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Peripheral Blood Cells in the Crested Newt After Long-Term Exposure to the Fungicide Manganese Ethylene*bis*dithiocarbamate *(Maneb)*

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The ethylenebisdithiocarbamates (EBDC) are an agriculturally important group of fungicides. As a part of a study on the effects of long-term exposure to EBDC in the European crested newt (ARIAS and ZAVANELLA 1979, ZAVANELLA et al. 1979), the effects of maneb (manganese EBDC) on the peripheral blood cells of this amphibian have been investigated.

MATERIALS AND METHODS

The Maneb 80 (supplied by SIPCAM, Milan) containing sodium ligninsulphonate as dispersant and n-butylnaphthalene-sulphonate as wetting agent and 80% active ingredient was used. Adult newts (Triturus cristatus carnifex) of both sexes were exposed percutaneously to different maneb concentrations, chosen after preliminary toxicity studies (PACCES ZAFFARONI et al. 1978). Two series of experiments were carried out.

First experiment. The animals were collected near Perugia in November and kept in running water for a month prior to experimentation. They were divided into five groups, each of 30 animals of comparable sizes, and maintained in 30-L glass aquaria. Three groups were kept in maneb solutions with concentrations of 0.5, 2.5 and 5.0 parts per million (ppm). One control group was maintained in tap water, a second group was kept in an aqueous solution of the dispersant and wetting agent in the same concentrations present in the 5 ppm solution of maneb. Water and test solutions were renewed three times a week. The water temperature was 16°-21°C. All the animals were killed after 19-23 weeks of treatment. Throughout this period they were not fed.

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Second experiment. Two populations of newts collected in June in two different localities (one near Perugia, the other near Milan) were used. They were kept in running water for a month prior to experimentation. Each population was divided into two groups of animals of comparable sizes. One group served as control and was maintained in tap water, the other was kept in maneb solution at the concentration of 5 ppm. A total of 185 animals was used. The animals were exposed to the fungicide for four consecutive days per week, maneb solution being renewed after the first two days. Thereafter, they were kept in tap water and fed with chopped beef liver. The water temperature was 20° -25°C. During the experiment, there was high mortality among the treated and the control newts that had been caught near Milan. All the surviving animals were killed after 37-40 weeks.

The two experiments were terminated in the spring of two subsequent years. At that time, blood samples were collected by cardiac puncture. Differential leukocyte counts were made from May-Grünwald-Giemsa stained smears. Thrombocytes and circulating erythroblasts were also counted from the smears and expressed as per cent of the white and the red blood cells, respectively. Blood smears of good quality were obtained from a total of 120 animals in the first experiment and from a total of 84 animals in the second experiment. Total blood cell counts were not considered reliable because of the extremely variable results.

RESULTS AND DISCUSSION

Cold-blooded vertebrates exhibit considerable variation in their hematologic characteristics, so that some difficulty is encountered in establishing normal values. Accordingly, a high degree of individual variation in the blood picture of the crested newt was found in the present investigation.

The mean values for differential leukocyte counts in control and treated newts are given in Tables 1 and 2. In the control newts the percentages of the various white blood cells were within the ranges reported by other investigators (BABUDIERI 1930, DELLA CORTE 1961, GARAVINI 1970, AMIRANTE 1971). Only the relative

TABLE 1

Mean differential white cell counts in newts exposed to different concentrations of maneb (1st experiment) (No. of animals in parentheses)*

	Female					Male				
	Control**		maneb (ppm)			Control**		maneb (ppm)		
	I (13)	II (14)	0.5 (15)	2.5 (14)	5.0 (10)	I (8)	II (9)	0.5 (14)	2.5 (11)	5.0 (12)
neutrophils	19.3	21.8	18.4	17.2	15.2	27.9	20.0	16.3	15.1	16.4
eosinophils	9.3	6.8	6.8	5.9	4.8	7.2	3.1	4.8	1.5	2.3
basophils	6.3	6.9	5.1	5.6	3.9	4.5	6.0	5.7	4.7	8.9
lymphocytes	61.0	61.5	67.8	67.2	73.7	55.1	65.5	69.0	76.2	65.4
monocytes	4.1	3.0	1.9	4.0	2.4	5.3	5.4	4.1	2.4	7.0

* Newts collected near Perugia

** Control I: tap water; control II: solution of dispersant and wetting agent

TABLE 2

Mean differential white cell counts in two populations of newts exposed to 5 ppm of maneb (2nd experiment) (No. of animals in parentheses)

source	Perugia area				Milan area			
	Female		Male		Female		Male	
	Control (15)	maneb (23)	Control (13)	maneb (13)	Control (10)	maneb (3)	Control (4)	maneb (3)
neutrophils	22.1	21.6	27.3	35.2	31.6	26.4	27.9	32.5
eosinophils	16.6	11.8	3.6	12.3	17.5	8.3	17.1	2.1
basophils	5.1	6.5	6.5	7.1	5.0	5.2	5.3	5.8
lymphocytes	54.7	58.5	56.6	43.4	40.5	57.6	42.7	55.2
monocytes	1.5	1.5	5.9	2.0	5.2	2.5	7.0	4.4

number of eosinophils was somewhat higher than that reported by others. This might be due to the high incidence of parasitic infestations of the liver and of the gut. Relative lymphocytosis has been reported to be present in the newt Notophthalmus viridescens after prolonged starvation (HIGHTOWER and HAAR 1978). However, no appreciable differences were detected between the unfed animals of the first experiment and the fed controls of the second experiment, if the animals collected near Perugia are considered.

No significant changes in the relative proportions of the white blood cells were found after exposure to maneb at any of the concentrations tested (Table 1 & 2). Percentages of thrombocytes in maneb-treated newts were also within the ranges observed in the control groups.

With respect to the effects of maneb treatment on erythropoiesis, a 40-60% decrease in number of circulating erythroblasts was observed at the concentration of 5 ppm. This change was clearly seen in the male newts, which are more susceptible than females to the toxic effects of maneb (PACCES ZAFFARONI et al. 1978). However, the decrease was significant only in the starved animals of the first experiment ($P < .05$), not significant in the animals of the second experiment (one-way analysis of variance).

No conclusion may be drawn about the effects of maneb treatment in the population collected near Milan, due to the low number of surviving animals and to the occurrence of widely metastatizing tumors in most of the controls as well as in the treated animals (unpublished).

So far, only scattered information is available on the effects of EBDC and of their degradation products on peripheral blood cells in other animal species. KURBAT (1968) observed leukopenia in rabbits given maneb and zineb (zinc EBDC). However, our results are not directly comparable to KURBAT's because we did not count the leukocytes. Instead, our result are consistent with those obtained by other investigators in rats, mice and dogs after long-term treatment with other dithiocarbamates, such as zineb, nabam (sodium EBDC), ferbam (iron EBDC) and thiram (tetramethylthiuram disulfide)(BLACKWELL SMITH et al. 1953, LEE et al. 1978) and with ethylenebisisothiocyanate sulfide,

which is a degradation product of EBDC (FREUDENTHAL et al. 1977). These investigators did not detect variations in either differential leukocyte counts or in the number of blood cells per cubic millimeter.

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