in the preleptotene spermatocytes suggests that there is no *myc* expression in the premeiotic G_1 period. One possible explanation for this is that the somatic cells examined (lymphocytes, fibroblasts, and Sertoli cells) (*1, 4*) continue to divide until either senescence or a specific signal causes cell division to cease. In contrast, most of the spermatogonia are in the middle of a developmental pathway that has only a tightly prescribed number of cell divisions before the onset of meiosis and terminal differentiation (*8, 12*). Therefore, it may be that the initiation of these final developmental steps sets up a series of mitotic divisions in which there is no obligatory requirement for *myc* transcription; in this respect germ cells may not be unusual. Perhaps all cells committed to the final few mitotic divisions before terminal differentiation do not need to express *myc*. Support for this last hypothesis is given by the observation that promyelocytic HL60 (*14*) cells and F9 teratocarcinoma (*15*) cells that have been induced to undergo terminal differentiation also have low amounts of *myc* transcripts, although in these examples the diminution in *myc* transcripts may be due to a decrease in cell turnover.

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Unequivocal Delayed Hypersensitivity in Mast Cell–Deficient and Beige Mice

Abstract. *It has been suggested that reserpine blocks expression of delayed hypersensitivity in mice because it depletes stores of the vasoactive amine serotonin in mast cells. To determine whether mast cell serotonin or other mast cell–derived mediators are essential for delayed hypersensitivity, responses to contact sensitizers in mast cell–deficient* W/W^v *or* Sl/Sl^d *mice were studied. Because blood platelets represent another potential source of serotonin in delayed hypersensitivity responses, beige mice, whose platelets contain less than 1 percent of the normal levels of serotonin, were also examined. By the criteria of tissue swelling, infiltration of iodinated leukocytes, or histology, mast cell–deficient or beige mice expressed delayed hypersensitivity reactions whose intensity generally equaled or exceeded that of reactions in littermate controls. In addition, reserpine blocked delayed hypersensitivity in* W/W^v *and beige mice, suggesting that effects on mast cell or platelet serotonin cannot explain this drug's action in delayed hypersensitivity.*

Delayed hypersensitivity (DH) is a complex expression of cellular immunity involving antigen-dependent changes in lymphocyte traffic, recruitment of leukocytes without immunologic specificity, and alterations in vascular permeability and blood flow (*1*). It has been proposed that products derived from tissue mast cells, in particular serotonin, are required for the emigration of antigen-specific T cells into sites of DH reactions in mice (*2, 3*). This idea is provocative because it points to unexpected parallels between DH and immediate hypersensitivity, a form of immunologic reactivity long known to involve mast cells (*4*). However, much of the evidence offered in support of an important role for mast cells in the leukocyte emigration associated with DH is indirect. For example, pharmacologic agents that deplete or antagonize serotonin have been reported to diminish DH (*2, 3*), but this may not necessarily reflect an effect on mast cells. Similarly, measurement of the tissue swelling associated with DH (*2, 3*) does not directly evaluate leukocyte emigration (*5*).

To examine more directly the specific role of mast cells in the leukocyte emigration associated with DH, we immunized mast cell–deficient mice and their littermate controls with the contact sensitizer picryl chloride and then measured the infiltration of leukocytes labeled with [^{125}I]5-iodo-2-deoxyuridine ([^{125}I]IDU) into cutaneous sites (ears) challenged with picryl chloride (*6*). We also determined the ratio of weights of challenged and control ears for each mouse as a measure of tissue swelling associated with DH (*6*). Reasoning that very high doses of antigens may interfere with the detection of a mast cell requirement in DH, we determined the response of sensitized or control C57BL/6J mice [which are semisyngeneic to W/W^v and Sl/Sl^d mast cell–deficient mice (*6*)] to different concentrations of picryl chloride (experiment 1 in Table 1). We chose 0.5 percent picryl chloride, which produced a suboptimal but readily detectable response in sensitized mice and little or no reaction in unsensitized mice, for tests of mast cell–deficient mice.

We first studied W/W^v mice, whose genetic defect results in a severe macrocytic anemia, an absence of cutaneous melanocytes, sterility, and a virtual lack of tissue mast cells (*7*). These mice expressed DH responses to picryl chloride that were statistically indistinguishable from those of heterozygous littermate controls (experiments 2 and 3 in Table 1). The DH reactions of W/W^v and heterozygous control mice were also histologically similar (Fig. 1), except for the virtual absence of mast cells in W/W^v skin (*7*). In an experiment comparing W/W^v mice with homozygous (+/+) litter-

mate controls, leukocyte infiltration into sites of DH reactions in mast cell–deficient mice exceeded that in controls, whether judged by infiltration of [^{125}I]IDU-labeled cells (experiment 4 in Table 1) or by histological appearance. Similar results were also obtained with *Sl/Sl^d* mice (experiment 5 in Table 1), whose mast cell deficiency is due to mutations on a chromosome different than that involved in *W/W^v* mice (*8*).

Blood platelets represent a potential alternative source of serotonin in murine DH and other inflammatory processes (*3*, *9*). To evaluate whether platelet-derived serotonin is required for DH, we tested beige (*bg/bg*) mice. Beige mice have giant cytoplasmic granules in many cell types, including mast cells, granulocytes, and melanocytes (*10*). In addition, beige mouse platelets contain less than 1 percent of the normal level of serotonin, probably because they lack cytoplasmic dense bodies (*11*). Infiltration of [^{125}I]IDU-labeled leukocytes into DH reaction sites in *bg/bg* mice was not statistically different from that in *bg/+* controls (experiments 6 and 7 in Table 1). DH reactions in *bg/bg* and *bg/+* mice were also histologically similar, except that beige mouse mast cells and granulocytes contained giant cytoplasmic granules.

We next evaluated reactions to a different contact sensitizer, oxazolone (*12*), and tested the sensitivity of these reactions to reserpine (Serpasil, Ciba; 5 mg/kg in a volume of 0.1 ml) injected intraperitoneally 18 hours before the antigen-

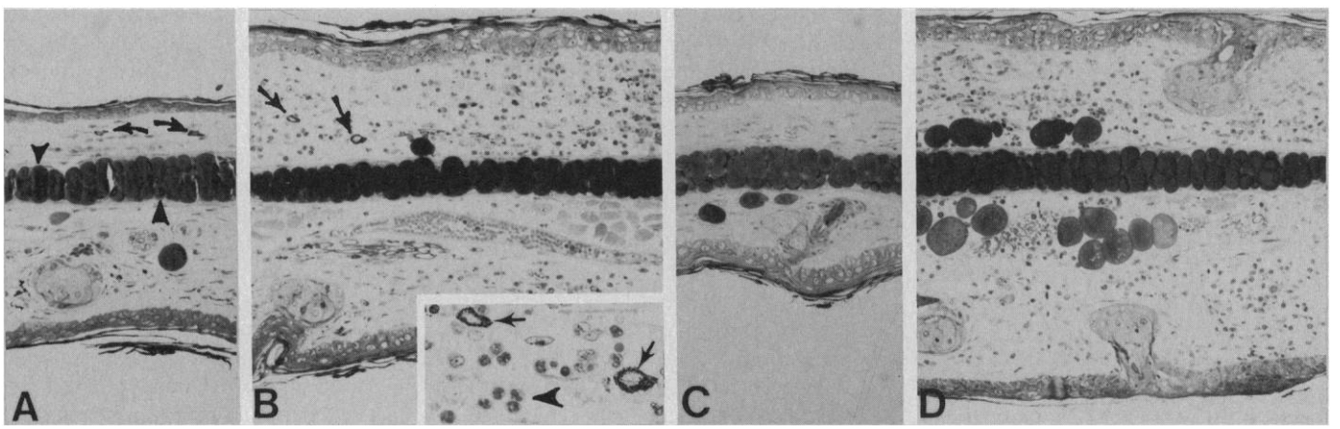

Fig. 1. Photomicrographs of sections of ears of *W^v/+* mice (A and B) or *W/W^v* mice (C and D) used in experiment 2 (Table 1). Ears from unsensitized mice (A and C) do not show any swelling or leukocytic infiltrates after challenge with 0.5 percent picryl chloride. In (A) arrowheads indicate cartilage and arrows mast cells. Ears from sensitized mice (B and D) challenged with 0.5 percent picryl chloride exhibit vascular dilatation, dermal swelling (note increased ear thickness), and leukocytic infiltration. Mast cells in *W^v/+* skin (arrows in B) are also shown in the inset (arrows), as are many leukocytes (arrowhead). All sections were taken from the midline of the ears (magnifications: A to D, ×128; inset, ×320).

Table 1. Expression of DH reactions to picryl chloride in *W/W^v*, *Sl/Sl^d*, and *bg/bg* mice and littermate controls. Leukocyte emigration is expressed as the ratio of ^{125}I radioactivity (counts per minute) in ears challenged with antigen (picryl chloride) and in the contralateral control ears, a measure of antigen-dependent infiltration of [^{125}I]IDU-labeled leukocytes (*6*). Tissue swelling is expressed as the ratio of weights of antigen-challenged ears and control ears (*6*). Mice immunized with picryl chloride (sensitized) and control mice (not sensitized) were tested. Values are means ± standard errors. N.D., not done.

Experiment	Mice	Picryl chloride challenge (%)	Radioactivity ratios				*P**	Weight ratios		*P**
			Sensitized	*n*	Not sensitized	*n*		Sensitized	Not sensitized	
1	C57BL/6J	1.5	6.78 ± 2.02	7	1.42 ± 0.14	6	<0.001	1.81 ± 0.17	1.07 ± 0.06	<0.001
	C57BL/6J	0.5	4.54 ± 0.64	6	1.09 ± 0.13	6	<0.001	1.33 ± 0.08	1.08 ± 0.06	0.047
	C57BL/6J	0.2	3.04 ± 0.57	7	N.D.			1.31 ± 0.16	N.D.	
	C57BL/6J	0.05	1.57 ± 0.18	6	N.D.			1.09 ± 0.04	N.D.	
2	*W/W^v*	0.5	2.42 ± 0.31	5	1.18 ± 0.13	3	0.012	1.37 ± 0.08	0.92 ± 0.18	0.024
	W/+, W^v/+	0.5	3.28 ± 0.28	5	0.99 ± 0.08	3	0.018	1.38 ± 0.05	0.87 ± 0.10	0.018
3	*W/W^v*	0.5	2.37 ± 0.60	5	N.D.			1.25 ± 0.13	N.D.	
	W/+, W^v/+	0.5	2.15 ± 0.34	5	N.D.			1.26 ± 0.16	N.D.	
4	*W/W^v*	0.5	2.68 ± 0.18†	10	N.D.			1.24 ± 0.08	N.D.	
	+/+	0.5	1.88 ± 0.21	4	N.D.			1.25 ± 0.16	N.D.	
5	*Sl/Sl^d*	0.5	2.80 ± 0.26‡	10	1.18 ± 0.17	9	<0.001	1.34 ± 0.07	1.00 ± 0.04	<0.001
	+/+	0.5	1.59 ± 0.15	8	0.89 ± 0.09	9	<0.001	1.25 ± 0.05	1.00 ± 0.05	<0.001
6	*bg/bg*	0.5	2.48 ± 0.30	4	1.08 ± 0.06	4	0.014	1.38 ± 0.16	1.08 ± 0.04	0.056
	bg/+	0.5	1.65 ± 0.21	5	0.79 ± 0.11	4	0.008	1.47 ± 0.15	1.00 ± 0.06	0.008
7	*bg/bg*	0.5	3.48 ± 0.57	9	1.27 ± 0.20	9	<0.001	1.27 ± 0.03	1.08 ± 0.03	<0.001
	bg/+	0.5	3.14 ± 0.35	9	1.47 ± 0.07	7	<0.001	1.21 ± 0.06	1.07 ± 0.02	>0.05

*Comparison between value for sensitized mice and corresponding value for unsensitized mice (Mann-Whitney *U* test, one-tailed). †Significantly different from corresponding value for +/+ mice ($P < 0.02$; Mann-Whitney *U* test, two-tailed). ‡Significantly different from corresponding value for +/+ mice ($P < 0.002$; Mann-Whitney *U* test, two-tailed).

ic challenge. Reserpine has been reported to deplete serotonin and catecholamine stores and to inhibit DH reactions in mice with normal numbers of mast cells (*2*). As shown in Table 2, W/W^v and *bg/bg* mice had DH responses to oxazolone that were similar in intensity to those in controls. At the higher dose of oxazolone (2.5 percent), DH-associated ear swelling in W/W^v mice was slightly reduced (experiment 1 in Table 2) and [^{125}I]IDU ratios were lower (experiments 1 and 4 in Table 2) than in +/+ mice. However, none of these differences were statistically significant. By contrast, at the lower dose of oxazolone (0.25 percent), W/W^v mice developed statistically significant DH-associated swelling whereas +/+ controls did not (experiment 2 in Table 2). Histological examination showed weak reactions in sensitized W/W^v and +/+ mice challenged with 0.25 percent oxazolone, but the ratios of radioactivity (counts per minute) were low and did not differ significantly from values in unsensitized animals. Reserpine blocked DH reactions to oxazolone in W/W^v, +/+, *bg/bg*, and *bg*/+ mice, whether determined by ear swelling, [^{125}I]IDU ratios (experiments 1 to 3 in Table 2), or histology. "Nonspecific" responses to oxazolone in unsensitized animals also appeared to be diminished by reserpine, particularly in *bg/bg* and *bg*/+ mice.

Previous efforts to determine whether mast cells are required for expression of DH produced conflicting results. Thomas and Schrader (*13*) recently reported that W^f/W^f mice developed DH responses to oxazolone or picryl chloride that were equivalent or slightly greater than those in +/+ littermates, as judged either by ear swelling or, in one experiment, by infiltration of [^{125}I]IDU-labeled leukocytes. But these experiments did not exclude a role for mast cells in DH. The W^f/W^f mice had ~10 percent as many skin mast cells as +/+ controls and retained significant mast cell function, as judged by their ability to express passive cutaneous anaphylaxis (PCA) responses (*13*). The investigators also performed one experiment with the more severely mast cell–deficient W/W^v mouse, which does not express detectable PCA (*3, 13*). They reported that W/W^v mice developed as much DH-related ear swelling as did +/+ controls and that the DH responses in these W/W^v and +/+ mice were histologically similar (*13*). Reed *et al.* (*14*) also did not find a defect in DH in W/W^v mice. By contrast, Askenase *et al.* (*3*) found that 18- or 24-hour DH reactions to picryl chloride in five W/W^v and five Sl/Sl^d mice were undetectable or less pronounced than those in +/+ controls. However, DH was evaluated solely by measuring ear swelling; leukocyte emigration into these reactions was not determined. In an experiment reproducing the conditions of picryl chloride sensitization and challenge used by Askenase *et al.*, we did not find any statistically significant differences in DH between W/W^v and +/+ mice on the basis of weight ratios or [^{125}I]IDU ratios (*15*).

We found that W/W^v and Sl/Sl^d mice, which virtually lack mast cells and cannot express PCA (*3, 13*), developed approximately as much swelling in association with DH as did littermate heterozygous or +/+ controls. More important, the infiltration of leukocytes usually equaled and sometimes exceeded that observed in control mice, whether judged by migration of [^{125}I]IDU-labeled leukocytes or by semiquantitative histological observations in coded sections. These findings, obtained in eight experiments with 153 mast cell–deficient mice, offer little support for the hypothesis that mast cell–derived serotonin, or any other

Table 2. Experiments 1 to 3: effect of reserpine on expression of DH reactions to oxazolone in W/W^v and *bg/bg* mice and littermate controls (*6, 12*). In each experiment reserpine treatment significantly blocked the swelling and the infiltration of [^{125}I]IDU-labeled cells provoked by oxazolone in sensitized mice ($P < 0.01$ to 0.001, one-tailed Mann-Whitney U test). Reserpine treatment also appeared to interfere with the development of "nonspecific" reactions to oxazolone in unsensitized mice, as judged by significant differences in radioactivity ratios in W/W^v mice in experiment 1 ($P = 0.037$) and significant differences in radioactivity and weight ratios in *bg/bg* and *bg*/+ mice in experiment 3 ($P < 0.05$ to 0.001). Experiment 4: kinetics of DH response to oxazolone in W/W^v mice and +/+ littermate controls. Mice were sensitized and challenged as described in (*12*), but were killed for determination of DH-associated leukocyte emigration (radioactivity ratios) and swelling (weight ratios) 18, 48, or 72 hours later. Mice received [^{125}I]IDU 17.5 hours (18-hour group) or 24 hours (48- and 72-hour groups) before being killed. At the three intervals tested, reactions in W/W^v and +/+ mice were not statistically different, whether judged by radioactivity or weight ratios. In W/W^v and +/+ mice, DH reactions at 72 hours had higher radioactivity ratios but less swelling than reactions at 18 hours ($P < 0.042$ to 0.001, two-tailed Mann-Whitney U test). Values are means ± standard errors. N.D., not done; N.S., not significant.

Experiment	Mice	Oxazolone challenge (%)	Hours after challenge	Reserpine	Radioactivity ratios					Weight ratios		
					Sensitized	*n*	Not sensitized	*n*	*P**	Sensitized	Not sensitized	*P**
1	W/W^v	2.5	24	No	2.58 ± 0.83	8	1.05 ± 0.11	7	<0.001	1.57 ± 0.08	1.04 ± 0.02	<0.001
	W/W^v	2.5	24	Yes	1.05 ± 0.10	10	0.76 ± 0.08	5	N.S.	1.01 ± 0.01	0.98 ± 0.03	N.S.
	+/+	2.5	24	No	2.76 ± 0.36	10	1.16 ± 0.09	7	<0.001	1.78 ± 0.11	1.04 ± 0.02	<0.001
	+/+	2.5	24	Yes	1.00 ± 0.08	10	0.88 ± 0.14	5	N.S.	0.99 ± 0.02	1.00 ± 0.02	N.S.
2	W/W^v	0.25	24	No	1.53 ± 0.20	8	1.10 ± 0.09	8	<0.1	1.27 ± 0.04	0.96 ± 0.02	<0.001
	W/W^v	0.25	24	Yes	0.90 ± 0.19	5	1.00 ± 0.14	10	N.S.	1.06 ± 0.01	1.02 ± 0.03	N.S.
	+/+	0.25	24	No	1.57 ± 0.18	10	1.16 ± 0.18	8	<0.1	1.13 ± 0.03	1.06 ± 0.04	~0.1
	+/+	0.25	24	Yes	0.81 ± 0.08	10	1.00 ± 0.10	8	N.S.	1.03 ± 0.01	1.00 ± 0.03	N.S.
3	*bg/bg*	2.5	24	No	5.23 ± 0.99	10	1.67 ± 0.26	10	<0.001	2.05 ± 0.12	1.12 ± 0.02	<0.001
	bg/bg	2.5	24	Yes	1.16 ± 0.14	9	1.05 ± 0.15	10	N.S.	0.99 ± 0.02	0.99 ± 0.02	N.S.
	bg/+	2.5	24	No	5.05 ± 0.89	10	1.84 ± 0.28	10	<0.001	1.99 ± 0.07	1.14 ± 0.02	<0.001
	bg/+	2.5	24	Yes	0.77 ± 0.12	9	1.21 ± 0.18	10	N.S.	0.99 ± 0.02	1.01 ± 0.02	N.S.
4	W/W^v	2.5	18	No	2.32 ± 0.25	6	N.D.			1.80 ± 0.06	N.D.	
	+/+	2.5	18	No	2.62 ± 0.30	7	N.D.			1.84 ± 0.13	N.D.	
	W/W^v	2.5	48	No	5.15 ± 1.21	7	N.D.			1.67 ± 0.10	N.D.	
	+/+	2.5	48	No	8.22 ± 0.73	6	N.D.			1.46 ± 0.04	N.D.	
	W/W^v	2.5	72	No	6.26 ± 1.99	6	N.D.			1.34 ± 0.05	N.D.	
	+/+	2.5	72	No	6.68 ± 0.85	7	N.D.			1.38 ± 0.04	N.D.	

*Comparison between value for sensitized mice and corresponding value for unsensitized mice (Mann-Whitney U test, one-tailed).

mast cell–derived products, are required for the leukocyte emigration or the swelling associated with DH. Furthermore, our results with *bg*/*bg* mice show that DH can be expressed despite a virtual absence of platelet serotonin.

Delayed hypersensitivity reactions in mast cell–deficient or beige mice, like those in appropriate controls, were blocked by reserpine. Reserpine or the serotonin antagonist methysergide has also blocked the swelling associated with DH to sheep red blood cells in W/W^v mice (*3*). Gershon *et al.* (*2*) presented evidence that reserpine's effect on DH required intact monoamine oxidase activity and did not depend on its ability to deplete catecholamines. They concluded that reserpine's effect on DH probably reflected its ability to deplete mast cell serotonin (*2*). If it is true that drugs such as reserpine or methysergide block DH (or immunologically nonspecific reactions) because of their effects on serotonin, then perhaps either mast cell or platelet-derived serotonin is sufficient for these responses. Alternatively, sufficient serotonin to support DH may be provided by a third source. Most of the serotonin in normal mouse skin appears to be associated with mast cells (*2*), suggesting that such a third source may represent a leukocyte recruited to the reaction site, for example basophils (*16*) or even certain T-cell populations (*17*). Occasional leukocytes with the light microscopic appearance of basophils were observed in some of the DH reactions we examined. Whether these cells constitute a significant source of serotonin remains to be determined. Finally, we cannot exclude the possibility that reserpine suppresses DH by mechanisms independent of serotonin, as by actions on effector cells or on mediators other than serotonin.

Mast cell degranulation has been observed at sites of DH in humans and mice (*18*). Several agents, including immunoglobulin E and T-cell products, may contribute to mast cell activation in DH, but the net results of mast cell mediator release in DH are not fully understood (*19*). Indeed, consideration of the known effects of individual mast cell mediators suggests that mast cell activation may augment or suppress various aspects of the DH response (*19, 20*). In our study, statistically significant differences between measures of DH in mast cell–deficient and control mice were observed in three of eight experiments; in all three the reactions were greater in the absence of mast cells.

Mast cell–deficient mice express multiple abnormalities (*7, 8*), which may include a defect in the ability to serve as recipients for systemic passive transfer of DH reactivity (*3*). We have not performed passive transfer experiments. Nor can we exclude the possibility that mast cells are essential for DH in normal mice, but that some genetically determined abnormality of W/W^v and Sl/Sl^d mice permits these two mutants to express DH after active sensitization despite their marked deficiency of recognizable mast cells. Nevertheless, we feel that the simplest and most likely explanation of our results is that, whatever their precise function in DH, mast cells are not essential for the induction and elicitation of the response.

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6. Mast cell–deficient mice and their littermates [(WB-W/+ × C57BL/6J–*W*-W^v/+, F_1 = W/W^v, $W/+$, $W^v/+$, +/+) and (WC/*Re*-*Sl*/+ × C57BL/6J-Sl^d/+, F_1 = Sl/Sl^d, +/+)], beige mice (*bg*/*bg* = C57BL/6J-bg^J/bg^J) and their littermates (C57BL/6J-bg^J/+), and C57BL/6J mice (all from Jackson Laboratory) 10 to 20 weeks old were immunized by applying 0.1 ml of 5 percent picryl chloride in ethanol to the shaved abdomen and thorax. Control mice were shaved but not sensitized. Sensitized and control mice were challenged 4 days later by applying 10 μl of 0.5 percent picryl chloride in methanol to the underside of the left ear. The right (control) ear received 10 μl of methanol alone. About 30 minutes later, all mice received 2 to 4 μCi of [^{125}I]IDU (2200 μCi/mmol; NEX-072, New England Nuclear) intraperitoneally in 0.1 ml of sterile 0.9 percent NaCl. Twenty-four hours later, the mice were killed by cervical dislocation and their ears were cut off at the hairline and weighed in a Mettler AC88 balance. To obtain material for morphologic analysis, weighed tissues were fixed overnight [H. F. Dvorak *et al.*, *J. Exp. Med.* **150**, 322 (1979)] and then washed several times in 0.1*M* cacodylate buffer to remove fixative and to deplete the tissues of unbound radioactivity (*5*). Tissue-associated ^{125}I was determined in a Tracor Analytic model 1185 gamma counter (5 minutes per specimen). Sections of full-thickness strips of tissue extending from the edge to the base of the ears at their midline were then embedded in Epon and stained with Giemsa. For each mouse the ratio of weights of challenged and control ears was calculated along with the ratio of radioactivity in challenged and control ears [(counts per minute in challenged ear minus background) divided by (counts per minute in control ear minus background); the former as a measure of swelling, and the latter as an objective, reproducible measure of leukocyte infiltration (*5*). Our results have been reported in abstract form [S. J. Galli and I. Hammel, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **43**, 1973 (1984)].
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12. Methods used were identical to those described in (*6*), except that mice were sensitized with oxazolone (4-ethyoxymethylene-2-phenyl oxazolone, BDH Chemicals) (3 percent in ethanol). On day 6, sensitized and control mice received 10 μl of 0.25 or 2.5 percent oxazolone in ethanol on the underside of the left ear. The right (control) ear received 10 μl of ethanol alone.
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15. Thirty-week-old W/W^v and +/+ mice (five to nine per group) were tested for DH as described in (*6*), except sensitization was achieved with 0.15 ml of 5 percent picryl chloride in ethanol and acetone (3 to 1 by volume) and challenge (4 days later) was made with 0.8 percent picryl chloride in ethanol, acetone, and olive oil (3 to 1 to 13), given in one drop to each side of the left ear with a 27-gauge needle. The right (control) ear received the vehicle only. Ratios of [^{125}I]IDU at 22 hours were 2.90 ± 0.64 and 0.83 ± 0.15 for sensitized and unsensitized W/W^v mice, respectively (P = 0.004); and 2.14 ± 0.24 and 0.97 ± 0.17 for sensitized and unsensitized +/+ mice ($P < 0.001$). Weight ratios were 1.26 ± 0.07 and 1.03 ± 0.02 for sensitized and unsensitized W/W^v mice (P = 0.004) and 1.11 ± 0.05 and 0.95 ± 0.01 for sensitized and unsensitized +/+ mice ($P < 0.001$).
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