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Absence of increase of histidine decarboxylase activity in mast cell-deficient *W/W* mouse embryos before parturition

(histamine/nonmast cell/mutant mouse)

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ABSTRACT **The histidine decarboxylase (L-histidine carboxy-lyase, EC 4.1.1.22) activity of whole *W/W* mouse embryos, which are devoid of mast cells, remained very low and did not show the rapid increase before birth (17–20 days of gestation) seen in wild-type +/+ embryos. During the same period, the histamine content also remained very low and no mast cells were detected in the *W/W* embryos, in contrast to the large increase in both histamine content and number of mast cells in wild-type embryos. These findings imply that the histamine in embryos is largely derived from mast cells. In +/+ mice, histidine decarboxylase activity decreased rapidly soon after birth without concomitant decrease in histamine content or number of mast cells, suggesting that the enzyme activity in mast cells is regulated by some unknown mechanism.**

Histamine plays various roles as one of the biogenic amines; it is probably liberated from both mast cells and nonmast cells. The histamine liberated from mast cells is involved in anaphylactic and allergic reactions, inflammation and so on, and that liberated from nonmast cells is involved in gastric acid secretion, neurotransmission, and rapid tissue proliferation, such as growth of rodent embryos, healing of wounds, and growth of certain experimental tumors (1–6). The idea that it is produced by nonmast cells was proposed by Kahlson and Rosengren (1, 2) and by Schayer (3), but the nature of these nonmast cells secreting histamine is unknown.

The most dramatic and best-known example of nonmast cell histamine is seen during the development of rodent embryos. Namely, a marked increase in urinary excretion of histamine occurs in pregnant rodents, such as rats, mice, and hamsters, in the last few days of pregnancy, with a return to low levels after delivery (1, 2); in parallel with these changes, the histidine decarboxylase (HisDCase; L-histidine carboxy-lyase, EC 4.1.1.22) activity of whole embryos shows a sharp increase after day 16 of gestation, reaches a plateau on day 18, and decreases rapidly after birth. Kahlson and Rosengren used the term "nascent histamine" (1, 2) to distinguish this histamine from histamine released from mast cells, and proposed that it is formed as a result of increase in histamine-forming capacity. Schayer noted a similar marked increase in HisDCase activity when certain tissues were exposed to irritants, endotoxins, or infectious agents (3). He referred to this increased HisDCase as "inducible HisDCase," and suggested that it was derived from nonmast cells. Histamine produced by inducible HisDCase in nonmast cells is not stored in the cells but released immediately after its synthesis, thus having a rapid turnover (3, 4). In contrast, histamine in mast cells has a slow turnover rate, is stored in granules, and is released upon stimulus (4, 5). Although the views of Kahlson and Rosengren (1, 2) and Schayer (3) are very interesting, they seem to be based on rather indirect evidence: mast cells are so ubiquitous in tissues that, in studies using normal tissues, it is impossible to exclude the contribution of mast cells. Until now, no system has been available for demonstrating the presence of histamine in nonmast cells.

Kitamura *et al.* (7) observed that a double gene dose of the mutant allele at the *W* locus had a profound effect on the development of mast cells. The number of mast cells in the skin, stomach, caecum, and mesentery of adult W/W^v mice is less than 1% of that in congeneic +/+ mice. Moreover, no mast cells are present in suckling *W/W* mice, which usually die of severe anemia within 10 days of birth (8). We found that most tissues of W/W^v mice have little histamine but that brain and stomach have appreciable amounts, implying that histamine can be derived from nonmast cells (9). Further studies showed that the whole bodies of suckling *W/W* and W/W^v mice had 20% and 40%, respectively, of the HisDCase activity of that in wild-type mice (10). Thus *W/W* and W/W^v mice seemed useful for solving the problem of whether increase in HisDCase activity and the histamine level in rodent embryos during pregnancy is due to mast or nonmast cells. Accordingly, in this work we compared the HisDCase activity, histamine content, and number of mast cells in embryos of *W/W* mice and in congeneic +/+ mice during the preparturition period and immediately after birth.

MATERIALS AND METHODS

Mice. WB-(*W*/+, +/+) mice were raised in our laboratory using parental stocks originally obtained from The Jackson Laboratory. *W/W* embryos were obtained by mating female WB-*W*/+ mice with male WB-*W*/+ mice. The day on which vaginal plugs were found was taken as the first day of pregnancy. Mice were killed by cervical dislocation between day 13 and day 20 of pregnancy, and the embryos were removed. *W/W* embryos were distinguished from their *W*/+ and +/+ litter mates by the color of their skin, because they were anemic (11). +/+ Embryos were obtained from WB-+/+ females mated with WB-+/+ males.

Analytical Procedures. Most embryos and newborn mice were stored at −80°C for analyses, but a few were fixed in 10% formalin in phosphate buffer, pH 7.2, for determination of the number of mast cells. The procedure for determining the number of mast cells in subcutaneous tissue of embryos has been described (12). Whole body extracts of embryos were prepared as described (10) with some modifications. Single or combined embryos at various times of gestation and newborn mice were

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Abbreviation: HisDCase, L-histidine decarboxylase, L-histidine carboxy-lyase (EC 4.1.1.22).

homogenized in 4 vol (wt/vol) of solution A in a Polytron (Kinematica) operated at maximum setting 3 times for 10-sec periods in an ice bath. Solution A consisted of 0.1 M potassium phosphate, pH 6.8/0.2 mM dithiothreitol/0.01 mM pyridoxal 5'-phosphate/1.0% polyethylene glycol (average M_r 300) containing protease inhibitors (chymostatin, leupeptin, antipain, and pepstatin) at 2 μg/ml each. The homogenate was centrifuged at 10,000 × *g* for 20 min at 4°C. One-tenth of the supernatant was added to 9 vol of cold 0.5 M perchloric acid/5 mM EDTA for histamine analysis. The remaining 90% of the supernatant was dialyzed overnight against 50 vol of solution A with two changes of the outer solution for measurement of HisDCase activity (10, 13) and protein. Protein was measured by the method of Lowry *et al.* (14) using bovine serum albumin as a standard.

HisDCase Assay. HisDCase activity was assayed by incubating samples for 60 or 120 min with 0.25 mM L-histidine in solution A but with protease inhibitors at 20 μg/ml (10) and 0.2 mM aminoguanidine to prevent further degradation of histamine formed during the reaction. Each assay was carried out in parallel with a mixture containing 1 nmol of histamine, as an internal standard, and a mixture containing no histidine, as a blank. The recovery of histamine was >90% in all cases. Histamine formed during the reaction was separated from histidine on a small column of Amberlite CG-50 and measured with *o*-phthalaldehyde as described by Shore *et al.* (15) using the autoanalyzer system developed in this laboratory (13).

Histamine Analysis in Tissue Extracts. As described above, 10% of the extract was mixed with 9 vol of 5% perchloric acid/5 mM EDTA and stored in an ice bath for 10 min. Then, the mixture was centrifuged at 10,000 × *g* for 20 min at 4°C. The supernatant was applied to a column of Bio-Rad AG-50 (4 × 50 mm) equilibrated with 0.2 M sodium phosphate buffer, pH 6.5. The column was washed with 5 ml of water and 10 ml of 2 M HCl, and then histamine was eluted with 6 ml of 3 M HCl. The solution was evaporated to dryness at reduced pressure, and the residue was dissolved in 0.5 ml of 0.05 M HCl. One hundred microliters of the solution was injected into a TSK-IEX 510 SP column (0.4 cm inside diameter × 25 cm) and the column was developed with 0.2 M sodium propionate, pH 4.0/5 mM EDTA/0.5 M NaCl/20% (vol/vol) methanol. Histamine in the eluate was converted to fluorescent products by the *o*-phthalaldehyde method (15, 16) using a continuous flow reaction system. The fluorescent intensity was measured by means of a fluoromonitor (JEOL JLC-FL, λ excitation 230–400 nm, λ emission 410–800 nm, volume of flow cell 8 μl), equipped with a recorder.

RESULTS

Table 1 shows the changes with age in body weight, amount of soluble protein, HisDCase activity, and histamine content of +/+ and *W/W* mouse embryos and newborn mice. The body weights and soluble protein contents of *W/W* embryos were significantly less than those of +/+ embryos. There were striking differences between the two in HisDCase activity and histamine content, particularly during gestation. On day 18 of gestation, the HisDCase activity and the histamine content of +/+ mice were 150 and 100 times higher, respectively, than those of *W/W* mice and, on day 19, the differences were even greater. The total HisDCase activity dropped sharply after birth. The wide variations in the activities on day 1 after birth were due to difficulty in knowing the exact time of birth. On the other hand, the histamine content stayed at the same high level after birth as on days 18 and 19 of gestation.

These characteristics were more clearly seen when the HisDCase activity and histamine content were expressed on the basis of protein content. The relationship of changes with time in specific HisDCase activity and histamine concentration per mg of soluble protein in +/+ and *W/W* mouse embryos and newborn mice and in the numbers of mast cells in the skin are shown in Fig. 1. The specific HisDCase activity of crude extracts of +/+ mouse embryos began to increase rapidly on day 16 reaching a peak on day 18 of gestation, when the enzyme activity was 105 times that on day 13 of pregnancy. The activity decreased sharply after birth, being 1/17th of the peak value by day 3. In parallel with the increase in HisDCase, the specific histamine content and the number of mast cells began to increase on day 16 of gestation but, unlike HisDCase activity, they did not decrease greatly after day 20, remaining higher than before day 16 of gestation. On the other hand, in *W/W* mouse embryos, neither the HisDCase activity nor the histamine content increased at all during the preparturition period. As predicted from genetic studies (7), no mast cells were detected in subcutaneous tissues of mutant embryos.

DISCUSSION

We have examined the changes of HisDCase activity, histamine content, and number of mast cells during development of

Table 1. Body weights, amounts of soluble protein, HisDCase activities, histamine contents, and numbers of mast cells in whole bodies of +/+ and *W/W* mice during gestation and after birth

	Body weight, g				Total soluble protein, mg		Total HisDCase, pmol/min		Total histamine, nmol	
Day	+/+	*n*	*W/W*	*n*	+/+	*W/W*	+/+	*W/W*	+/+	*W/W*
					Gestation					
13	0.24	2			3.35		0.6		0.26	
14	0.20	1			10.6		1.6		0.23	
15	0.23 ± 0.04	2			7.85 ± 1.31		10.2 ± 2.3		0.19 ± 0.02	
16	0.29 ± 0.05	3			11.2 ± 2.0		35.4 ± 10.2		1.41 ± 0.29	
17	0.49 ± 0.08	4	0.41 ± 0.05	3	19.1 ± 2.5	13.6 ± 2.8	293 ± 95	2.72 ± 0.96	9.57 ± 2.98	0.11 ± 0.05
18	0.75 ± 0.07	8	0.64 ± 0.05	3	29.4 ± 4.4	21.0 ± 2.4	827 ± 105	5.50 ± 0.99	41.6 ± 4.63	0.42 ± 0.09
19	1.03 ± 0.08	5	0.70 ± 0.04	3	41.1 ± 5.7	20.3 ± 5.4	926 ± 270	2.27 ± 0.16	48.3 ± 5.4	0.18 ± 0.05
20	1.20 ± 0.10	3			39.3 ± 7.8		514 ± 341		45.2 ± 10.7	
					After birth					
1	1.57 ± 0.07	5	1.23 ± 0.11	5	69.4 ± 10.6	50.5 ± 10.7	386 ± 150	8.62 ± 2.69	120 ± 13	1.33 ± 0.59
3	2.07 ± 0.09	3	1.33 ± 0.27	3	82.2 ± 5.3	44.3 ± 9.5	75 ± 17	13.3 ± 7.1	67 ± 5	5.58 ± 5.10
5			1.46 ± 0.36	3		54.7 ± 17.6		8.93 ± 3.20		8.91 ± 5.23
	2.50 ± 0.4*	4	1.60 ± 0.4*	4	75 ± 12*	35 ± 13*	81 ± 42*	9.4 ± 4.5*	160 ± 67*	2.18 ± 0.97*

Results represent mean ± SD.
* Taken from data in ref. 10.

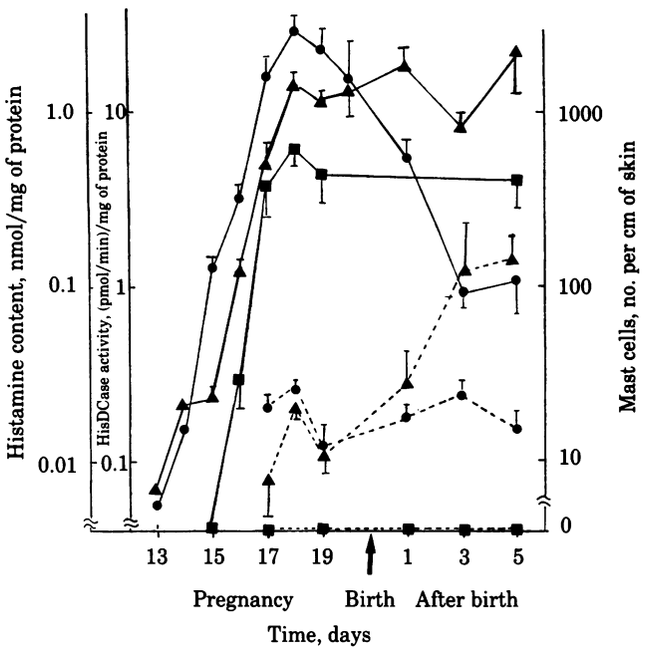

FIG. 1. HisDCase activity (●), histamine content (▲), and numbers of mast cells (■), in +/+ and *W/W* embryos and newborn mice. Three to 5 animals in each group were used to determine the number of mast cells. ——, +/+ mice; ----, *W/W* mice. Vertical bars show SDs.

+/+ and *W/W* mouse embryos. *W/W* mice are useful for determining whether histamine involved in fetal growth is derived from nonmast cells as nascent histamine (1, 2) because this mutant has no mast cells (7). In +/+ mouse embryos, we confirmed the results of Kahlson and Rosengren that HisDCase activity increased significantly between day 17 and day 20 of gestation (1, 2). Contrary to the idea that histamine formed by inducible HisDCase is not stored in cells but released and destroyed rapidly *in situ*, the histamine level of whole bodies of +/+ mice increased in parallel with the increase in HisDCase activity and the number of mast cells. Histamine has not previously been measured in whole embryos, but urinary histamine excretion in pregnant animals has been used as an indicator of histamine formation *in vivo* because nascent histamine has a fast turnover rate (1, 2). In *W/W* mouse embryos, which have no mast cells (7), the HisDCase activity and the histamine content remained low and did not increase between day 17 and day 19 of gestation. Thus, the increased histamine content observed in +/+ mouse embryos during the preparturition period is derived from mast cells of the embryos, not from nonmast cells, as suggested by Kahlson and Rosengren (1, 2). As *W/W* mice survived until sometime after birth even though they were smaller than +/+ mice during gestation and after birth, it seems unlikely that histamine is essential for maintenance and growth of embryos during gestation. The present study showed that rapid increase in HisDCase activity under certain conditions does not necessarily indicate that it occurs in nonmast cells. However, we have to test other conditions using the W/W^v mouse system, because there still remains the possibility that HisDCase deficiency and mast cell deficiency are independent in this mutant mouse.

It is interesting that, in +/+ mouse embryos, HisDCase activity reaches a peak on days 18 and 19 of gestation, begins to decrease on day 20, and becomes very low after birth, whereas the histamine content and the number of mast cells, which increase at the same times as the HisDCase activity, do not decrease later, but remain high even after birth. Two possible explanations for this dissociation of HisDCase activity from the histamine and mast cell contents can be considered. (*i*) HisDCase, which is induced on day 16 of gestation, in parallel with the increase in mast cells, may be destroyed by day 20 by some protease. This is consistent with the observation that HisDCase is very sensitive to protease (17). In fact, mast cells have a potent serine protease, as reported by Woodbury *et al.* (18). (*ii*) HisDCase activity may be masked in mast cells by some unknown factor(s)‡ that is removed when HisDCase activity is necessary. This possibility is consistent with the observation that the reaction of HisDCase in mast cells proceeded linearly only when a limited amount of protein was used, a large amount of protein causing strong inhibition (17). Histamine synthesized in mast cells is stored in granules and released when necessary. Thus, unless mast cells are depleted of histamine, they will not need to synthesize more histamine. Radioimmunoassay of HisDCase using anti-HisDCase antibody should be useful for determining which of these possibilities is correct.

L-ornithine decarboxylase activity increases during development of embryos (19). Recently, Fozard *et al.* (20) reported that inhibition of ornithine decarboxylase in mouse embryos *in vivo* by DL-α-difluoromethylornithine, an enzyme-activated irreversible inhibitor of the enzyme, resulted in arrest of embryonic development. Relative to the above discussion, it would be particularly interesting to test the effect of L-α-fluoromethylhistidine, a suicide inhibitor of HisDCase (21), on growth of mouse and rat embryos, because increase in HisDCase in mouse and rat embryos during gestation is associated with its increase in the skin and liver, respectively (1, 2, 22).

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‡ Heparin at up to 400 units/ml was not inhibitory to fetal rat HisDCase (M. Yamada, personal communication).

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