mammary duct growth than did 2.0 μg of estrogen alone.

1. Silver, M., *J. Endocrinol.*, 1953, v10, 35.
2. Lyons, W. R., Johnson, R. E., Cole, R. D., Li, C. H., In *Hypophyseal Growth Hormone, Nature and Actions,* McGraw-Hill, New York, 1955.
3. Kirkham, W. R., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 139.
4. Smith, T. C., *Endocrinology,* 1955, v57, 33.
5. Macdonald, G. J., Reece, R. P., *J. Dairy Sci.,* 1960, v43, 1658.
6. ———, *ibid.,* 1961, v44, 1192.

Received May 21, 1962. P.S.E.B.M., 1962, v110.

Histidine Decarboxylase Activity in the Walker Carcinosarcoma 256* (27606)

GEORGE A. HALLENBECK AND CHARLES F. CODE
Sections of Surgical Research and Physiology, Mayo Clinic and Mayo Foundation, Rochester, Minnesota

The hypothesis of Kahlson(1) that the increased rate of formation of histamine associated with some rapidly growing tissues may mean that histamine metabolism plays a role in growth, has led to investigation of the histidine decarboxylase activity of certain tumors. A transplantable hepatoma, F-hep, in rats of the August strain has been found to be rich in histidine decarboxylase activity, and as the tumor grows in females the quantity of histamine excreted in the urine increases(2,3,4). When the tumor was removed, the rate of excretion of histamine returned to normal. A mouse mastocytoma has been found to possess active histidine, 5-hydroxytryptophane, and desoxyphenylalanine decarboxylases(5). The present experiments were done to learn whether the Walker carcinosarcoma 256 in the Sprague-Dawley rat has the ability to form histamine *in vitro* and whether its presence causes increased excretion of histamine in the urine.

Methods. Female Sprague-Dawley rats were used. *Determination of excretion of histamine in the urine.* Four rats were kept in metabolism cages designed to permit separate collection of urine and feces. Two milliliters of normal HCl was added daily to each collecting flask to keep the pH of the mixture low enough (1.8 to 3.0) to preserve histamine.

* This investigation was supported in part by a grant from the Louis W. and Maud Hill Family Foundation.

Urine was collected during successive periods of 4 days and was pooled and refrigerated. The female rat, in contrast to the male, excretes free histamine almost exclusively (rather than conjugated histamine) in the urine(6,7). For bioassay, such urine requires only neutralization and dilution with Tyrode's solution and may then be introduced directly to the guinea pig gut. The assay procedure used was a modification(8) of the method of Barsoum and Gaddum. Urinary excretion of histamine was measured in 4 rats before and after the Walker tumor was implanted and during recurrence after an excision.

Management of tumors. Portions of fresh Walker carcinosarcoma 256 were ground with saline solution, using a mortar and pestle; 0.3 ml of the resulting suspension of tumor particles was injected subcutaneously into 6 rats. On the twelfth day after implantation, the animals were anesthetized with ether and the tumors were removed. In the 4 animals in which urinary excretion of histamine was being measured, the operative wounds were closed and excretion of histamine in the urine was measured for 8 more days, during which time the tumors recurred.

Determination of histidine decarboxylase activity of tumors. The method used was essentially that of Waton(9). Preliminary tests indicated production of more histamine at pH 6.5 than at pH 7.2 (Table I); pH 6.5 was used in subsequent experiments. A pre-

TABLE I. Histamine Content and Histidine Decarboxylase Activity in the Walker 256 Transplantable Tumor in the Female White Rat.

Rat	Tissue frozen or fresh	No. of days frozen	Histidine decarboxylase activity, μg histamine/g tumor/hr pH 6.5	pH 7.2	Histamine content, μg/g tumor
A	Fresh	—	.23	.10	.95
B	Frozen	1	.34	.13	.84
1	"	1	.29	—	1.25
2	"	1	.38	—	2.50
3	"	4	.33	—	1.70
4	"	4	.30	—	1.35

liminary test gave no evidence that freezing the tissue altered the results unfavorably, and freezing was subsequently done in all cases for convenience.

Each system contained a homogenate of 400 mg of tumor tissue, 60 μg of pyridoxal, 0.5 mg of aminoguanidine, and enough buffer solution to make 5.0 ml. Two tubes were set up for each specimen of tumor; 7.5 mg of histidine was added to one, and the other served as the control or blank.

The tumor tissue was not dialyzed, and nitrogen was not used during incubation. After 3 hours of incubation, the reaction was stopped by adjusting the pH to 2.5 with 2 or 3 drops of 2 N HCl and boiling for 5 minutes. Each tube was then centrifuged; the supernatant fluid was decanted and frozen. Three to 5 days later, the material was thawed, its pH adjusted to 7 to 8 with 2 or 3 drops of 2 N NaOH, and its histamine content determined by the same method used for the urine. Results were expressed as micrograms of histamine formed per gram of tumor tissue per hour of incubation.

Results. The histidine decarboxylase activity of tumor tissue was measurable, although it was very low, ranging in 6 cases from 0.23 to 0.38 μg of histamine per gram of tumor per hour (Table I). In accord with this finding, urinary excretion of histamine was not greatly or consistently changed while the tumors were growing (Fig. 1). Variability was greater among rats bearing the tumor than among 3 normal rats, but the changes did not take place consistently in either direction when the tumor was growing rapidly or after it was excised.

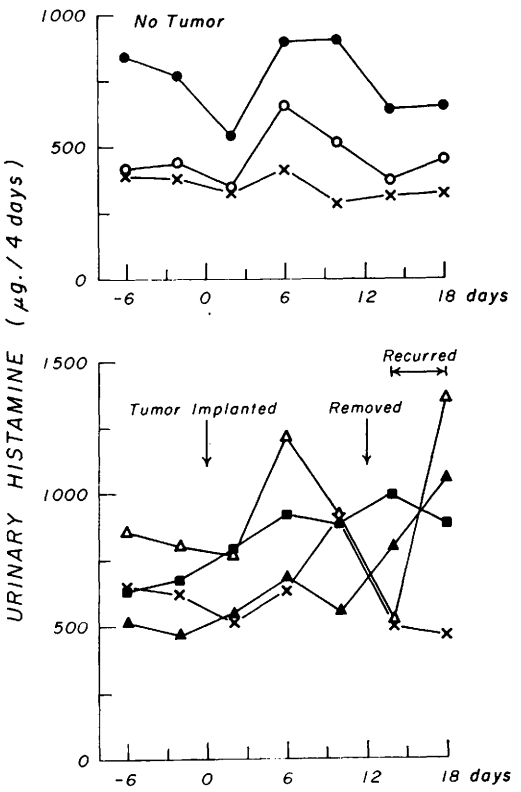

FIG. 1. Urinary excretion of histamine, μg/4 days, by female rats. Upper graph: data from 3 normal rats; lower graph: data from 4 rats given the Walker 256 tumor.

Comment. The lower limit of detectability of histidine decarboxylase activity by the method used in these experiments is about 0.1 μg of histamine formed per gram of tissue per hour. Normal pyloric stomach of female rats yielded a mean value of 18.7 μg per gram per hour (S.E. ± 1.95)(10). Comparable values for rat liver have been 0.5 to 1.5 and for fetal liver, 139.3 (S.E. ± 14.13)(10). It can be seen that the histidine decarboxylase activity in the Walker tumor was barely detectable by the method used; it was much less than that found in this laboratory for some normal tissues and that reported by others for the transplantable hepatoma and the mastocytoma. Hakansson(11), measuring the C^{14}-labeled histamine formed from C^{14}-labeled histidine as described by Shayer, found the histamine-forming capacity of "Walker rat mammary carcinoma 256" taken from 4 female albino rats to be, respectively, .031, .032, .045, and

.048 μg of C^{14}-histamine per gram of tissue per hour; these values are smaller than, but similar to, those being reported here. Hakansson compared these values for histamine-forming capacity in the Walker tumor with the absence of this activity in normal rat mammary tissue and concluded that "The present observations are in line with reports . . . which suggest that rapidly formed intracellular histamine actively and locally promotes certain types of tissue growth."

An alternative interpretation is that the low level of histidine decarboxylase activity in the Walker 256 tumor and the absence of increased urinary excretion of histamine by the female rat while the tumor is growing make it unlikely that histamine metabolism is specifically concerned with the growth of this tumor.

Conclusions. 1. Histidine decarboxylase activity in the Walker carcinosarcoma 256, although measurable, is very low. 2. No consistent change occurred in excretion of histamine in the urine of female rats when the tumors were implanted or growing rapidly, or after their removal and subsequent recurrence.

1. Kahlson, Georg, *Lancet,* 1960, v1, 67.
2. MacKay, D., Marshall, P. B., Riley, J. F., *J. Physiol.,* 1960, v153, 31.
3. MacKay, D., Riley, J. F., Shepherd, D. M., *J. Pharm. and Pharmacol.,* 1961, v13, 257.
4. Kameswaren, Lalitha, West, G. B., *ibid.,* 1961, v13, 191.
5. Hagen, P., Weiner, N., Ono, S., Lee, Fu-Li, *J. Pharmacol. and Exp. Therap.,* 1960, v130, 9.
6. Leitch, J. L., Debley, V. G., Haley, T. J., *Am. J. Physiol.,* 1956, v187, 307.
7. Gustafsson, B., Kahlson, G., Rosengren, E., *Acta physiol. scand.,* 1957, v41, 217.
8. Code, C. F., *J. Physiol.,* 1937, v89, 257.
9. Waton, N. G., *Brit. J. Pharmacol.,* 1956, v11, 119.
10. Code, C. F., Hallenbeck, G. A., *J. Physiol.,* 1961, v159, 66P.
11. Hakansson, R., *Experientia,* 1961, v17, 402.

Received May 28, 1962. P.S.E.B.M., 1962, v110.