed for testing all isopropanol papers, and in the case of the acetone-insoluble part, rat uterus also

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TABLE I. Serotonin-Forming Activity of Carcinoid and "Normal" Tissue.

Tissue	5-Hydroxytryptophane decarboxylase activity* (μg 5-HT formed per g per hr)	Serotonin in fresh tissue (μg per g)
Appendiceal carcinoid	840	952
Normal appendiceal tissue	23	1

* In terms of serotonin formed from 4×10^{-4} *M* 5-hydroxytryptophane under the standard conditions established by Gaddum and Giarman(9).

was used. Not only were the paper strips examined for fluorescent material(13) but also the extracts were examined for their fluorescence characteristics in an Aminco-Bowman Spectrophotofluorometer. The extracts and standard materials were examined routinely in a 0.1 *N* HCl-medium. In some cases the fluorescence spectrum in 0.1 *N* HCl was compared with that in a 3 *N* HCl, because activation of serotonin, at 295 mμ in dilute acid results in a peak fluorescence emission at 330 mμ, while this shifts to 550 mμ in 3 *N* HCl (14).

Results. Table I shows the comparison between natural serotonin content and serotonin-forming capacity of each tissue. Thus, it is clear that the carcinoid contained nearly 1000 times as much serotonin as normal appendiceal tissue, and had about 35 times the capacity of the normal appendix to form serotonin.

After surgery the blood of the patient contained 3.7 μg of serotonin per 100 ml, a value within the wide range of normal values obtained in this laboratory(15). A 24-hour sample of urine, obtained 2 days post-operatively, gave a negative test for carcinoid, as determined by the urinary level of 5-hydroxyindole acetic acid(4).

Lysergic acid diethylamide (LSD-25), which specifically inhibits the stimulatory effect of serotonoin on the uterus of the rat(16) completely blocked the effect of the acetone extract of the carcinoid on this preparation. On both the clam heart and the guinea pig ileum there was only one spot of activity on paper strips developed with the 3 solvent systems and this spot corresponded to the R_f values of a sample of serotonin, chromatographed concomitantly, either separately or mixed with the extract. Bufotenine (dimethylserotonin hydrochloride) migrated faster in all solvent systems and differed qualitatively from serotonin and the extract in its action on the clam heart and showed a persistent effect in contrast with the evanescent effects of serotonin and the extract. Only the pharmacologically active area of the paper on which the extract was chromatographed showed fluorescence. Similarly, the acid extract of the carcinoid showed activity on the heart, uterus, and ileum, and the R_f values of the active spots corresponded to the R_f values of serotonin. The acid extract showed no bufotenine-like activity on the clam heart. LSD-25 prevented response of the uterus to the acid extract.

Spectrophotofluorometric studies indicated that serotonin was present in both the acetone and acid extracts of the carcinoid and of the normal appendix. Other fluorescent materials, probably not indoles, were found in the acid extracts. Table II summarizes these data.

Discussion. It is now a well established fact that carcinoid tumors contain relatively massive concentrations of serotonin(6,7,8). We have obtained data to substantiate this and have shown further, by direct estimation, that the increase in serotonin parallels an augmented capacity of the tissue to decarboxylate 5-hydroxytryptophane. This agrees with the previous finding that carcinoid tissue was

TABLE II. Spectrophotofluorometric Characteristics of Extracts of a Carcinoid Tumor.

	Peak fluorescence (mμ)			
	0.1 *N* HCl			3 *N* HCl
Activation wavelength (mμ)	295	310	325	295
Substance studied				
Serotonin	330			550
Carcinoid				
Acetone extract	330			550
Acid extract*	330	630	670	550
Normal appendix				
Acetone extract	330			550
Acid extract*	330	650		550

* Of acetone insoluble portion.

more active than kidney cortex and liver in decarboxylating 5-hydroxytryptophane (17). That the blood levels of serotonin and the urinary levels of 5-hydroxyindole acetic acid were in the normal range post-operatively in the case described indicates that the tumor removed was the primary one and that metastases retaining the capacity to form serotonin are not likely to have been present.

Of special interest have been the reports by Lembeck that carcinoids contained, in addition to serotonin, a second pharmacologically active substance, which was not identical with substance P(6,7,8). However, study failed to reveal a second substance, and all the pharmacological effects and fluorescence characteristics of our extracts could be attributed to their content of serotonin. It is important to point out in this connection that not all the carcinoids studied by Lembeck were found to contain the second active substance. It would seem worthwhile to continue to seek such a substance in other carcinoids.

Summary. The pharmacological activity and fluorescence characteristics of extracts of an appendiceal carcinoid tumor could be accounted for entirely by its serotonin content. The amount of serotonin in the carcinoid tissue was about 1000 times that of normal tissue, and its enzymatic ability to form serotonin from 5-hydroxytryptophane was about 35 times that of normal human appendiceal tissue.

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