cell in culture or its attachment (or lack thereof) to a solid substrate influences the response of the cell to growth factors and serum (*13*). Rounded, unattached cells in soft agar appear to respond to different growth factors than those attached to plastic or glass (*5*). The possibility cannot be excluded that the postreceptor pathways activated by GI or TGFβ lead to a stimulatory response when the cells are rounded and unattached and to an inhibitory response when the cells are attached and flattened. Another possible explanation for the differences in response is cell-type differences. The growth of certain cells is significantly inhibited by the GI whereas the growth of others is inhibited minimally or not at all (*2*, *3*). Type β TGF stimulates certain cells to grow in soft agar but not others (*14*). BSC-1 cells are epithelial whereas the AKR-2B cells are mesenchymal, and for a single growth-modulating polypeptide to exhibit growth stimulation of mesenchymal cells while inhibiting epithelial cells could have some physiological significance.

Although the GI and TGFβ bind to the same cell surface receptor, it is not known whether the cell surface receptors for GI or TGFβ that lead to growth stimulation are the same receptors that lead to growth inhibition. Different receptors may be involved in the different actions. Alternatively, if the same receptors are involved, a primary action of GI or TGFβ, such as an effect on Na^+ fluxes (*15*), may be growth inhibitory in one setting and growth stimulatory in another.

RONALD F. TUCKER
GARY D. SHIPLEY
HAROLD L. MOSES*
Mayo Foundation and Medical School, Rochester, Minnesota 55905
ROBERT W. HOLLEY
Salk Institute, San Diego, California 92138

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16. Supported by National Cancer Institute grants CA 16816 and CA 27217 (H.L.M.), CA 09441 (H.L.M. and G.D.S.), and CA 11176 (R.W.H.) and by American Cancer Society grant CD 59J (R.W.H.). R.W.H. is an American Cancer Society Professor of Molecular Biology.
* To whom correspondence should be addressed at the Department of Cell Biology, Guggenheim-5, Mayo Clinic/Foundation, 200 First Street S.W., Rochester, Minnesota 55905.

5 June 1984; accepted 9 August 1984

Transcription and Promoter Usage of the *myc* Gene in Normal Somatic and Spermatogenic Cells

Abstract. *In somatic cells the level of* myc *transcription is not restricted to particular cell types but correlates closely with the rate of cell division. Such transcription involves the use of two active* myc *promoters and produces two messenger RNA species that are differentially represented among the transcripts of different tissues. In apparent contrast to somatic cells, mitotically and meiotically dividing germ cells have very few* myc *transcripts and appear to proliferate, at least for a few divisions, in the absence of* myc *transcription. These results raise interesting questions regarding the role of the* myc *gene product in terminally differentiating cells, particularly of the germ line series.*

The *myc* gene is transcriptionally active in cells of the hematopoietic lineage, yet its expression is low in liver and fibroblasts (*1*). The apparently limited number of cell types expressing *myc* transcripts at high levels has led to the suggestion that the *myc* gene may be preferentially expressed in lymphocytes (*2*) or else may play a role in cellular differentiation (*3*). The *myc* gene has been shown to be transcriptionally activated in both fibroblasts and lymphocytes by various competence-inducing and mitogenic agents, suggesting that the gene product may function during the transition through the G_1 phase of the cell cycle (*4*); this observation, in contrast to the hypothesis relating *myc* function to differentiation, suggests a more fundamental role for the gene product. In consideration of the concept of *myc* being a participant in cell division, we have examined its transcriptional activity in normal tissues by means of a sensitive S1 nuclease protection assay.

The probe used in this assay extends from near the 3′ end of the first exon of the *myc* gene to beyond the two promoter-like sequences that have been identified at the 5′ end of the first exon (*5*) (Fig. 1). This probe allows assessment of whether both promoters are used as well as determination of the total amount of *myc* transcripts. The results (Fig. 1) indicate that *myc* transcripts can be detected in all the tissues and organs examined, with high levels occurring in the ovaries, uterus, preputial gland, and thymus. The other tissues had fewer transcripts, and in some tissues, such as skeletal muscle, transcripts were barely detectable. Although it appeared as though both promoters were active, there were tissue-specific differences in the relative abundance of the transcripts. In tissues such as spleen and thymus the ratio of longer to shorter transcripts was approximately 1:4, whereas in the preputial gland the ratio was closer to 1:20. It has been suggested that the two different transcripts may behave differently in their processing, stability, or efficiency of translation (*6*, *7*).

There appeared to be a general correlation between the number of *myc* transcripts and the overall rate of cellular proliferation within each tissue. For example, in the thymus and ovaries the relatively high degree of cell division coincided with substantial amounts of *myc* RNA. In contrast, skeletal muscle, which has few dividing cells, had barely detectable *myc* transcripts. Because it is extremely difficult to obtain accurate estimates for the total rate of cell division within a whole tissue or organ, we were not able to rule out the possibility that factors other than cellular proliferation contributed to the degree of *myc* expression. One apparent exception to this correlation is adult testes, which have substantial numbers of actively proliferating cells and low amounts of *myc* messenger RNA (mRNA). This apparent discrepancy has been examined further by measurements of changes in the amount of *myc* RNA during sexual maturation of the mouse testis (*8*). After a brief collagenase treatment, the developing testes were separated into two fractions: the

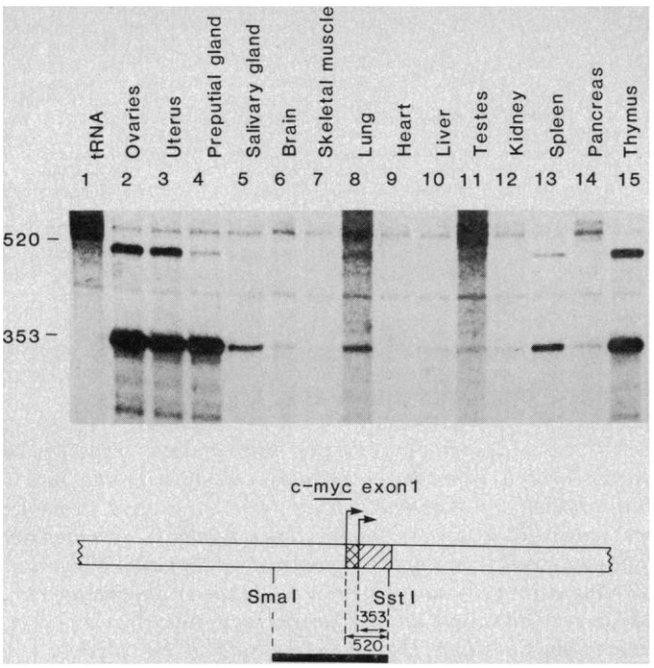

Fig. 1. Transcriptional activity and promoter usage in normal mouse tissues. The tissues listed were dissected from 8-week-old, out-bred CD-1 mice (Charles River Laboratories), rinsed in Hanks balanced salt solution at 4°C, and RNA purified as described (*16*). A Sma I–Sst I mouse *myc* M13 subclone (*17*) that extends from the 3′ end of the first *myc* exon to a Sma I site 422 bases 5′ of the first transcriptional start site was used to assay 10 μg of total RNA from each tissue in a modified S1 nuclease protection assay (*18*). Each hybridization reaction contained an excess of isotopically labeled probe (10^5 count/min; specific activity, 10^8 count/min per microgram), 10 μg of total RNA, 75 percent formamide, 400 m*M* NaCl, 20 m*M* tris-HCl (*p*H 7.5), 1 m*M* EDTA, and 0.1 percent sodium dodecyl sulfate. After incubation overnight at 58°C the probe that had not hybridized was digested with 1500 U of S1 nuclease (Boehringer Mannheim) at 37°C for 1 hour. The undigested material was precipitated with ethanol and subjected to electrophoresis on an 8*M* urea–5 percent acrylamide sequencing gel, and the protected fragments were visualized by autoradiography with the use of Kodak X-AR film and an intensifying screen for 3 days. Sizes were estimated from an end-labeled Eco RI–Hinf I pBR322 digestion that appeared on the same gel. The protected fragment 353 bases long represents transcripts initiated at the *myc* gene proximal start site, and the protected fragment 520 bases long represents transcripts initiated at the distal start site. The band at approximately 400 bases (seen faintly here and more strongly in Figs. 2 and 3) probably results from the probe reannealing to itself. Abbreviation: tRNA, transfer RNA.

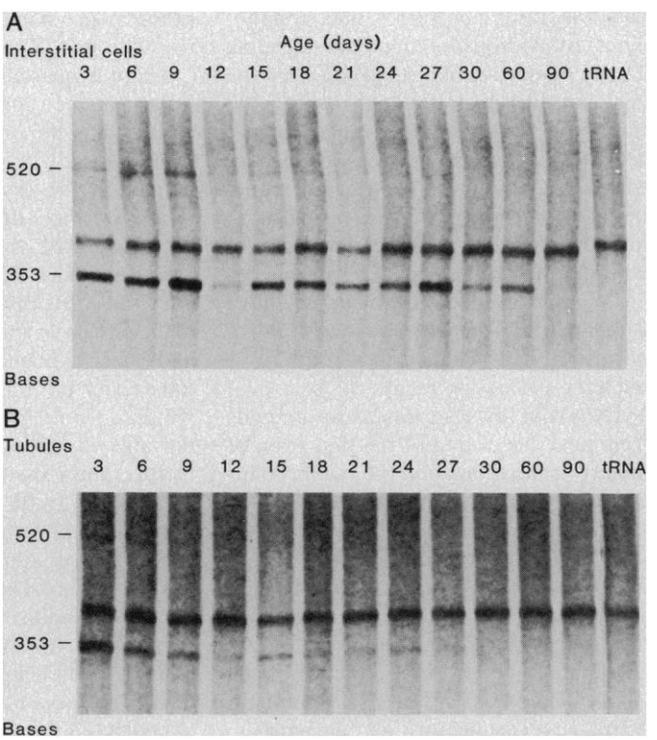

Fig. 2. Changes in *myc* expression in interstitial cells and in seminiferous tubules during testicular development. Testes from CD-1 mice at the indicated ages were removed and decapsulated, and the interstitial cells and seminiferous tubules were separated as described (*9*). RNA was purified from each of these preparations, and the amounts of *myc* transcripts were determined in an S1 nuclease protection assay as described in the legend to Fig. 1. In both preparations the amount of *myc* RNA (as revealed by the protected fragments at 353 and 520 bases) decreased with increasing age, with the rate of decrease being more rapid in the seminiferous tubules. The dark band that also is seen in the tRNA lane probably results from self-annealing of the probe. The dried gel was exposed to Kodak X-AR film with an intensifying screen for 5 days.

interstitial tissue, composed primarily of Leydig cells, peritubular myoid cells, and the vascular endothelial cells; and the seminiferous cords or tubules, comprised of Sertoli cells and the differentiating germ cells (*9*).

During the first several days of postnatal development the interstitial cells (principally Leydig cells) actively divide. After a few days of diminished cellular proliferation, further divisions of the Leydig cells and the vascularizing endothelial cells continue to late puberty (60 days), at which time cell division drops again to a low rate (*8*). This pattern of cell division correlated closely with the level of *myc* transcription (Fig. 2A).

The amounts of *myc* RNA in the isolated seminiferous tubules also exhibited age-dependent changes, being highest at day 3 and then gradually diminishing until day 30 when the *myc* transcript was no longer detectable. This result correlated with the developmental changes in the proliferation of Sertoli cells, which divide actively only during late gestation and early postnatal life (*8*). The low amounts of *myc* mRNA present after day 12, when Sertoli cell division ceases (*8*), was probably due to a limited number of peritubular cells adhering to the seminiferous epithelium. This period also corresponds to the maximum expression of the seminiferous growth factor, a mitogen known to induce proliferation of Sertoli cells in vitro (*10*). Significantly, the subsequent decline in *myc* transcripts did not correlate with the high rate of germ cell division that occurs during this period. A comparison was made between the number of *myc* transcripts in seminiferous tubules taken from 18- and 24-day-old postnatal mice and that in the normal spleen (Fig. 3A). In the day 24 seminiferous tubules, the Sertoli cells had ceased dividing and the proliferating type A and type B spermatogonia comprised 10 percent of the total cells (*11*). However, even though the latter divide every 27 to 30 hours (*12*), there were no detectable *myc* transcripts. In the spleen, the percentage of proliferating cells was difficult to estimate because of uncertainties such as the possibility of antigenic stimulation. While the proportion of proliferating cells was probably not in excess of 10 percent, the degree of *myc* activity was at least 20 times that detected in the day 24 seminiferous tubules. This result suggests that mitotically dividing germ cells have an extremely low amount of *myc* mRNA.

A comparison of the levels of *myc* expression was made between discrete

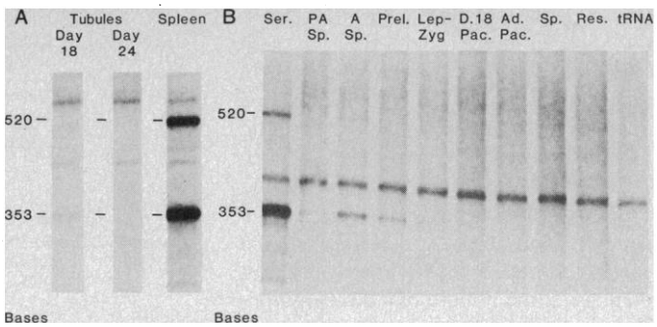

Fig. 3. A comparison between the degrees of *myc* expression in day 18 and day 24 seminiferous tubules and in the spleen (A) and an analysis of *myc* expression in partially purified testicular cell populations (B). (A) By means of the same procedure as that described (*9*) but in a different experiment, seminiferous tubules were prepared from day 18 and day 24 CD-1 mice; RNA was extracted from these two preparations as well as from the pooled spleens of five CD-1 mice, and 10 μg of RNA was analyzed as described in the legend to Fig. 1. (B) The following purified cell populations were prepared. Sertoli cells (S) and primitive type A spermatogonia (PA sp), both from 6-day-old mice; type A spermatogonia (A sp) from 8-day-old mice; preleptotene spermatocytes (Prel); leptotene-zygotene spermatocytes (Lep-Zyg) and pachytene spermatocytes (D-18 Pac), both from 18-day-old animals; and pachytene spermatocytes (Ad. Pac.), spermatids (sp), and residual bodies (Res.) from adult mice. RNA was extracted from each of these fractions, and the *myc* transcripts were assayed in an S1 nuclease protection experiment as described in the legend to Fig. 1. The lanes in (A) and (B) were exposed for 3 and 5 days, respectively, to Kodak X-AR film with an intensifying screen.

populations of Sertoli cells, proliferating spermatogonia, differentiating spermatocytes, and spermatids (Fig. 4). The rapid proliferation of day 6 Sertoli cells (*8*) was reflected by a large number of *myc* transcripts. Both the primitive type A spermatogonia and the type A spermatogonia also underwent rapid mitotic divisions (cell-cycle periods of 27 to 30 hours) (*12*), yet the amount of *myc* mRNA in these populations was 3 percent and 7 percent, respectively, of that seen in Sertoli cells at the same developmental age. The preleptotene and leptotene-zygotene spermatocyte pouplations also had low but still detectable amounts of *myc* RNA. No transcripts were detected in the pachytene spermatocytes, round spermatids, or residual bodies.

The low level of *myc* expression in seminiferous tubules of mice beyond the prepuberal age (Fig. 3A) indicated the possibility that the mitotically dividing germ cells have unusually low numbers of *myc* transcripts. Because the assay detected stable *myc* mRNA, a possible interpretation of this phenomenon is that the results were caused by preferential instability of *myc* RNA in germ cells. An alternative possibility is that *myc* expression in germ cells does differ from somatic cells, leaving the question as to whether *myc* is expressed at all during the mitotic and meiotic divisions of germ cells. For *myc* expression to be completely absent in these cells, it becomes necessary to explain the low level of *myc* transcripts in the purified spermatogonia and preleptotene spermatocytes.

From Nomarski differential interference microscope examination, the contamination of Sertoli cells in the primitive type A spermatogonia and the type A spermatogonia populations was 2 percent and 8 percent, respectively (*13*). This degree of somatic cell contamination would be sufficient to account for the *myc* transcripts in the two spermatogonial populations. Comparable analyses of the spermatocyte population taken from day 18 animals also suggested that the small amounts of *myc* mRNA may have been due to contaminating somatic cells. From the amount of *myc* mRNA present in the day 18 seminiferous tubules (Fig. 2C), it is probable that the differentiated Sertoli cells contaminating the preleptotene and leptotene-zygotene spermatocyte populations contained very few *myc* transcripts. However, the known low degree of peritubular cell contamination [~2 percent (*13*)] would be sufficient to account for the amount of *myc* mRNA in the populations of early spermatocytes.

These results imply that the proliferating spermatogonia express *myc* at amounts significantly below those for normally dividing somatic cells. Furthermore, the apparent absence of *myc* RNA

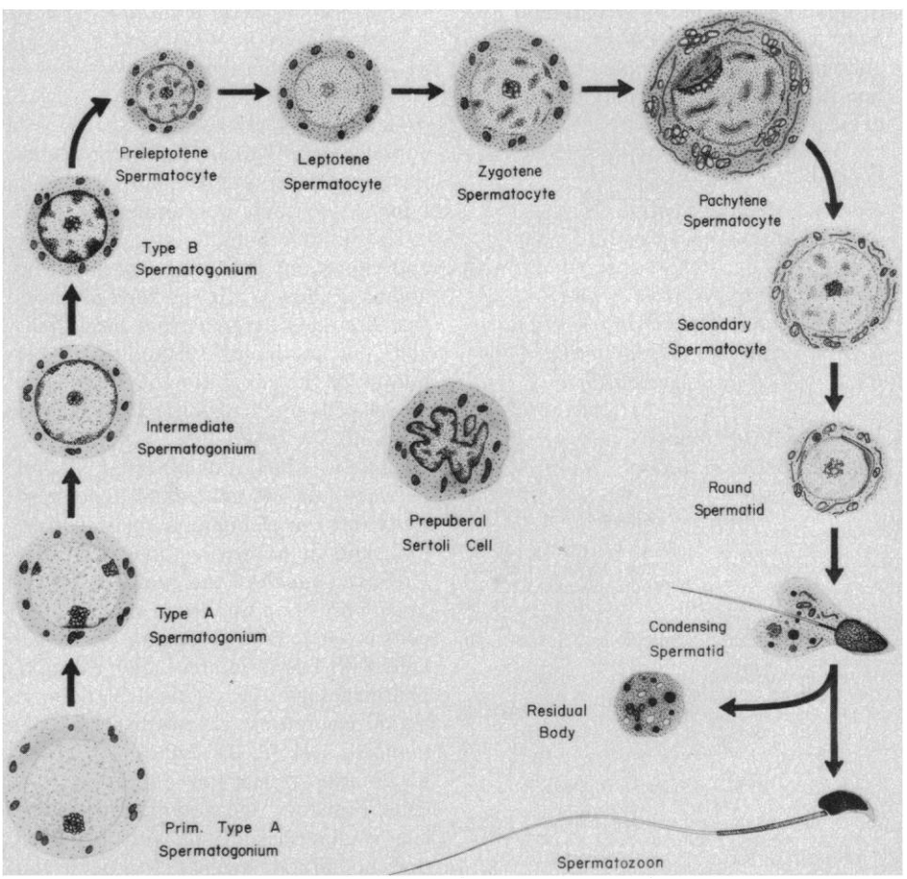

Fig. 4. Diagram of spermatogenesis in the developing mouse testis depicting the successive stages of germ cell proliferation and differentiation. Mitotically quiescent primitive type A spermatogonia, when induced to proliferate 3 to 4 days after birth, initiate the sequence of cell divisions that yield renewing stem cells (not shown) and the differentiating spermatogonia, including types A_{1-4}, intermediate, and type B (vertical). The type B spermatogonia divide to form preleptotene spermatocytes that undergo DNA synthesis and then, without dividing, enter meiotic prophase (horizontal). Advanced primary spermatocytes enter the first meiotic division to yield secondary spermatocytes and at the second and final division yield the spermatids. These haploid cells differentiate to form the mature spermatozoa. [Courtesy of *J. Cell Biol.*]

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.

TIMOTHY A. STEWART
Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115
ANTHONY R. BELLVÉ
Department of Physiology and Biophysics and *Laboratory of Human Reproduction and Reproductive Biology, Harvard Medical School*
PHILIP LEDER
Department of Genetics, Harvard Medical School

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18. We thank T. Broderick, B. Burnham, and P. Mayhew for technical assistance. This work was supported by E. I. Du Pont de Nemours and Co., American Business for Cancer Research Foundation, EMTECH Corporation, and NIH grants HD08270 and HD06645.

26 April 1984; accepted 12 July 1984

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-