Eosinophil Rhythm in Splenectomized Mice.* (22044)

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As an extension of related work on rats(1), the possible effects of splenectomy upon eosinophil counts and especially upon the 24-hour eosinophil rhythm of mice(2-5) were studied. Twenty-three B_1 males, 2½ months of age, were splenectomized, using Nembutal for anesthesia, while 22 comparable mice were subjected to a sham-operation. Purina Fox Chow and tap water were available to these mice *ad libitum* since weaning and throughout the experiment. For at least 7 days prior to experiment, the mice were singly housed and kept in a room illuminated by artificial light only. A clock-controlled switch turned the lights on at 06:00, and off at 18:00. In order to eliminate effects of previous handling upon the circulating eosinophil level, each group of mice was divided into 2 sub-groups. One subgroup from both the splenectomized group and the sham-operated group was used for sampling of tail blood at one of the 2 times of day chosen for study, namely between 07:00 and 08:30, and between 21:00 and 22:30. The methods employed for eosinophil counts have been described(6).

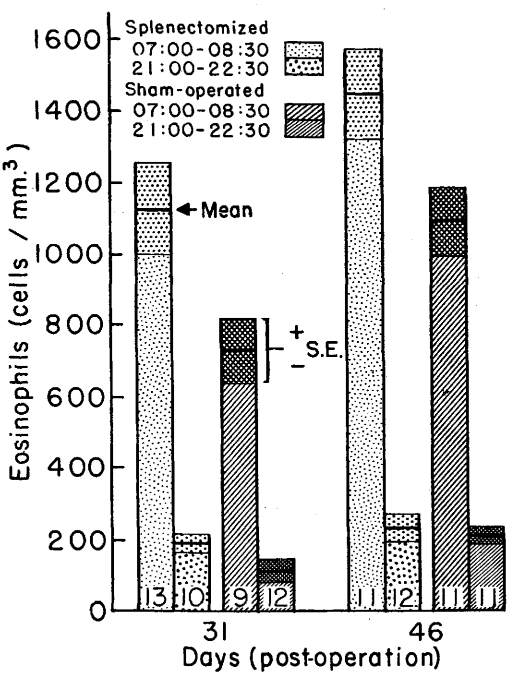

FIG. 1. Eosinophil levels of splenectomized and sham-operated B_1 male mice, at 2 times of day, 31 and 46 days after operation.

TABLE I. Comparison of Eosinophil Levels in Splenectomized Mice and in Sham-Operated Mice, at Several Post-Operative Time Points and at Several Times of Day.

Days after operation	Time of day	Diff. in mean eosinophil count*	t	P
31	7- 8:30	+399	2.41	.026
	21-22:30	+ 87	2.49	.022
46	7- 8:30	+361	2.18	.04
	21-22:30	+ 19	.42	>.60
68	2:50- 4:45	+285	3.12	<.01

* Mean eosinophil count of splenectomized group *minus* the corresponding value of sham-operated group.

The *results*, summarized in Fig. 1, reveal that at 31 and at 46 days after operation, the morning counts were consistently higher than the corresponding evening counts for both the splenectomized group and the sham-operated group ($P<0.001$ in all instances). Clearly, in mice as well as in rats, the spleen may not be necessary for the maintenance of the eosinophil rhythm.

A comparison of counts made at the same time of day in sham-operated mice and splenectomized mice (Fig. 1) also suggests the occurrence of a post-splenectomy eosinophilia, a phenomenon analyzed in Table I. This table includes additional data obtained 68 days after operation, and reveals that the magnitude of post-splenectomy eosinophilia is to some extent a function of the time of day at which the counts were done. But, at the daily times of high eosinophil levels (07:00-08:30),

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the eosinophilia occurring during the second month after splenectomy in mice stands out with unmasked clarity and is statistically significant.

Summary. At several post-operative time points, eosinophil counts were made on tail blood from splenectomized and from sham-operated male B_1 mice, kept under standardized environmental conditions. The occurrence of post-splenectomy eosinophilia was noted. Furthermore, the mean eosinophil counts of all of the groups of mice studied were consistently higher by day than by night. In B_1 mice and rats the presence of the spleen is not essential for the maintenance of eosinophil rhythm.

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Methemoglobinemia Induced by X-Irradiation. (22045)

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Oxidation reactions in aqueous media are commonly enhanced by exposure to ionizing radiation. The iron of the circulating hemoglobin, both reduced hemoglobin and oxyhemoglobin, is in the ferrous or reduced state. Ferric or oxidized hemoglobin occurs in methemoglobin, which amounts normally to 1 to 2% of the total blood pigment(1,2). Fricke and Peterson(3) found that oxyhemoglobin in solution disappears when it is irradiated. They concluded, from spectroscopic observations, that methemoglobin is the principal reaction product. This reaction has been studied to ascertain its usefulness as a quantitative biologic indicator of radiation exposure.

Normal human values. Methemoglobin was determined at various times in 8 apparently healthy laboratory workers, 7 male and one female, by the method of Evelyn and Malloy (4). Total blood pigment was determined after its conversion to methemoglobin by potassium ferricyanide.

The results are summarized in Table I. In our hands, this method was reproducible to $\pm$ 0.04% methemoglobin. The mean value of 33 determinations was 1.53 $\pm$ 0.30%. The normal methemoglobin level in a given individual was found to vary from time to time; the mean standard deviation of individual variations was 0.33% methemoglobin.

In vitro studies. Hemolyzed washed human red blood cells were mixed with M/60 phosphate buffer, pH = 6.5, in the proportions 1:1, 1:10, 1:20, 1:40, and irradiated with 260 kvp x-rays filtered by 1.0 mm Al and 0.5 mm Cu. The results of a typical experiment are shown in Fig. 1. For doses below 20000 r, the formation of methemoglobin was found to be a linear function of dose. Methemo-

TABLE I. Normal Levels of Methemoglobin (%).

E. R.	J. W.	J. T.	L. W.	W. K.	R. D.	G. S.	S. G.
1.53	.57 2.05 2.06	2.48 1.53	1.77	.53	1.29	1.93	.93
1.62	1.60 2.83 2.74	.82 2.50	1.58	1.82	1.03	1.70	1.23
1.92	1.14 .78 .86	1.06 1.23	2.32	.84	1.22	2.01	1.66
1.69 $\pm$ .17	1.66 $\pm$ .78	1.60 $\pm$ .66	1.89 $\pm$ .31	1.06 $\pm$ .55	1.18 $\pm$ .11	1.88 $\pm$ .13	1.27 $\pm$ .30