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# DIFFERENCES IN TURNOVER OF ENDOGENOUS AND EXOGENOUS 5-HYDROXYTRYPTOPHAN, 5-HYDROXYTRYPTAMINE, AND HISTAMINE IN NEOPLASTIC MAST CELLS IN CULTURE*

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**Abstract**—Neoplastic murine mast cells in culture turned over exogenous 5-hydroxytryptophan (5-HTP), 5-hydroxytryptamine (5-HT), and histamine at a slower rate than did their endogenously produced counterparts. Whereas endogenous 5-HTP could not be detected in these cells, exogenous 5-HTP had a half-life of about 24 hr. In the same cell-line, X-1, endogenous 5-HT had a half-life of 18 hr whereas exogenous 5-HT had a half-life of 60 hr. Endogenous histamine had a half-life of 27 hr, but exogenous histamine, after an initial loss, persisted unchanged in these cells for at least 3 days. Another cell-line, X-2, which contains lower endogenous levels of these amines, turned over the exogenous amines more slowly than did X-1. Two pools for amines are postulated: one, containing endogenous amines and a mechanism for the elimination of the amines, is of only limited accessibility to the exogenous amines, most of which are held in a separate pool.

A MAST cell tumor of the mouse[1] has been grown[2–4] and maintained in culture in this laboratory for nearly 4 years, during which time the cells have continued to synthesize histamine, 5-hydroxytryptamine, and heparin. In the formation of the amines, the cells first concentrate the precursor amino acids which are then catabolized.[5, 6] Preformed amines, including 5-HT and histamine, are also taken up by these cells against a concentration gradient.[5, 7, 8] One cell line—a polyploid, called X–1—excelled in synthesizing the amines, whereas the near-diploid X–2 cell, which was less active in synthesizing the amines, had a greater capacity to take up the preformed amines.[7, 8] Neither cell line significantly catabolized either endogenous or exogenous amines.

In the formation of $^{14}$C-HT from $^{14}$C-tryptophan, it was noted that the intermediate metabolite, $^{14}$C-5-hydroxytryptophan ($^{14}$C-5-HTP) was decarboxylated almost as rapidly as it formed: endogenous $^{14}$C-5-HTP could be demonstrated (presumptively) in only one experiment and then in small amounts;[5, 6] in similar experiments Schindler[9] was unable to show $^{14}$C-5-HTP. This rapid decarboxylation of *endogenous* $^{14}$C-5-HTP contrasted with the comparatively slow decarboxylation of *exogenous* $^{14}$C-5-HTP, which, although rapidly taken up and converted to $^{14}$C-5-HT, persisted in these cells for long periods.[6] Work presented in this paper provides further evidence that endogenous and exogenous $^{14}$C-5-HTP turn over at different rates.

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Evidence is also presented that exogenous 5-HT and histamine turn over at rates different from endogenously formed 5-HT and histamine. A preliminary report of this work has appeared.[5]

## METHODS

The methods and medium for culture of the mast cells have been described.[2–4] In experiments in which radioactive histidine or tryptophan was used, unlabeled histidine or tryptophan was omitted from the medium The isotopic compounds were obtained from the California Corp. for Biochemical Research: DL-indolyl (alanine-3-$^{14}$C), 6·0 mc/mmole; DL-5-hydroxyindolyl (alanine-3-$^{14}$C), 2·4 mc/mmole; 5-hydroxy-3 ($\beta$-aminoethyl-$\beta$-$^{14}$C), 6·36 mc/mmole; L-histidine-2-*ring*-$^{14}$C, 2·62 mc/mmole; and histimine-2-*ring*-$^{14}$C, 1·48 mc/mmole.

To measure turnover of the compounds, cells were grown in 60 ml of culture medium containing 1 $\mu$c of the radioactive material. After 24 hr and every 24 hr thereafter, cells were collected by centrifugation, counted, and washed three times with an ice-cold solution of 0·9% NaCl. The cell population was then divided into two portions. One aliquot was extracted and fractionated, and radioactivity was measured in a liquid scintillation counter. For each point on the curves, extracts of 3 to 10 million cells were counted for 3 to 10 min. The loss of radioactivity with time was corrected for cell multiplication. An analogous procedure has been used to measure the turnover rate of radioactive sulfate and glucosamine in heparin.[3] When X–1 and X–2 cells were to be compared, the experiments were run in parallel. The total concentration of amines did not change significantly during the experimental period.

Cells were extracted by methods that have been described in detail.[6, 8] Free tryptophan, 5-HTP, and 5-HT were extracted with 70% acetone in water (v/v), histidine and histamine with 0·1 N HCl. The acetone-insoluble tryptophan was demonstrated to be in protein.[6] 5-HT was separated from tryptophan and 5-HTP by chromatography on a carboxylic acid resin,[10] and all three compounds were separated by paper chromatography.[6] Histamine was separated from histidine by solvent extraction[11] and identified by paper chromatography.[6]

The medium was fractionated by first precipitating the protein in 70% acetone, taking the extract to dryness, dissolving the residue in a small amount of 70% acetone, and identifying the radioactive material by paper chromatography.[6] Known compounds that were run as markers were dissolved in solutions prepared in the same manner. Radioactivity was detected on paper chromatograms with a Geiger–Müller tube.

## RESULTS

### *Turnover of free $^{14}$C-tryptophan, endogenous $^{14}$C-5-HTP, and endogenous $^{14}$C-5-HT*

In X–1 cells, the half-life of free $^{14}$C-tryptophan was 24 hr and of endogenously formed $^{14}$C-5-HT, 18 hr (Fig. 1). No $^{14}$C-5-HTP could be detected in these cells at any interval, nor could it be demonstrated in the pooled samples. No $^{14}$C-5-HTP could be found in the medium that contained only $^{14}$C-tryptophan and $^{14}$C-5-HT. Attempts to demonstrate labeled tryptamine, indolyl-3-acetic acid, and 5-hydroxyindoleacetic acid in the cells and medium were unsuccessful.

### *Turnover of exogenous $^{14}$C-5-HTP*

In X–1 cells that had been grown in a medium containing $^{14}$C-5-HTP, $^{14}$C-5-HTP was detectable in the cell for 2 days (Fig. 2). This exogenous $^{14}$C-5-HTP had a half-

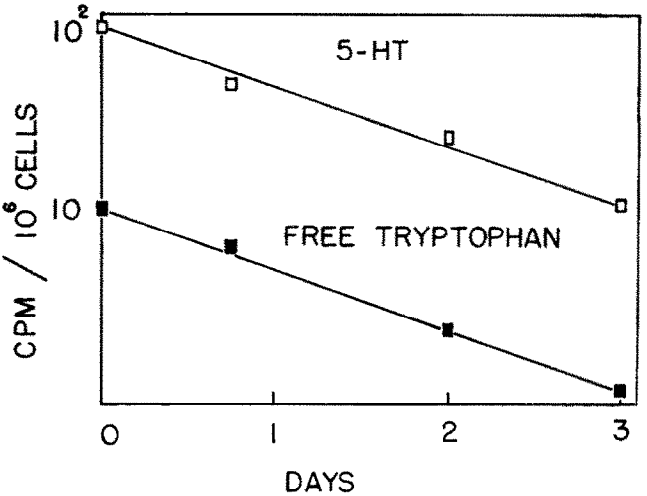


FIG. 1. The turnover of $^{14}$C-tryptophan and endogenous $^{14}$C-5-HT in X–1 cells in culture. No $^{14}$C-5-HTP could be detected.

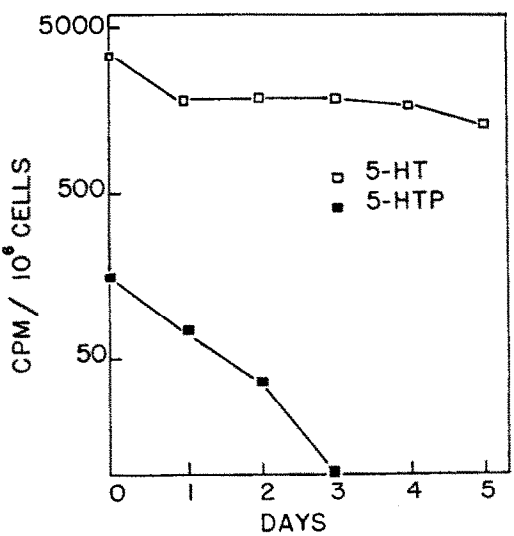


FIG. 2. The turnover of exogenous $^{14}$C-5-HTP and the formation of $^{14}$C-5-HT in X–1 cells in culture.

life of about 24 hr. The $^{14}$C-5-HT that was formed from $^{14}$C-5-HTP declined initially and was then maintained until the third day. After this time the $^{14}$C-5-HT began to decline slowly. The fall in intracellular levels of $^{14}$C-5-HTP was reflected in the appearance in the medium of radioactive material which consisted of $^{14}$C-5-HTP and $^{14}$C-5-HT. In neither the medium nor the cells could 5-HIAA be demonstrated.

*Turnover of exogenous $^{14}$C-5-HT*

Exogenous $^{14}$C-5-HT had a half-life of 60 hr in X–1 cells and about 84 hr in X–2 cells (Fig. 3). The loss of $^{14}$C-5-HT from the cells was accompanied by a gain of this

in the medium. No radioactive compound other than 5-HT was demonstrable in either the cells or the medium.

*Turnover of endogenous $^{14}$C-histamine*

The half-life of endogenous $^{14}$C-histamine in X–1 cells was 27 hr (Fig. 4). With the loss of radioactivity from the cell, the medium gained $^{14}$C-histidine and $^{14}$C-histamine;

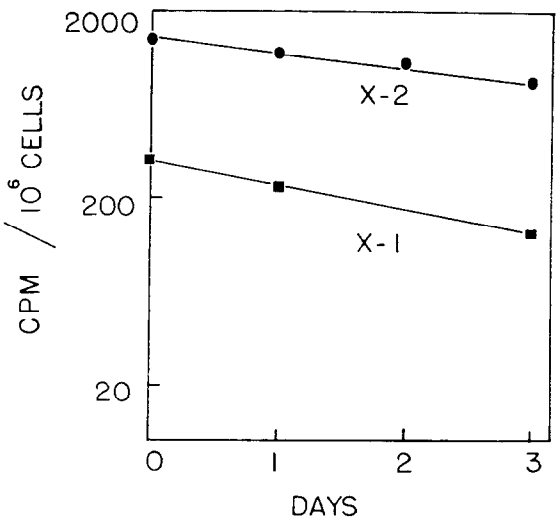


FIG. 3. The turnover of exogenous $^{14}$C-5-HT in X–1 and X–2 cells in culture.

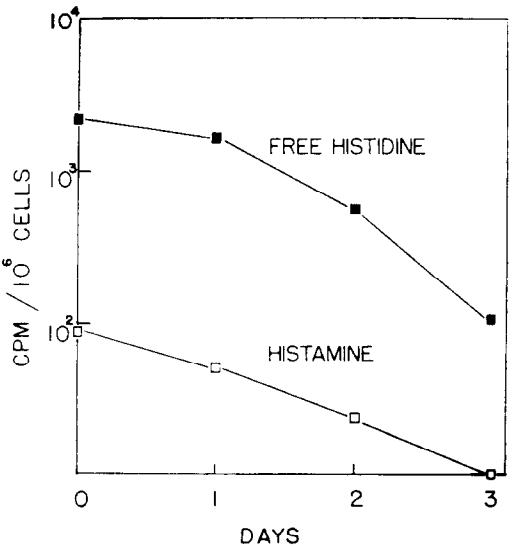


FIG. 4. The turnover of $^{14}$C-histidine and endogenously formed $^{14}$C-histamine in X–1 cells in culture.

no other radioactive substance could be demonstrated in either the medium or the cells.

*Turnover of exogenous $^{14}$C-histamine*

X–1 cells lost $^{14}$C–histamine for the first 24 hr, this portion of histamine turnover having a half-life of 12 hr. After this initial loss, radioactivity was retained by the

cells until at least the third day (Fig. 5). The initial loss of $^{14}$C-histamine was slower in the X–2 cells, showing a half-life of 42 hr. This loss persisted over a 3-day period, after which radioactivity was retained by these cells for at least 2 days.

The loss of radioactive material from the cells coincided with the appearance of radioactive material in the medium. Only $^{14}$C-histamine could be detected in the cells and the medium.

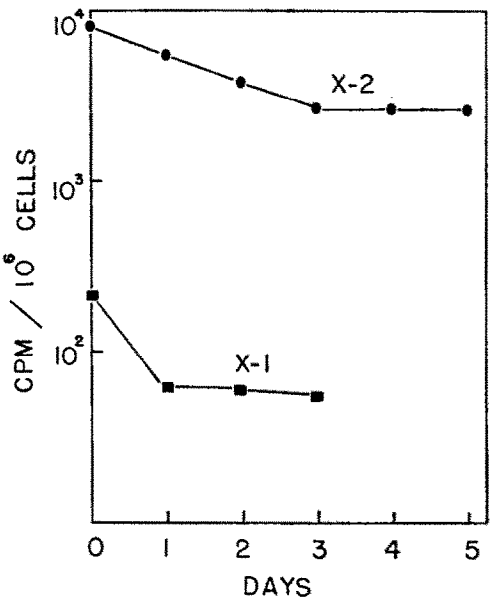


FIG. 5. The turnover of exogenous $^{14}$C-histamine in X-1 and X–2 cells in culture.

## DISCUSSION

Endogenously formed 5-HTP was not demonstrable in these cells; paradoxically, exogenous 5-HTP had a half-life of 24 hr. The absence of endogenous 5-HTP may rest on the fact that the rate of formation of 5-HTP from tryptophan does not exceed the capacity of the cell to decarboxylate 5-HTP. When the rate-limiting step—the hydroxylation of tryptophan—is circumvented by providing the cell with preformed 5-HTP, 5-HTP persists, in part because the cell concentrates 5-HTP more rapidly than it can be decarboxylated. It is likely, however, that reasons more complicated than simple kinetics will have to be invoked to explain fully the persistence of exogenous 5-HTP in these cells; perhaps a mechanism analogous to that postulated below can account for the different rates of turnover of endogenous and exogenous 5-HTP.

Certainly the slower turnover of exogenous, as compared with endogenous, amines cannot be explained by the kinetics of metabolizing enzymes since, as shown earlier[5, 6, 8] and confirmed in the present work, neither histamine nor 5-HT is significantly catabolized by these cells. The amines are instead lost to the medium, the endogenous amines being eliminated from the cell more rapidly than are the exogenous amines. The slow turnover of exogenous amines cannot be explained by postulating that the cell took up more amines than it could eliminate, for the exogenous amines, notably histamine, persisted in the cells for several days. Furthermore, the loss of exogenous

histamine appeared to occur from at least two separate pools, an obvious, although not inevitable, implication of the experiment shown in Fig. 5.

The existence of separate pools may, in fact, explain the apparent ability of the cell to treat these endogenous and exogenous amines in a different manner. The endogenous amines in these (and other) cells are stored mainly in one particulate compartment.[12] It is postulated that this compartment, being nearly saturated with endogenous amines, can accommodate only limited amounts of exogenous amines, the bulk of which is held in another cellular compartment. Those amines, either endogenous or exogenous, that are held in the particulate compartment are continually being displaced by newly synthesized amines. This exchange results in a turnover of amines, perhaps by an eliminative process that is associated with the storage mechanism, namely, the particulate material. In the postulated second compartment where most of the exogenous amines are localized, there is only slight mixing or exchange with the endogenously produced amines, and there is also no efficient mechanism for the elimination of the amines; these circumstances permit the exogenous amines to persist in the cells. Accordingly, the initial loss of exogenous histamine (Fig. 5) occurs from the particulate compartment, while the exogenous histamine in the other compartment is retained.

If it is true that the exogenous amines can mix with the endogenous amines, it would be predicted that cells with a limited ability to synthesize endogenous amines should show a slow turnover of exogenous amines, since the exogenous amine would be only slowly replaced by newly synthesized amines. In fact, X–2 cells, which synthesize 5-HT and histamine much more slowly than do X–1 cells,[3, 6] have a slower turnover of exogenous 5-HT (Fig. 3) and a slower initial turnover of exogenous histamine (Fig. 5). Again, if the exogenous amines do indeed have access to the stores for endogenous amines, a likely consequence would be that cells with a low content of endogenous amines should have a high capacity to take up exogenous amines, since these cells would have more free sites available for the binding of exogenous amines. Such a relationship has been established in experiments in which X–1 and X–2 cells were compared. The latter have lower levels of endogenous amines than have X–1 cells[3, 6] and a far greater capacity to concentrate amines[7, 8] (also Figs. 3 and 5). Direct evidence has recently been obtained that these cells contain two pools for amines and that the extent to which exogenous amines enter the pool containing the endogenous amines is determined by the levels of endogenous amines.[13]

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