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HISTIDINE TRANSPORT BY ISOLATED RAT PERITONEAL MAST CELLS

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Abstract—Kinetic constants for the transport of [^{3}H]histidine into isolated rat peritoneal mast cells were determined. The value of K_m for histidine transport was 44.0 μM; the value of V_{max} under the same conditions was 18.9 pmoles·min^{-1}·(10^6 cells)$^{-1}$. These parameters did not change in value after the addition of exogenous histamine. The uptake of histidine and its decarboxylation to histamine were relatively rapid processes compared to the transfer of the newly formed histamine into mast cell granules, so that nascent histamine appeared transiently in the cytoplasm. Amino-acid competition experiments support the assignment of L system transport for the bulk of histidine uptake by mast cells. Metabolic inhibitors that deplete cellular ATP did not inhibit the uptake process.

The role of the mast cell in the inflammatory process has been addressed extensively [1]. With appropriate stimulation, these cells release granule-bound histamine, as well as other chemical mediators of the inflammatory response, into the extracellular medium. The mechanism of the secretory degranulation process has been studied by a variety of laboratories (for a review, see Ref. 1). The biogenesis and the metabolism of unprovoked mast cells, however, have suffered apparent neglect. In particular, cellular transport of histidine, the amino-acid precursor of histamine, has not been a subject of detailed study. Although several laboratories have measured mast cell uptake of dopamine, norepinephrine, 5-hydroxytryptamine, and histamine [2–4], these experiments employed long periods of incubation relative to the initial entry rate of amine into the cell. Schayer [5] has provided the only report on the uptake of histidine and its conversion to histamine by rat peritoneal mast cells. The past emphasis on amine uptake is surprising, because the concentration of histidine is many times higher than histamine in the extracellular medium *in vivo*, and a soluble, pyridoxal phosphate-dependent enzymatic activity that decarboxylates histidine has been demonstrated in both mouse mastocytoma and rat peritoneal mast cells [5–8]. The present study describes the characteristics of histidine uptake by purified mast cells during incubation periods that are suitable for the determination of kinetic parameters.

MATERIALS AND METHODS

Materials. Materials were obtained from the suppliers indicated: L-[2,5-^{3}H]histidine (42 Ci/mmole) (Amersham/Searle, Arlington Heights, IL); DL-B-2-aminobicyclo-[2,2,1]-heptane-2-carboxylic acid (New England Nuclear Corp., Boston, MA); L-histidine hydrochloride, histamine dihydrochloride, L-phenylalanine, L-homoarginine, α-(methylamino)-isobutyric acid, 2,4-dinitrophenol, 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide, β-methylumbelliferone, ATP (disodium salt, from equine muscle), choline chloride, and FLE-50 buffered firefly lantern extract (Sigma Chemical Co., St. Louis, MO); Path-O-Cyte 4 albumin (Miles Laboratories, Inc., Elkhart, IN); 3A70 scintillation fluid (Research Products International, Elk Grove Village, IL); and Polygram DEAE-cellulose thin-layer chromatography sheets (Brinkmann Instruments, Inc., Westbury, NY).

Cell isolation. Mast cells were obtained from the peritoneal cavities of male Sprague–Dawley rats (retired breeders obtained from Hilltop Laboratory Animals, Scotdale, PA) as described previously [9]. The purity of mast cell fractions thus obtained was always higher than 80 per cent (and typically above 90 per cent). The cells that remained at the albumin–buffer interface were collected as the "non-mast cell fraction". Mast cell and non-mast cell fractions were washed once in a balanced salt solution, pH 7.2, that also contained 0.5% albumin (BSSA, Ref. 9), were collected by centrifugation at 900 *g* for 5 min, and were resuspended in the same buffer to give a final cell density of approximately 10×19^6 cells/ml. Cell densities were determined with a Neubauer hemocytometer.

Histidine uptake experiments (general protocol). Aliquots of approximately 0.5×10^6 cells (10×10^6 cells/ml) were incubated at 37° in the presence of 5 μCi of [^{3}H]histidine (42 Ci/mmole) and sufficient carrier histidine to give the concentration indicated in a final incubation volume of 75 μl. Unless otherwise stated, final histidine concentration was 216.3 μM and incubation time was 1 min. Parallel incubations at 4° were performed to correct for non-

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specific uptake of histidine by the cells. Correction was also made for the non-mast cell contribution to mast cell uptake. Following incubation for the stated time interval, the cells were quickly centrifuged (11,500 *g* for 0.5 min), thrice washed with several assay volumes of BSSA buffer, and finally extracted with 25 μl of either 2.5% trichloroacetic acid or 5% perchloric acid (ATP depletion experiments). This extract was centrifuged and a 10-μl aliquot of the supernatant fraction was neutralized with NaOH and counted in 10 ml of 3A70 scintillation fluid in a Beckman LS-250 liquid scintillation spectrometer.

Intracellular distribution experiments. In the experiments in which the distribution of uptake between cytoplasmic and granule fractions was measured, approximately 1×10^6 mast cells (10×10^6 cells/ml) were incubated in the presence of [^{3}H]histidine (10 μCi; 216.3 μM) in a final volume of 0.15 ml for the time intervals indicated, centrifuged, and washed thrice with several assay volumes of BSSA buffer. The cell pellets were then resuspended in 0.5 ml of the same buffer, and lysed by sonication in a Sonogen bath sonicator (Branson Instruments, Inc., Stamford, CT) for a period of time sufficient to give 90–100 per cent cell lysis (0.75 to 1.5 min). The supernatant and granule fractions were separated by centrifugation at 8,730 *g* for 3.5 min in a Beckman Microfuge B. Each fraction was extracted with trichloroacetic acid (final concentration, 2.5%) and subsequently analyzed for incorporation of radioactive material. An aliquot of the extracted granule fraction was further analyzed for relative amounts of [^{3}H]histamine and [^{3}H]histidine by t.l.c. (below). To correct for the radioactivity distribution resulting from granule membrane breakage during sonication, the activity of the granule marker enzyme *N*-acetyl-β-D-glucosaminidase was determined (below) in each fraction prior to acid extraction, and the appropriate corrections were made.

Amino acid competition and sodium replacement experiments. Stock solutions of L-phenylalanine (150 mM), α-(methylamino)isobutyric acid (300 mM), and L-homoarginine (300 mM) were prepared in water. A stock solution of DL-B-2-amino-bicyclo-[2,2,1]-heptane-2-carboxylic acid (100 mM) was prepared in water-methanol (1:1). Aliquots of approximately 0.5×10^6 cells (10×10^6 cells/ml) were incubated with [^{3}H]histidine (5 μCi; 1.4 to 1.6 μM) as described in the general protocol above in the absence or presence of these amino acids at the final concentrations indicated in Table 3. In the sodium replacement experiment, a buffer containing 0.154 M choline chloride, 0.68 mM $CaCl_2$, K^+-phosphate (54 mM K^+, 30.7 mM phosphate, pH 7.3), and 0.5% albumin replaced the normal assay buffer [9]; prior to assay, cells were washed three times and resuspended in this sodium-depleted buffer. The cells were then incubated as described in the general protocol (above).

ATP depletion experiments. Stock solutions of 26 mM 2,4-dinitrophenol and of 5.2 and 26 μM antimycin A were prepared in ethanol. To deplete cellular ATP, 50-μl aliquots (10×10^6 cells/ml) of mast cells were preincubated for 10 min at 37° in the presence of the respective inhibitor at the final concentrations indicated in Table 4. Following preincubation, the remaining incubation components were added, and [^{3}H]histidine uptake was measured as described in the general protocol above. ATP content was determined as described in "Chemical assays" (below). The final incubation volume was 77 μl (maximum ethanol concentration, 2.6%). Control experiments showed that 2.6% ehtanol had no effect on either [^{3}H]histidine uptake or ATP content under the conditions of these experiments.

Separation of [^{3}H]*histidine and* [^{3}H]*histamine.* Aliquots (typically 10 μl) of cellular trichloroacetic acid extracts were spotted on Polygram DEAE-cellulose t.l.c. sheets and separated with a solvent system of isopropanol–2.5 M aqueous ammonia (80:12) [10]. Migration of each fraction was determined by visualization of authentic standards with ninhydrin spray reagent (2.5 mg/ml in acetone–pyridine, 9:1). The portions of the chromatogram corresponding to histidine ($R_f = 0.09$) and histamine ($R_f = 0.58$) were scraped into scintillation vials, extracted with 0.25 ml of 2.5% trichloroacetic acid, neutralized with NaOH, and counted in 10 ml of 3A70 scintillation fluid.

Chemical assays. The amount of *N*-acetyl-β-D-glucosaminidase activity released into the supernatant fraction of lysed cell preparations was assayed as described previously [11], except that the assay was terminated with 0.5 M sodium carbonate, pH 10.75.

A luciferase/luciferin assay based on the methodology of Stanley and Williams [12] was used to determine cellular ATP content. To a 6 × 50 mm tube was added 50 μl of 10 mM phosphate buffer (pH 7.4) containing 4 mM $MgSO_4$, 50 μl of water, 50 μl of 100 mM sodium arsenate buffer (pH 7.4) containing 40 mM $MgSO_4$, and 1 μl of either cellular perchloric acid extract or ATP standard solution (containing 1–5 pmoles ATP in 5% perchloric acid). Light intensity was measured 10 sec after the addition of 100 μl of buffered firefly lantern extract (prepared according to supplier's instructions) with an Aminco fluoro-colorimeter/Chem–Glow photometer.

RESULTS

Because there is no published information concerning mast cell uptake of histidine at short time intervals, we first determined the time range during which uptake of this amino acid was linear. Figure 1 indicates that, for mast cells supplemented with 1.59 μM histidine, this period was short (on the order of 1 min). A similar profile was obtained for mast cells supplemented with 216.3 μM histidine (not shown). Thus, unless otherwise indicated, all histidine uptake experiments were performed using an incubation time of 1 min. Presumably, measurements of amino-acid uptake at a short time interval have the advantage of minimizing any contribution of efflux of labeled precursor into the extracellular medium.

After mast cells were incubated for 1 min in the presence of tritiated histidine at a variety of histidine concentrations, and the incorporated tritiated material was separated by t.l.c. into [^{3}H]histidine and [^{3}H]histamine fractions, most of the labeled precursor was found to be histamine (Table 1), in agree-

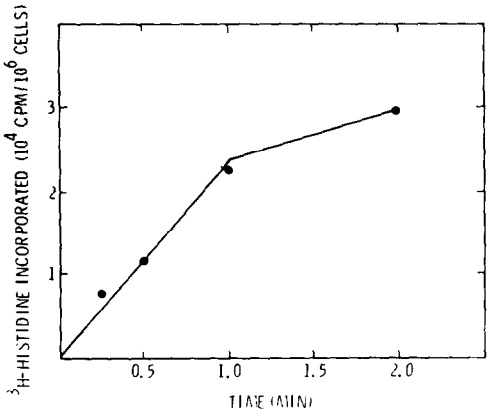

Fig. 1. Incorporation of [^{3}H]histidine (1.59 μM; 5 μCi/assay) into rat peritoneal mast cells as a function of time. Cells were assayed and extracted as described in Materials and Methods.

Table 1. Incorporation of [^{3}H]histidine and conversion to histamine in mast cell and non-mast cell fractions of rat peritoneal fluid*

Fraction	Histidine conc. (μM)	% Incorporation† Histidine	Histamine
Mast cells	1.59	9.6†	90.4‡
	10.2	14.0 (3.7)	86.0
	216.3	22.0 (7.0)	78.0
Non-mast cells	10.2	93.3 (2.8)	6.7
	216.3	97.7 (0.6)	2.3

* Mast cell and non-mast cell fractions were isolated and assayed as described in Materials and Methods. Incubation time was 1 min at the indicated histidine concentration (5 μCi/assay).

† Per cent of incorporated material as histidine or histamine, as separated by t.l.c. (Materials and Methods). Numbers in parentheses indicate standard deviations of three or more replicate determinations.

‡ One determination only.

ment with the report of Schayer [5]. The conversion of histidine to histamine by mast cells was a highly efficient process, yielding approximately 80–90 per cent histamine in a 1-min (Table 1) or longer incubation. Even at incubation intervals of 0.25 to 0.5 min (at a histidine concentration of 1.59 μM), the conversion of histidine to histamine was greater than 90 per cent (data not shown). In contrast, although the cells of rat peritoneal fluid that constitute the non-mast cell fraction (predominantly monocytes and macrophages) transport [^{3}H]histidine, they lack the capacity to decarboxylate this material to histamine.

To further characterize the kinetic behavior of [^{3}H]histidine uptake by mast cells, the velocity of uptake was measured as a function of extracellular histidine concentration. This process was shown to be saturable and, from a standard analysis of the linearized data (Fig. 2), the kinetic constants shown in Table 2 were obtained. The value of K_m (44 μM; Table 2) was much lower than that reported by Christensen [13] of 2.6 to 4.0 mM for histidine transport by Ehrlich tumor cells. Histidine transport was not affected by excess amounts of exogenous histamine. Further, a Hill plot (Fig. 3) of the data presented in Fig. 2 (no added histamine) yielded a slope of unity, within experimental error, providing support for the notion of a single, independent site for mast cell histidine transport. The data of Cabut and Haegermark [2] suggest an approximate K_m for mast cell histamine transport of 2 to 2.5 mM,

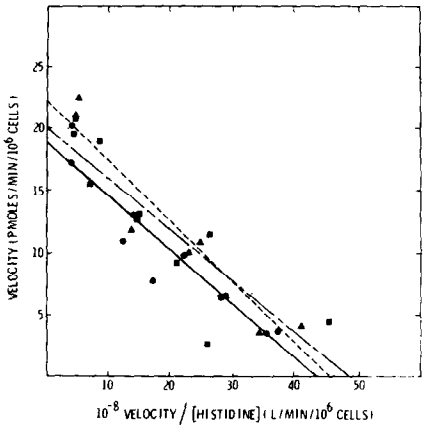

Fig. 2. Augustinsson plot of velocity of mast cell uptake of [^{3}H]histidine as a function of histidine concentration. Incorporation was measured as described in Materials and Methods. Additions: (●——●) none; (■– – –■) 300.0 μM histamine; and (▲– – –▲) 600.1 μM histamine. Best-fit lines were obtained by the method of least squares.

although the process did not seem to be saturable at histamine concentrations as high as 6–7 mM. These authors further observed that histidine did not block histamine uptake in mast cells. Agreement of values of V_{max} by these different laboratories cannot be easily determined, since velocities measured in each report are not in directly comparable contexts.

Table 2. Kinetic constants for histidine uptake by mast cells*

Additions	K_m (μM)	V_{max}[pmoles·min^{-1}·(10^6 cells)$^{-1}$]
None	44.0 (6.0)	18.9 (1.4)
Histamine (300.0 μM)	41.5 (8.7)	20.1 (1.8)
Histamine (600.1 μM)	49.0 (6.8)	22.2 (1.6)

* Mast cells were isolated and assayed as described in Materials and Methods. Kinetic constants were derived from analysis of the Augustinsson plot (Fig. 2). Numbers in parentheses represent standard errors of the kinetic constants.

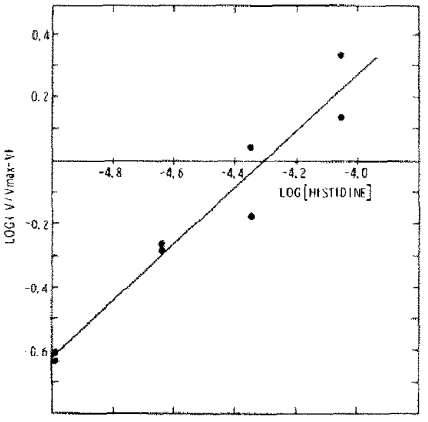

Fig. 3. Hill plot of the data presented in Fig. 2. Slope of the best-fit line (method of least squares) was 0.894 (S.E. 0.090).

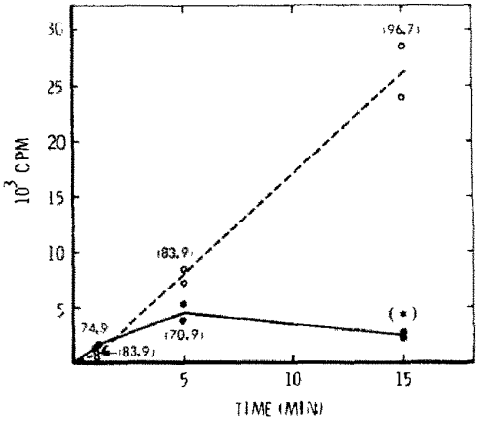

Fig. 4. Incorporation of radioactivity from [^{3}H]histidine into cytoplasmic (●) and granule (○) fractions of whole mast cells as a function of time. Incubations, cell lysis, and separation and analysis of cytoplasmic and granule fractions are described in Materials and Methods. The average percentage of histamine in each fraction is given in parentheses above the respective sample points. An asterisk (*) indicates no detectable histamine.

Since both the uptake of [^{3}H]histidine and the decarboxylation to [^{3}H]histamine were rapid processes, we next tested the ability of the mast cell granules to transport the nascent [^{3}H]histamine as a function of time. Figure 4 shows the distribution of tritiated precursor that was incorporated into whole mast cells at various time intervals, as described in Materials and Methods. The bulk of the radioactive material found in the granule fraction was histamine, although small amounts of histidine were also present. In agreement with the data presented in Table 1, the tritiated material in the cytoplasmic fraction was also predominantly histamine. However, although there was a transfer of [^{3}H]histamine from cytoplasm to granules with increasing time, this was apparently a slow process relative to the initial histidine uptake and decarboxylation. Only after approximately 15 min did the proportion of incorporated label in the granule fraction exceed 90 per cent.

To determine whether mast cell histidine uptake could be classified as either an A or an L transport system [14], the experiments presented in Table 3 were performed. A low concentration of histidine was used in these assays to maximize competitive effects. Transport of histidine by the so-called A system can be inhibited by the model substrate α-

Table 3. Inhibition of [^{3}H]histidine uptake by amino acid competition and sodium depletion*

Additions or replacements	Histidine conc. (μM)	[^{3}H]Histidine incorporation†
MeAIB (18.7 mM)	1.5	85.7 ± 5.4
Phe (9.4 mM)	1.5	25.4 ± 7.5
Phe (9.4 mM) + MeAIB (18.7 mM)	1.4	31.1 (28.3, 33.8)
BCH (6.25 mM)	1.5	27.6 ± 3.3
Homoarg (18.7 mM)	1.5	71.6 ± 8.6
Choline chloride replaces sodium chloride	1.6	47.8 ± 12.8

* Mast cell [^{3}H]histidine incorporation was measured at the indicated histidine concentration in the absence or presence of α-(methylamino)isobutyric acid (MeAIB), L-phenylalanine (Phe), DL-B-2-aminobicyclo-[2,2,1]-heptane-2-carboxylic acid (BCH) or L-homoarginine (Homoarg), or with choline chloride replacing sodium chloride as described in Materials and Methods.

† Per cent of control value ± S.D. for three or more replicate experiments; individual values for experiments with fewer than three values are given in parentheses.

(methylamino)isobutyric acid (MeAIB) [13, 15]. Mast cells incubated with [^{3}H]histidine and this model amino acid at levels of MeAIB comparable to those reported previously [15] showed no such inhibition (Table 3). Conversely, phenylalanine (Phe) or DL-B-2-aminobicyclo-[2,2,1]-heptane-2-carboxylic acid (BCH), which are known to inhibit L system transport [13, 16, 17], reduced mast cell histidine uptake significantly. MeAIB did not reverse the effect of phenylalanine under the conditions of these experiments. Thus, mast cells transported histidine predominately by the L pathway under the conditions employed. The residual uptake in the presence of both phenylalanine and MeAIB may have resulted from a cationic transport system for which L-homoarginine is a competitive substrate [17] (Table 3), a sodium ion-dependent ASC system described by Christensen *et al.* [15], or an as yet undescribed transporter. Christensen [13] has reported that the contribution of the L system to histidine transport by Ehrlich tumor cells is approximately 66 per cent.

Because the L transport system has been reported to be independent of sodium ion concentration, we tested the ability of mast cells to transport histidine in medium depleted of sodium. Under our experimental conditions, replacement of the sodium chloride with choline chloride yielded an inhibitory effect (52.2 per cent; see Table 3) larger than is consistent with the amino acid competition data, which suggests a contribution of an L transport system of approximately 70–75 per cent (Table 3). We are at a loss to explain this inconsistency, although a toxic effect of a high concentration of extracellular choline on mast cells cannot be ruled out. Alternatively, the lower extracellular phosphate concentration (which was necessary to attain both complete depletion of sodium and the same potassium content as in the normal balanced salts buffer [9] may have been a complicating factor.

Finally, the effect of depletion of cellular ATP on histidine uptake was measured. Table 4 shows that, in cells that had been incubated with either 2,4-dinitrophenol or antimycin A at concentrations sufficient to reduce mast cell ATP levels substantially, there was no inhibitory effect on histidine transport activity. Independence of uptake of histamine and other amines on cellular ATP has been indicated by Cabut and Haegermark [2] and Heisler and Uvnas [3], whose data demonstrate little effect of metabolic poisons such as cyanide or 2,4-dinitrophenol on amine uptake by mast cells.

DISCUSSION

Levels of human blood histidine are on the order of 74 μM [18], whereas the corresponding value for histamine is approximately 4 nM [19]. If the ratio of histidine to histamine in rodent tissue fluids reflects the ratio for the blood levels of these amines in humans, the histidine concentration to which mast cells are exposed would be approximately four orders of magnitude above that of histamine. The large difference between these values and between the K_m for histidine (44 μM; Table 2) and that for histamine (2 to 2.5 mM; from data in Ref. 2) transport indicate that histidine is by far the preferred extracellular source of mast cell histamine via uptake and decarboxylation. The value of the K_m for histidine uptake reported in this paper is in the same concentration range as that of circulating histidine, so that mast cell histamine content is potentially susceptible to the influence of circulating histidine levels.

Our value for K_m is unlikely to be a valid estimate for the dissociation constant of the carrier–substrate complex, since it is almost certainly perturbed by subsequent steps in the intracellular handling of histamine, i.e. decarboxylation and sequestration in the granule. The discrepancy between the value of K_m in Table 2 and that reported by Christensen [13] for Ehrlich tumor cell histidine uptake (2.6 to 4 mM) under circumstances in which histidine is neither converted to histamine nor sequestered is therefore not surprising. Measurements of mast cell transport by histidine analogs that are not processed further following their initial uptake would be expected to provide a more reliable indication of initial uptake parameters; such analogs, however, are not presently available in radiolabeled form.

Histidine uptake by rat peritoneal mast cells was insensitive to excess levels of histamine in the extracellular medium (Table 2), and thus, even if high local levels of histamine should occur following degranulation, there would be no inhibition of his-

Table 4. Effect of ATP depletion on [^{3}H]histidine uptake by mast cells*

Additions	[^{3}H]Histidine uptake†	ATP content†
None	100	100
DNP (670 μM)	90.3 (80.7, 99.9)	22.9 (14.8, 31.0)
Antimycin A 135 nM	113 ± 33.5	23.5 ± 4.1
338 nM	114 ± 35.0	11.5 ± 1.8

* Mast cell uptake of [^{3}H]histidine in the absence and presence of the indicated inhibitors, as well as analysis of cellular ATP content, is described in Materials and Methods. DNP = 2,4-dinitrophenol.

† Per cent of control values ± S.D. for three or more replicate experiments; individual values for experiments with fewer than three values are given in parentheses.

tidine uptake. In fact, levels of histamine in the venous effluents of areas in human patients subject to urticarial reactions have been reported to be approximately 80–320 nM [20], and in only one patient, who had experienced repeated hypotensive episodes, were peak histamine levels in the micromolar range [20].

The mast cells rapidly converted transported histidine to histamine (Table 1). Although other laboratories [5–8] have reported that mast cell histidine decarboxylase appears in the soluble enzyme fraction, the tight coupling that we have observed between uptake and decarboxylation suggests the possibility of a membrane-bound decarboxylase activity associated with the transport assembly. If such an enzyme were peripherally and loosely held to the membrane, then the cell disruption necessary for isolation of the crude activity could be sufficient to dissociate enzyme from membrane.

Relative to the efficient uptake and conversion of histidine by mast cells, transfer of the nascent decarboxylated amine into the mast cell granules appears to have been a somewhat slower process (Fig. 4). Although transport of amines by chromaffin granules has been widely studied [21, 22], the mechanism by which mast cell granules concentrate histamine has not been elucidated. Further, it is not clear which aspects of cell organization are necessary for optimal granule transport of histamine in mast cells. The apparent presence of some [^{3}H]histidine in the granule fraction in these experiments should not be construed as strong evidence for this occurrence, because limited artifactual binding of histidine to granule matrices that have lost their membranes has not been excluded by either the methods or the analysis of the data employed.

The mast cell histidine uptake system shares a number of properties with related transport systems. As in Ehrlich tumor cells [13], the predominant transporting mechanism for histidine at neutral pH was of the L type. Although histidine and histamine evidently are transported by independent systems in the mast cell, both processes proceed normally in the presence of low levels of intracellular ATP. The implication of this independence of cell ATP has been considered in detail by Christensen [23].

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