Increase in Red Cell Volume in Response to Long Exposure to Cold.* (23179)

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Although blood and plasma volume changes have been given some attention in studies involving exposing animals to cold environments, very little mention has been made regarding the red cell picture. Bass and Henschel(1) have recently reviewed the subject of volume changes in cold environments. Baxett *et al.*(2) report a decrease in blood volume and total circulating hemoglobin in humans after prolonged exposure to cold. However, Brown *et al.*(3) observed that the blood volume of the Eskimo was high and that it fell in the summer months and suggested that this was associated with cold acclimatization. In the rat, Deb and Hart(4) observed increased blood and plasma volume after 5 weeks exposure to an environment of 6°C.

Since it is well substantiated that oxygen consumption is increased in rats exposed to a cold environment(5,6), and since one of the functions of hemoglobin is the transport of oxygen, it would seem pertinent that the state of erythropoiesis should be reinvestigated. Use of labelled red cell dilution technic was employed here to see if there is any net gain in total red cell volume in rats exposed to a cold environment.

Methods. The rats were males of the Long-Evans strain, fed a complete laboratory diet.† Thirty-six rats, approximately 170 days of age, were divided into 2 groups so that the average weight of each group was the same at the beginning of the experiment. The control group was maintained at room temperature and the cold-exposed group was kept at 3°C ± 1°C. At the end of 30 days, blood volumes were determined by use of Fe^{59} labelled red cell dilution technic(7). The labelled red cells were obtained by cardiac puncture from a donor animal previously injected with Fe^{59}. *Blood volume determinations* were done under ether anesthesia. Injections of tagged blood were made through the saphenous vein which was exposed by a small skin incision. A specially calibrated 0.25 ml tuberculin syringe was used so that a constant volume of donor blood was injected into each animal. Using this syringe, the same volume of donor blood was put into a 10 ml volumetric flask, which when diluted and an aliquot pipetted and counted, gave a measure of the amount of Fe^{59} activity which was injected into each animal. After injection, 6 minutes were allowed for the donor blood to mix, at the end of which time the abdomen was opened, and as much blood as would flow freely was drawn from the vena cava into a heparinized syringe. A portion of this blood was used for an hematocrit and hemoglobin determination and an aliquot was counted directly in a scintillation counter adapted for vial counting(8). The amount of radioactivity injected was divided by the amount of radioactivity/unit of recipient blood to obtain "blood volume." The total "blood volume" multiplied by the hematocrit gave total red cell volume. The total circulating hemoglobin was obtained by multiplying total blood volume by the hemoglobin concentration.

Results. A summary of the data is presented in Table I. The differences between normal and exposed rats observed in the hematocrits and hemoglobin concentrations were not statistically significant. However, total red cell volume and total circulating hemoglobin of the cold exposed rats increased by 12.9 and 10.9% respectively. These values are significant with a "P" value of less than 0.001.

Stullken and Hiestand(9) observed no change in either hemoglobin concentration or erythrocyte number in mice after a 5 day ex-

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† The diet was 'Simonsen Green Diet' obtained from Simonsen Laboratories, Gilroy, Calif.

TABLE I. Summary of Hematological Data of Rats Exposed to Cold Environment for 30 Days; 18 Rats/Series.

	Body wt, g		Hematocrit, %	Hemoglobin conc., g/100 ml blood	Total blood vol, ml	Total red cell vol			Total circulating hemoglobin		
	Initial	Final				ml	% change	P*	g	% change	P
Controls	400	428	45.5	13.2	20.22	9.20	12.9	.001	2.67	10.9	.001
Cold exposed 30-days	400	413	47.2	13.4	22.01	10.39			2.96		

* Fisher's "t" test(10).

posure to cold. However, they suggest that possibly 5 days is not a long enough time in which to expect acclimatization to appear. The present study would also suggest that concentration studies alone are perhaps not as conclusive as the observation of a net increase in red cell volume in response to a particular stimulation.

That an increase in red cell volume is observed in animals exposed to a cold environment is not altogether unexpected when one considers that it is accompanied by a physiologically increased requirement of oxygen. Such a stimulation of red cell production, possibly secondary to an increased oxygen consumption, is interesting since in many different endocrine states variations in the red cell picture are seen accompanied by variations in oxygen consumption.

Summary. An increase in total red cell volume and total circulating hemoglobin was observed in rats exposed to a cold environment for a period of 30 days.

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Bromsulphalein Dye Retention Test in Toxicological Investigations. (23180)

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Routine toxicologic investigations demand evaluation of liver function in the experimental animal as an indication of possible hepatotoxicity. The method employed should give results which can be read quantitatively. This report describes a procedure by which concentrations of the bromsulphalein dye in the blood are obtained which can easily be determined by standard electrophotometric methods. Moses *et al.*(1) have reported that bromsulphalein at doses of 5, 10 and 20 mg/kg intravenously in dogs yielded reproducible and quantitatively similar curves. Gornall *et al.*(2) showed that a 10

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