Histamine Production by Transplantable Argyrophilic Gastric Carcinoid of Praomys (Mastomys) natalensis

Abstract. *A transplantable argyrophilic gastric carcinoid of* Praomys (Mastomys) natalensis *contained appreciable amounts of histamine. Dialyzed, microsome-free supernatant of tumor tissue produced definite amounts of histamine in the presence of* L-*histidine and pyridoxal phosphate. These findings may relate to hypersecretion of gastric acid and formation of multiple ulcers in the stomach and duodenum of* Mastomys *bearing carcinoids.*

The spontaneous occurrence of cancers arising in the body of the glandular stomach of *Mastomys* was first reported in 1957 by Oettlé (*1*). These tumors, which are found in approximately 40 percent of old *Mastomys*, have recently been characterized as malignant argyrophilic carcinoids because they contain argyrophil but not argentaffin granules and are transplantable to other *Mastomys (2). Mastomys* is a distinct subgenus of rodent, and is intermediate in size between the mouse and the rat. It was formerly called *Rattus* (*Mastomys*) *natalensis*, but according to the new classification by Davis (*3*), it should be properly named *Praomys* (*Mastomys*) *natalensis*. For brevity, we continue to speak of the animal simply as *Mastomys*. The fact that, in addition to gastric carcinoids, *Mastomys* develops other lesions such as a severe form of degenerative joint disease (*4*), glomerulonephritis similar to the type seen in man (*5*), thymomas, and thymic hyperplasia (*6*) indicates that its pattern of disease is somewhat different from that of other laboratory rodents.

A few large primary gastric carcinoids, especially those that have metastasized, thus increasing the bulk of tumor tissue, may be associated with ulcers of the stomach and duodenum. Animals in which the transplanted tumors grow after being transplanted into the muscles of the thigh suddenly begin to lose weight when the tumor measures approximately 1.0 cm in diameter; within 5 days the host becomes emaciated and dehydrated, and it will die unless watched carefully and killed. Those that do die and some that are killed are found to have massive hemorrhage into the gastrointestinal tract or perforated ulcers of the duodenum. Ulcers are usually present in the stomach and jejunum as well.

Because of the similarity of this tumor to the human carcinoid, we initially tried to demonstrate 5-hydroxyindoles in the tumor tissues, but we failed to detect 5-hydroxytryptamine (serotonin) and its precursor, 5-hydroxytryptophan, by the method of Davis, Huff, and Brown (*7*) and by a modification of Udenfriend's method (*8*), respectively.

The body of the stomach, which is the site of the primary argyrophilic carcinoids in *Mastomys*, contains a greater concentration of histamine than the antrum (*9*), whereas the antrum is a main source for gastrin extraction (*10*). This, and the finding that some human gastric carcinoids produce fair amounts of histamine (*11*), prompted us to examine the possibility that histamine is produced by the transplanted gastric carcinoid of *Mastomys*. First we determined the endogenous amounts of histamine in the tumor tissue as well as in the various organs of normal *Mastomys*. The argyrophilic carcinoids contained 12.7 ± 2.37 μg of histamine per gram of wet tissue (range, 9.3 to 15.3 μg), an amount much higher than that in any organ examined from normal *Mastomys* (Table 1). The tumor tissue contained a very limited number of mast cells as determined by microscopic observation of formalin-fixed paraffin sections stained with toluidine blue.

Next, two slices from both ends of each tumor nodule from two *Mastomys* were examined for histamine content. The histamine concentrations of the two slices from each tumor were not significantly different: one tumor had

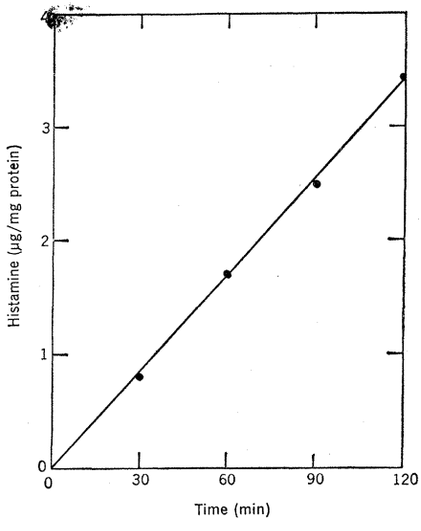

Fig. 1. Relationship of reaction time and histidine decarboxylase activity. Tumor tissue (0.6 g) was added to 5.4 ml of Hanks solution and homogenized in a Teflon homogenizer. The homogenate was centrifuged at 105,000*g* for 60 minutes. The clear portion of the supernatant was dialyzed in a cellophane tube at 4°C for 24 hours against 200 volumes of 0.025*M* phosphate buffer solution (*p*H 7.0) with three changes of external fluid and used as a source of enzyme. The reaction mixture contained 0.3 ml of the supernatant (containing 1.65 mg of protein), 0.1 ml of $5 \times 10^{-2}M$ L-histidine adjusted to *p*H 7.0, 0.1 ml of $2 \times 10^{-3}M$ pyridoxal phosphate, and 0.5 ml of 0.3*M* phosphate buffer solution (*p*H 7.0). At specified times each 1 ml of incubation mixture was added to 4 ml of 0.4*N* perchloric acid and then centrifuged. A 4-ml sample of the supernatant fluid was transferred to a 25-ml glass-stoppered shaking tube containing 0.5 ml of 5*N* NaOH, 1.5 g of solid NaCl, and 10 ml of *n*-butanol. The tube was shaken for 5 minutes to extract the histamine into the butanol, and, after centrifugation, the aqueous phase was removed by aspiration. The organic phase was then shaken for 2 minutes with 5 ml of salt-saturated 0.1*N* NaOH. The tube was then centrifuged, and an 8-ml sample of the butanol was transferred to a 40-ml glass-stoppered shaking tube containing 4 ml of 0.1*N* HCl and 15 ml of *n*-heptane. After being shaken for 2 minutes, the tube was centrifuged. Two milliliters of the aqueous layer was transferred to a test tube, and 0.4 ml of 1*N* NaOH was added followed by 0.1 ml of *o*-phthalaldehyde (1 percent solution in methanol). After 4 minutes, 0.2 ml of 3*N* HCl was added, and the contents of the tubes were thoroughly mixed after each addition. The solution was then transferred to a cuvette, and the fluorescence at 440 nm resulting from excitation at 350 nm was measured in a Hitachi EPE 2 type fluorescence spectrometer.

Table 1. Contents of histamine in argyrophilic carcinoids and in various organs of normal *Mastomys*. Histamine was determined by the method of Shore *et al.* (*14*) and its content was expressed as micrograms of base. *Mastomys* bearing the 11th generation transplant of the carcinoid were killed 2 months after the tumor had been transplanted into thigh muscle.

Tissue	Animals (No.)	Histamine (μg/g of wet tissue)
Argyrophilic carcinoid	5	12.7 ± 2.37*
Glandular stomach	3	6.6
Thigh muscle	3	2.8
Lung	3	2.4
Kidney	3	0.5
Liver	3	0.3

* Mean ± standard error.

13.5 and 11.5 μg of histamine per gram of wet tissue; the other had 10.7 and 9.3 μg of histamine per gram of wet tissue.

In order to determine the histamine production by the tumor tissue, we prepared a dialyzed microsome-free supernatant from the tumor and used it as a source of histidine decarboxylase. When this extract was incubated at 37°C in the presence of L-histidine and pyridoxal phosphate, definite amounts of histamine were formed. The relationship between enzyme activity and time was linear (Fig. 1). We could not measure the histidine decarboxylase activity of normal gastric mucosa of *Mastomys* because the mucosa freed from the muscularis is so small that a microchemical technique for its measurement must be developed.

From these results it is apparent that the transplantable argyrophilic carcinoid elaborates histamine, and this may relate to the hypersecretion of gastric acid. Our data also suggest that this tumor is a kind of atypical carcinoid because it secretes histamine rather than 5-hydroxyindoles. Measurable gastrin (198 and 116 ng per gram of tissue) was found in two transplanted tumors from two *Mastomys* with the most severe gastrointestinal lesions (*12*).

Comparison of the amount of histamine in the urine or blood of normal and tumor-bearing *Mastomys* was impossible because the minute amounts of urine (0 to 0.5 ml/day) and blood obtainable from the host animals were below the sensitivity of the present method for histamine determination.

Very recently histamine-containing epithelial cells, which gave the argyrophil but not the argentaffin reaction, have been found in murine gastric mucosa; 5-hydroxytryptamine as well as dopamine could be demonstrated in the cells only when exogenous monoamine precursors were supplied (*13*). Therefore, it is possible that the gastric carcinoid tumor of *Mastomys* may be derived from argyrophilic histamine-containing epithelial cells in the gastric mucosa.

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Phenylthioacetate: A Useful Substrate for the Histochemical and Colorimetric Detection of Cholinesterase

Abstract. *Cholinesterase from frozen sections of honey bee* (Apis mellifera, *L.*) *brain hydrolyzes phenylthioacetate and acetylthiocholine to give virtually identical patterns of enzyme distribution with and without the use of paraoxon. In addition, phenylthioacetate is an economical substrate for in vitro and in vivo studies of cholinesterase. Phenylthioacetate offers the advantages of being easily synthesized and lipoid soluble, and appears to penetrate membranes easily.*

The use of acetylthiocholine (ATCh) as a substrate for cholinesterase has become the basis for the histochemical localization of these enzymes in tissues by precipitation of copper sulfide (*1*, *2*). It was demonstrated a number of years ago that phenylthioacetate (PT) was an excellent substrate for cholinesterase and could be used in the histochemical method (*3*). More recently ATCh has been widely used in a rapid colorimetric assay for cholinesterase based on the formation of a yellow dye by reaction of the liberated thiocholine with 5,5′-dithio-bis-2-nitrobenzoate (*4*).

We wish to call attention to the advantages of phenylthioacetate over acetylthiocholine as a histochemical, electrophoretic, and colorimetric substrate for detecting cholinesterase activity in honey bee brain. Acetylthiocholine has retained its success largely because of its specificity for cholinesterase in mammals (*2*). While the original method of Koelle and Friedenwald (*1*) has undergone several modifications, its accuracy of localization is still limited by its use of ATCh. In particular the electronic properties of the quaternary ammonium moiety may cause poor penetration and the crystalline nature of the primary reaction product decreases its usefulness (*5*). In addition, ATCh is highly water soluble and therefore would not offer optimum membrane penetration. The thiocholine analogs are very expensive to purchase commercially; they are hygroscopic, and are difficult to synthesize routinely in the laboratory.

Phenylthioacetate can be prepared very easily and inexpensively by refluxing benzene thiol (thiophenol) with acetyl chloride, or by shaking it in ice water with acetic anhydride (*3*, *6*). Phenylthioacetate, a clear liquid (boiling point at 6 mm, 99° to 100°C; $[n]_D^{30}$, 1.5655), is collected by distillation. The compound is of lower water solubility (about 0.007 mole/liter at 37°C) and would not be expected to diffuse away from the site of localization. It also does not have the formally charged quaternary ammonium structure of the choline salts and therefore penetrates readily through nerve sheaths and membrane barriers (*3*).

Honey bee heads were selected and prepared for histochemical methods by a slightly modified procedure previously reported (*7*). Honey bee head sections (18 μm) (*7*) were incubated for 30 minutes at 27°C in distilled water or $1 \times 10^{-6} M$ paraoxon dissolved in acetone and water prior to incubation with PT or ATCh for 1 hour at 37°C. Eighty milligrams of PT were dissolved in 3 ml of acetone or 3 ml of 100 percent ethanol, after which 27 ml of Gomori's solution (*8*) was added to form a milky suspension. Acetylthiocholine methods have been previously described (*7*).

The acrylamide gels for the electrophoresis experiments were prepared according to Davis (*9*), with the exception that sodium barbital buffer was used in place of the tris buffer. The staining procedure was a modification of Wieme's technique (*10*).

Figure 1, A and C, shows the distribution of cholinesterase activity