20 to 30% as much H^3-DFP as the blood PMN neutrophils is consistent with the previous interpretation of *in vivo*, DFP^{32} granulocyte disappearance curves(3).

Conclusions. Uptake of tritiated diisopropylfluorophosphate (H^3-DFP) by various morphologic types of leukocytes after labeling *in vitro* and *in vivo* has been studied. When leukocytes from normal subjects and patients with leukemia were labeled *in vitro*, myelocytes labeled most intensely. Metamyelocytes and polymorphonuclear (PMN) neutrophils contained about half as many grains/cell as the myelocytes. Lymphocytes, eosinophils, and basophils did not bind significant amounts of DFP under the conditions of this study while a few monocytes were lightly labeled. Granulocytes in both blood and bone marrow were labeled after intravenous injection of H^3-DFP. Relative degree of labeling was as follows: blood PMN neutrophils, 1.0; marrow PMN neutrophils, 0.3; marrow metamyelocytes, 0.2; and marrow myelocytes, 0.6. None of the other formed elements in either blood or bone marrow contained significant amounts of the label.

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Haematologic and Serum Protein Alterations in the Hypothermic Rat.* (26646)

JOHN R. BEATON
Defence Research Medical Laboratories, Toronto, Ont., Canada

During studies on blood metabolite and electrolyte alterations in the hypothermic rat(1,2) we observed that, in animals with rectal temperature reduced to 15°C, there is a significant increase in hematocrit. Increased hematocrit in hypothermia has been reported by others(3,4) although the explanation is not clear. It has been suggested that the increased hematocrit may result from decreased plasma volume(5) or by splenic discharge of red cells(3). Another possible explanation would be an increase in mean corpuscular volume of erythrocytes. The present experiments were undertaken to study hematocrit, hemoglobin, blood cell and serum protein levels in hypothermic rats, seeking in particular, further information on the cause of the increased hematocrit values.

Methods. In all experiments, male albino rats of the Wistar strain, weighing 200-250 g and maintained on fox chow and water *ad libitum,* were used. Cooling was carried out as in previous experiments(1,2), by placing unanesthetized animals in individual, cylindrical screen cages under crushed ice for a period of approximately 30 minutes at which time rectal temperature was 15°C. At this rectal temperature, respiratory movements

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TABLE I. Haematologic Alterations in Hypothermic (15°C Rectal Temperature) Rats. Results expressed as mean ± stand. error of the mean for 10 rats.

	Control	Hypothermic	Probability, P*
Hematocrit, %	46 ± 1.5	55 ± 1.8	<0.01
Hemoglobin, g %	13.0 ± 0.53	13.7 ± 0.35	—
Erythrocyte count, millions/mm³ blood	8.03 ± 0.12	8.48 ± 0.52	—
Mean corpuscular volume, μ^3	57.7 ± 1.3	65.4 ± 2.0	<0.01
Mean corpuscular hemoglobin content, $\mu\mu g$	16.2 ± 0.43	16.1 ± 0.52	—
Leukocyte count, thousands/mm³ blood	11.9 ± 1.0	7.6 ± 1.2	<0.01
Neutrophiles, %	24 ± 0.63	10 ± 1.7	<0.001
Lymphocytes, %	75 ± 0.63	89 ± 1.6	<0.001
Monocytes, %	0	0	—
Eosinophiles, %	<1	<1	—
Basophiles, %	0	0	—

* Calculated by application of Student's "t" test.

had almost ceased, cardiac activity, as measured by electrocardiogram, markedly reduced and the animals were lethargic. Artificial ventilation was not provided during the cooling procedure. Blood samples were withdrawn by syringe from the exposed heart (right ventricle) following intraperitoneal administration of sodium pentobarbital in 0.85% aqueous sodium chloride to provide 5 mg/100 g body weight. In the first experiment, heparin was added to blood as the anticoagulant.

In the first experiment, the following determinations were made on individual blood samples from 10 control and 10 hypothermic rats: hematocrit by the method of Wintrobe (6), hemoglobin by the method of Collier (7), erythrocyte count, leukocyte count and differential leukocyte count using Wright's stain. Mean corpuscular volume and mean corpuscular hemoglobin content were calculated from these data.

In a second experiment, blood samples from 10 control and 10 hypothermic rats were allowed to clot, the sera were separated, and total protein, albumin and globulin concentrations were determined by the method of Gornall *et al.*(8). In addition, the starch gel zone electrophoretic procedure of Smithies(9) was used to study serum proteins of 12 control and 12 hypothermic rats.

Results. The results of blood cell count, hematocrit and hemoglobin measurements are shown in Table I. The increased hematocrit of hypothermic rats(1) was confirmed and was accompanied by a significant increase in mean corpuscular volume of erythrocytes. In the hypothermic rat there was a neutropenia, a lymphocytosis and a net leukopenia. Hypothermia had no apparent effect upon erythrocyte count, hemoglobin concentration or mean corpuscular hemoglobin content.

The results of the serum protein analyses are shown in Table II. Values obtained for control animals are comparable to those obtained previously using this analytical method(10). It is apparent that hypothermia in the rat, under these conditions, results in a significant increase in total serum protein concentration and in an increased albumin: globulin ratio. Starch gel zone electrophoresis showed no qualitative differences between the sera of the control and of the hypothermic rats.

Discussion. Barbour *et al.*(5), on the basis of changes in plasma proteins and chloride, concluded there is a decrease in

TABLE II. Serum Protein Concentrations in Hypothermic Rats (15°C Rectal Temperature). Results expressed as mean ± stand. error of the mean for 10 rats.

	Control	Hypothermic	Probability, P*
Total serum proteins, g %	5.78 ± 0.044	6.30 ± 0.073	<0.001
Serum albumin, g %	3.74 ± 0.070	4.76 ± 0.044	<0.001
Serum globulin, g %	2.04 ± 0.083	1.54 ± 0.070	<0.001
Albumin: globulin ratio	1.83 ± 0.15	3.09 ± 0.28	<0.001

* Calculated by application of Student's "t" test.

plasma volume of hypothermic monkeys and rats. Our results of serum protein analyses seem to support this conclusion, as does our earlier observation(2) of a decrease in plasma water content in hypothermic rats. Swan *et al.*(3) reported increased hematocrit with little change in plasma volume which they suggested as being indicative of splenic discharge of cells. The data reported here show an increase of 13.3% in mean corpuscular volume which, although insufficient alone to account for the 19.5% increase in hematocrit, is no doubt a contributing factor. This increase in mean corpuscular volume may result, in part, from the 7.7% increase in erythrocyte water content which we have observed in hypothermic rats(2). It would appear that the increased hematocrit of our hypothermic rats is a consequence of more than one factor, including a decreased plasma volume and an increased mean corpuscular volume.

Villalabos *et al.*(11) have observed a marked decrease in leukocyte and platelet counts of hypothermic dogs and provided evidence that these decreases are due to sequestration rather than to destruction. If this is true in the hypothermic rat, the relative increase in lymphocytes and relative decrease in neutrophiles are difficult to explain unless neutrophiles are preferentially sequestered.

The increase in serum albumin concentration more than accounts for the increase in total serum proteins, since there is a decrease in serum globulin concentration. Indeed, if plasma volume is reduced in hypothermia (5), the decrease in serum globulin content is more marked than is indicated by the concentration values. This decrease might well be the cause of the increased serum albumin: globulin ratio.

Summary. As a consequence of hypothermia in the rat (15°C rectal temperature), there are significant increases in hematocrit, mean corpuscular volume, serum total protein and albumin concentrations and in the serum albumin:globulin ratio. Hypothermia does not alter erythrocyte count, hemoglobin concentration or mean corpuscular hemoglobin content. These observations are discussed in relation to the causes of the increased hematocrit. In addition, the hypothermic rat has a leukopenia with a relative decrease in neutrophiles and a relative increase in lymphocytes.

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