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Erythroid Homeostasis in Normal and Genetically Anemic Mice: Reaction to Induced Polycythemia

Abstract. *After hypertransfusion, normal* ww *and anemic* WW^v *mice show the same increase in red-cell volume, decrease in reticulocytes, and temporary cessation of blood formation. The time required for the red cell volume to return to the value observed before treatment is the same for both groups of mice. The cell volume at which new erythrocytes are again released into the circulation depends upon the genotype of functioning blood-forming tissue. Injections of erythropoietin stimulate red cell formation in polycythemic* ww *mice, but have much less (if any) effect upon polycythemic* WW^v *mice.*

Erythroid homeostasis of *WW^v* mice, afflicted with a genetically induced macrocytic anemia, differs from that of normal mice. Under normal atmospheric conditions, 1 mm^3 of blood from an untreated adult *WW^v* mouse contains 6 or 7 million erythrocytes, that from a normal mouse 11 or 12 million cells (*1*). These red blood cells form a smaller proportion of the total blood volume in *WW^v* mice (38 to 40 percent) than in *ww* mice (46 to 49 percent). The differing ratios of cell volume to total blood volume (hereafter called hematocrit levels from their method of determination) depend upon the genotype of functioning blood-forming tissue, since *WW^v* mice implanted with isologous *ww* blood-forming tissue acquire and maintain a hematocrit percentage of 46 to 49 percent (*2*). The anemic mice respond normally to oxygen deprivation, but injections of purified erythropoietin have little or no influence upon their blood picture (*3*). In studies of the mechanism of erythroid homeostasis, polycythemic normal mice have frequently been used, since they cease forming new erythrocytes until their hematocrit levels have dropped markedly, or until they have been stimulated by the hormone erythropoietin. They provide both a sensitive tool for assay of erythropoietin activity (*4*) and a favorable site for study of the kinetics of differentiation and multiplication of red cell precursors (*5*).

We have used *ww* and *WW^v* mice, highly congenic with the WB × C57BL/6 F_1 hybrid genetic background (*6*), in a series of experiments to gain further evidence concerning the basis for observed differences in erythroid homeostasis. In each experiment, normal (*ww*) and anemic (*WW^v*) adult littermate pairs were treated identically; they were plethorized to a hematocrit level of at least 70 percent and observed after the same intervals to determine the duration of polycythemia and the absence of new blood formation.

Approximately 2 percent of the red cells of untreated *ww* mice, and 3 to 4 percent of those of untreated *WW^v* mice, are young incompletely hemoglobinized erythrocytes with a recognizable reticulum; following plethorization, these reticulocytes disappear from the circulation. Table 1 shows results of four experiments, each involving a specified difference in conditions. All mice, normal and anemic, were injected on each of 4 consecutive days with 0.5 ml of a red cell suspension (70 to 90 percent vol/vol in physiological saline). Determinations of the ratio of cell volume to total blood volume were made by the microhematocrit method (*7*). Each reticulocyte count was based on at least 500 cells, stained by the method of Brecher (*7*). In experiments 1, 3, and 4, the donors were retired C57BL/6J breeders and in experiment 2, they were 4- to 7-month old WB-B6F_1 hybrid *WW^v* mice. The *ww*-implanted *WW^v* mice in experiment 3 all had typical *ww* blood pictures before and after the period of polycythemia. Erythropoietin (*8*) was injected intraperitoneally on days 1 to 8 after completion of plethorization.

In each of these experiments, *ww* and *WW^v* mice responded similarly to plethorization, although the extent of cell-volume increase, resulting from equal volumes of added red cells, tended to be greater in nonimplanted *WW^v* recipients (mean increase, 36 percent) than in *ww* recipients (mean increase, 30 percent). In experiments 1 to 3, there was almost complete suppression of erythropoiesis (absence of reticulocytes) throughout the period of polycythemia (17 to 22 days). For a brief period after the cell volume returned to normal level, elevated reticulocyte percentages (8 to 10 percent, sometimes higher in *WW^v* mice) were always observed.

Experiment 2 demonstrates that plethora induced by addition of macrocytic *WW^v* erythrocytes persists at least as long as that induced by addition of an equal volume of normocytic *ww* erythrocytes, indicating that *WW^v* erythrocytes have a normal lifespan.

In experiment 3, *ww* and *WW^v* mice implanted with *ww* blood-forming tissue gave identical responses to plethorization, including the reappearance of reticulocytes when the hematocrit level

Table 1. Responses of normal *ww* and anemic *WW^v* mice to plethorization with or without other treatment. Ht., hematocrit; Ret., reticulocyte; Geno. (No.), genotype and number of plethorized mice. Results in mean ± S.E.

Geno. (No.)	Before transfusion		Peak (day 0)		Interm. (day 8)		Polyc. days
	Ht. (%)	Ret. (%)	Ht. (%)	Ret. (%)	Ht. (%)	Ret. (%)	
Expt. 1. Plethorization only with C57BL/6J ww *RBC*							
ww (10)	46 ± 0.3	2 ± 0.3	76 ± 1.0	0.2 ± 0.1	63 ± 1.6	0.2 ± 0.1	18
WW^v (10)	36 ± 0.9	4 ± 0.3	73 ± 1.1	0.6 ± 0.1	59 ± 1.3	0.2 ± 0.1	19
Expt. 2. Plethorization only with isologous WW^v *macrocytic RBC*							
ww (3)	49 ± 1.0	3.5 ± 0.3	76 ± 1.2	0.2 ± 0.1	69 ± 1.0	0.2 ± 0.1	21
WW^v (3)	39 ± 1.0	4.5 ± 0.5	78 ± 1.2	0.2 ± 0.2	66 ± 2.0	0.2 ± 0.1	22
Expt. 3. Established implant ww *cells, plethorization with* ww *RBC*							
ww (4)	48 ± 1.0	3 ± 1.0	72 ± 1.5	0.4 ± 0.2	61 ± 1.0	0.2 ± 0.1	17
WW^v (4)	48 ± 1.1	3 ± 0.9	74 ± 0.8	0.2 ± 0.1	64 ± 0.7	0.2 ± 0.2	17
Expt. 4. Plethorization with ww *RBC, plus injection of erythropoietin*							
ww (5)	47 ± 0.6	2.7 ± 0.7	80 ± 1.5	0.8 ± 0.4	73 ± 1.4	3.2 ± 0.5	24
WW^v (5)	41 ± 1.2	4.0 ± 0.6	77 ± 0.6	1.1 ± 0.8	65 ± 0.6	0.6 ± 0.1	23

had dropped to 48 percent. Thus the genotype of blood-forming tissue alone determines the ww/WW^v difference in the ratio of red cell volume to total blood volume.

In experiment 4, the presence of 3 percent reticulocytes in blood of *ww* mice just after completion of eight daily injections of erythropoietin indicates a typical erythropoietic response under plethorization (*4*). The low percentage of reticulocytes in erythropoietin-treated plethorized WW^v mice demonstrates a defect in response to erythropoietin. However, the slight elevation of reticulocyte number between day 4 (0.1 percent, not shown in table) and day 8 (0.6 percent) is sufficiently suggestive of a minimal incomplete erythropoietic response to warrant further testing. The similar reaction of normal *ww* and anemic WW^v mice to plethorization, combined with their differing reaction to erythropoietin injection, suggests that further studies combining these treatments may yield valuable information on the cellular and biochemical basis of the delay in erythroid maturation characteristic of WW^v macrocytic anemia (*9*).

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8. Purified erythropoietin kindly supplied by Drs. Keighley and Lowy of the California Institute of Technology.
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Juvenile Hormone Activity: Effects of Isoprenoid and Straight-Chain Alcohols on Insects

Abstract. *A wide variety of alcohols, applied topically or injected into pupae of the yellow mealworm,* Tenebrio molitor *L., exhibit juvenile hormone activity. Included among these compounds are saturated and unsaturated alcohols with 8 to 15 carbon atoms, in straight- or branched-chains. Ether derivatives of several of these alcohols further enhance the activity.*

The juvenile hormone of insects was first demonstrated to be present in a lipid extract of the abdomens of the adult male cecropia moth, *Hyalophora cecropia* (L.) (*1*). Since this initial work, a number of biological extracts prepared from sources other than insects have been shown to possess juvenile hormone activity (*2*). Recently, several isoprenoid alcohols and their derivatives were demonstrated to possess similar activity (*3–5*). However, others (*6*) have shown that the activity of these alcohols is considerably less than that observed for semipurified extracts of *H. cecropia,* except for the methyl ether of farnesol which has been shown recently to possess several hundred times the activity of the free alcohol (*5*). We therefore attempted to determine whether these isoprenoid alcohols could be chemically modified so that their juvenile hormone activity would be enhanced or decreased. We also investigated certain other alcohols for activity.

A modification of the "*Tenebrio* test" (*4, 6*) was used in this investigation. All compounds were injected into the abdomen of newly molted pupae of the yellow mealworm, *Tenebrio molitor* L., not more than 24 hours old. Dilutions were made with purified peanut oil. Injection volumes were restricted to 0.6 μl; the solutions were injected by means of a micrometer-driven 50-μl Hamilton syringe. Two groups of six pupae were used in each test with different concentrations of the compounds. After injection the pupae were kept in small glass jars for 8 days, at which time the newly molted insects were removed and inspected under the microscope for the retention of pupal characters. The characters most sensitive to juvenile hormone activity are the pupal urogomphi and lateral abdominal gin traps (Fig. 1). The extent to which these characters were retained in the imago depended upon the activity of the compound injected. The normal adult has no gin traps, and only the male retains external remnants of the urogomphi. The greatest possible effect was retention of all pupal characters, which was achieved with one of the materials investigated; however, these insects died without further development. In most cases a positive test was signified by the retention of several gin traps and somewhat modified urogomphi (Fig. 1).

Farnesol (3,7,11-trimethyl-2,6,10-dodecatrien-1-ol), obtained commercially, was purified by column chromatography with Florisil (*7*). The fraction utilized consisted of 91 percent of the *trans trans* isomer and 9 percent of the *cis trans* isomer, as shown by comparison of the relative retention times of the four possible isomers of farnesol (*8*) in gas chromatographic analysis. All derivatives were made from farnesol so purified. Before use, other alcohols and their derivatives, examined by thin-layer and gas-liquid chromatography and infrared spectroscopy, were estimated as being more than 99 percent pure.

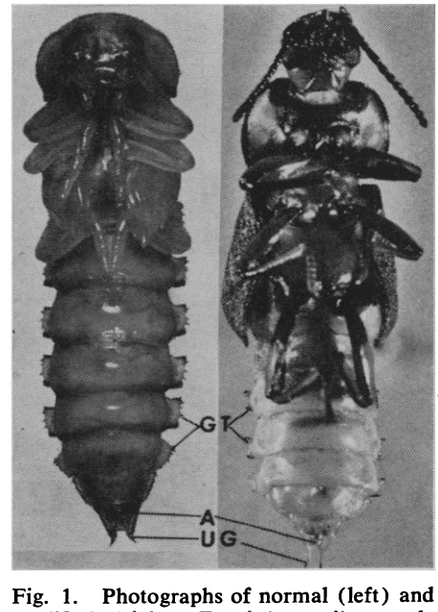

Fig. 1. Photographs of normal (left) and modified (right) *Tenebrio molitor* male beetles. In the modified beetle, the head and thorax are essentially adult, but the abdomen has pupal gin traps (*GT*), urogomphi (*UG*), and pupal cuticle on the posterior segments. The pupal aedeagus (*A*) is retained in the modified beetle.