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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Received September 6, 1955. P.S.E.B.M., 1955, v90.

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

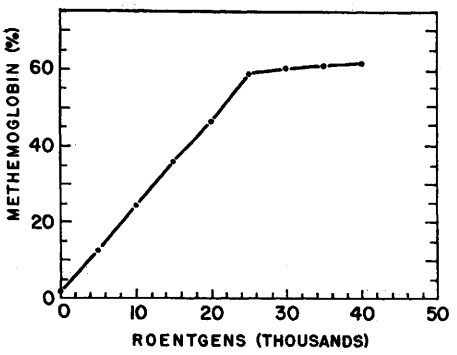

FIG. 1. Methemoglobin formed by irradiation of lysed washed RBC 1:40 in M/60 phosphate buffer, pH = 6.5.

globin was formed in the more dilute solutions at a relatively greater rate than would be expected from the concentration differences.

In vivo experiments. Ten adult male Sprague-Dawley rats were irradiated with 260 kvp x-rays filtered by 1.0 mm Al and 0.5 mm Cu at a rate of 50 r/min. Samples of tail blood for methemoglobin determinations were obtained pre-irradiation, during irradiation after 3000 r, and 6000 r and postirradiation after 1, 3, 5, and 8 hours. The results are depicted in Fig. 2. Variations in the normal methemoglobin values similar to the variations in the human determinations were observed.

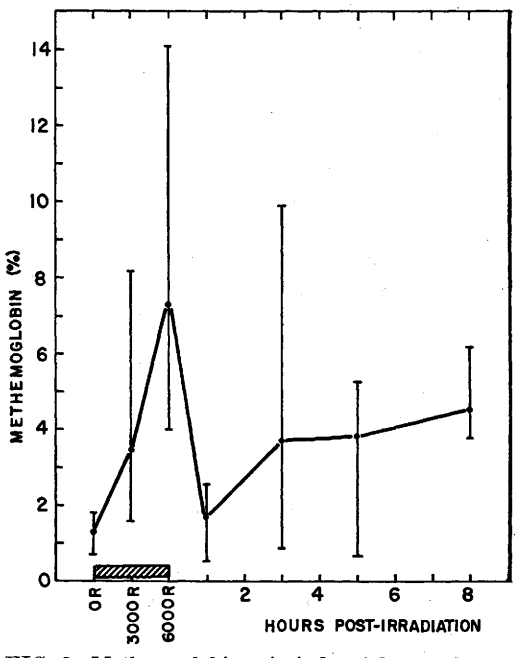

FIG. 2. Methemoglobinemia induced by 260 kvp x-rays in Sprague-Dawley rats with observations in the postirradiation period. Range of values and means are shown.

Discussion. The oxidation of the ferrous iron within the hemoglobin moiety is presumably analogous to the radiation induced oxidation of ferrous salts in aqueous solution. The latter reaction is the basis of one kind of chemical radiation dosimeter(5). The reaction is probably mediated by the oxidative free radicals which are formed upon irradiation of water. In the case of inorganic iron salts, pH and substrate concentration affect the reaction rate. These parameters are held within narrow limits in the circulating blood.

The low one-hour postirradiation methemoglobin level is less than might be expected from reconversion of the methemoglobin in drug induced methemoglobinemias. In experimental drug induced methemoglobinemias the rate of reconversion to hemoglobin was found to be 8-10% per hour(6,7).

Although the increase in methemoglobin was observed to be proportional to radiation exposure in both the intact animal and *in vitro* studies for moderate doses, the usefulness of methemoglobinemia as a quantitative biologic radiation dosimeter appears to have several limitations. Variation was found in the normal baseline levels from individual to individual, and in a given individual from time to time. The secondary postirradiation methemoglobinemia found in the intact animals is a complication. Appreciable methemoglobinemia is produced only after exposures in the lethal and supralethal ranges.

Summary. Methemoglobin forms in aqueous solutions of hemoglobin and in intact rats after exposure to ionizing radiation. For moderate doses, the degree of methemoglobinemia is a linear function of dose. In the sub-lethal dose range, the methemoglobinemia is comparable to the random variation in normal values. A secondary methemoglobinemia was observed in rats 3 to 8 hours postirradiation.

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Received September 7, 1955. P.S.E.B.M., 1955, v90.

Relation of Sex and Age to Resistance of Mice to Experimental Histoplasma Infections.* (22046)

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Preliminary studies from this laboratory indicated that age affected the resistance of white Swiss male mice to experimental histoplasmosis(1). Campbell and Saslaw(2) using 14- to 17-g white mice noted a greater mortality in males. Salvin(3) reported no sex differences in mortality of 35-day-old mice. The purpose of this report is to elucidate further the sex and maturity factors relative to histoplasma infections in mice.

Materials and methods. Five-week-old white Swiss mice of both sexes were obtained at one- or 2-week intervals and kept until there were groups representing these age intervals from 5 through 23 weeks. The numbers of mice used in each group varied according to availability. The mice were inoculated intraperitoneally with 23 or 35 million yeast phase *H. capsulatum* organisms in 0.5 ml of 5% hog gastric mucin. All mice were weighed on the day of inoculation, and at weekly intervals. The mice were observed for a 30-day period and necropsy performed on those dying during this interval.

Results. Table I summarizes the results of these studies. Column A in Table I demonstrates that 10- to 15-week-old male mice receiving 23 million organisms showed increased resistance to fatal infection as compared to those 5 and 9 weeks old. A further increase in resistance was noted in those 16 weeks or older. With the 35 million dose (column C) the increased resistance was first noted at 17 weeks of age. The parallel studies in female mice with the lower dosage (column B) showed a similar increase in resistance at 8 weeks as compared to 10 weeks in males and a further increase in survival rate after 15 to 16 weeks of age. With the 35 million dose (column D) the 2 periods of comparative increased resistance were noted at 9 and 17 weeks in females and males, respectively. In comparing the death rates in both sexes receiving 23 million organisms the 8-week-old male mice showed 19 of 22 deaths (86.5%) as compared to 14 of 25 female deaths (56%); in the 9-week-old group, 9 of 10 males (90%), and 7 of 11 females (63.3%) died. Thereafter the incidence was similar except at 15 weeks when 8 of 12 males, as compared to 4 of 10 females, died. In those mice receiving 35 million organisms the differences in resistance of sexes became apparent in the 9-week-old mice where 15 of 16 males (93.7%) as compared to only 9 of 15 females (60%) died. This difference became less apparent by the 13th week and no appreciable variation was noted thereafter. Combining the total mortalities under both sex groups the 8- to 14-week period represented the time interval when apparent sex differences in susceptibility occurred (Table II). As can be seen in Table II, when all of the age groups were combined no significant difference between sexes was noted following inoculation with 23 million organisms, since 174 of 337 females (51.6%) died as compared to 183 of 331 males (55.3%). However, with the 35 million dose, the males were significantly more susceptible in that 105 of 145 males (72.5%)

* This investigation was supported in part by research grant from the National Institute of Health.