TABLE II. Recovery of Fed Micellar Cholesterol-4-C^{14}-α,α-Methyl Ethyl Caproate (C^{14}-MEC).

Tissue*	% of C^{14} recovered as Free sterol-C^{14}	Ester-C^{14}	C^{14}-MEC	Total
Lymph (0-12 hr)†	Trace	Trace	0	Trace
Intestinal tissue†	"	"	0	"
Feces plus contents	0	0	98.1	98.1 ± 1.0

* Represents average of 4 animals ± S.E. Each lymph fistula rat received 6.8 mg cholesterol-4-C^{14} MEC (5.99×10^6 dpm) solubilized in phospholipid-bile salt micelles(5).

† The counts of free and esterified sterol-C^{14} from 2 ml lymph and approximately 1 g small intestine represented 13-36 cpm above background, and can be accounted for by 0.35% contamination of C^{14}-MEC.

initial transfer of cholesterol into mucosal cells is specific for free cholesterol, and that cholesterol esters are not absorbed from the intestinal lumen prior to hydrolysis.

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Received March 2, 1964. P.S.E.B.M., 1964, v116.

In vitro Uptake of Tritiated Thymidine by Erythrocytic Percursors Following Hemorrhage in the Dog.* (29290)

N. O. NIELSEN,† L. E. FEINENDEGEN,‡ V. P. BOND, N. ODARTCHENKO§ AND H. COTTIER‖

Medical Research Center, Brookhaven National Laboratory, Upton, L. I., N. Y.

Following hemorrhage, the bone marrow responds with an increased output of red cells which can reach 7 times normal values(1). The nature of the cellular response by which this is achieved has not been firmly established. An expansion of the mass of erythrocytic precursor cells has been reported, as well as an increase in reticulocyte count(2-4, 12). It has remained questionable whether the cellular response involves also an increase in rate of maturation, and change in rate of DNA synthesis.

The value of tritiated thymidine and autoradiography in the study of *in vivo* cell kinetics has been well established(5-7). *In vitro* studies have also been used(8-10). The incubation of freshly drawn bone marrow with tritiated thymidine *in vitro* results in incorporation of the isotope into the nucleus of those cells that are synthesizing DNA. They are recognized autoradiographically. The mean number of grains over nuclei of mor-

* Research supported by U. S. Atomic Energy Commission.

† College of Vet. Med., University of Minnesota, St. Paul.

‡ Euratom, Brussels, Belgium.

§ Centre Anti-Cancereux Romand, Hopital Cantonal, Lausanne, Switz.

‖ Inst. of Pathology, Univ. of Bern, Switz.

phologically similar cells reflects an approximation of the average rate of DNA synthesis for the group. The percentage of a cell sample which incorporates tritiated thymidine depends on the length of DNA synthesis time in relation to generation time. The percentage of labeled cells and the distribution of label intensity are similar after *in vitro* incubation and *in vivo* injection of H^3-thymidine and the results are interpreted with this assumption. The methods of analysis of data thus obtained have been reviewed by Cronkite *et al*(7).

This communication presents data on the effect of hemorrhage on red cell precursors in bone marrow of dogs, with particular emphasis on incorporation of tritiated thymidine *in vitro* at time intervals up to 52 hours after bleeding.

Methods. Four six-month-old dogs (one beagle, A, and 3 mongrel litter mates, B, C, D) were used. All were free of disease and were fed a standard laboratory diet.

Approximately 40% of the blood volume (32 ml/kg) was removed from experimental dogs C and D (under sodium pentothal anesthesia) *via* the femoral artery at 1 PM. Controls were anesthetized only. Bone marrow samples were taken immediately before bleeding and 12, 24, 30, 36 and 45 and 52 hours thereafter (12 and 30 hour samples were omitted for control dog A). Two ml of marrow were aspirated from either the pelvis or femur, under local anesthesia with xylocaine, into 0.15 ml of a 0.1% EDTA 0.7% saline solution. One ml of this marrow mixture was incubated with 5 μc of tritiated thymidine (specific activity of 1.9 curie per mM) for 40 minutes at 37°C. Slide preparations of marrow particles were air-dried and fixed in methanol for 20 minutes.

Autoradiograms were made with AR 10 Kodak stripping film(11). Exposure time was 5 days. The developed and dried preparations were stained with Giemsa solution (dilution 1:20) buffered at pH 5.75.

At least 500 nucleated erythrocytic precursor cells in each marrow sample were evaluated. They were grouped morphologically according to classical description(1) with the exception of the basophilic normoblasts which were divided into "large" and "small" categories. In addition, the ratio of erythrocytic precursors to the rest of the marrow was determined in at least 500 marrow cells, exclusive of lymphocytes and segmented granulocytes. This ratio is directly equivalent to the erythroid-myeloid ratio. Certain difficulties in classifying properly all cells could not be avoided. In addition, it should be noted that in the dog the cytoplasm of the "orthochromatic" normoblasts retains more basophilia than corresponding cells in human marrow. Tritiated thymidine is not incorporated into this cell type. Cells were scored as to labeling and the number of grains overlying labeled nuclei were counted. The grains over segmented neutrophilic granulocytes were used for background correction. Nuclear diameters were also estimated to the nearest micron with a calibrated eyepiece.

Results and discussion. The mean hemotocrit of the 2 bled dogs was 44 before bleeding and 30 on the day following bleeding. The procedure was tolerated well and no clinical problems developed during the experiment.

The autoradiographic preparations of the bone marrow smears were satisfactory for both morphological and grain count examination, except for a few which were discarded.

The differential counts of the nucleated red blood cell precursors (Fig. 1) did not show remarkable changes after bleeding throughout the time of observation, and the differences in the data are considered well within the limits of error of measurement. Furthermore, the number of erythrocytic precursor cells in relation to the remainder of the marrow was not appreciably altered (Table I). The mitotic index also did not deviate from the distribution of control values (Table I). However, the relative number of naked nuclei, normally about 5% of total red cell precursors, showed perhaps a tendency to increase 24-45 hours after bleeding.

Following bleeding a sudden increase in the more immature normoblasts or early loss of orthochromatic normoblasts should be expected to alter the distribution of erythrocytic cells in different stages of maturation, as expressed in the differential counts(12).

TABLE I. Effect of Bleeding on Erythrocytic Precursor Cells in the Dog Bone Marrow.

Dogs	Hr after bleeding	% labeled cells: Including orthochromatics	% labeled cells: Excluding orthochromatics	Mitotic index	Extruded orthochromatic nuclei per 100 cells	Erythroid/ Myeloid ratio
A Control	0	26.6	68.2	1.4	4.2	.49
	24	40.6	78.8	1.9	2.8	.49
	36	35.0	74.2	1.6	2.2	.43
	45	35.0	73.5	1.2	4.8	.57
	52	40.6	71.2	.8	3.0	.62
B Control	0	32.3	62.9	1.9	4.4	.43
	12	*	*	3.6	5.8	.41
	24	34.2	71.3	3.5	7.0	.53
	30	33.3	65.0	3.0	6.2	.50
	36	40.9	77.6	2.9	5.2	.52
	45	36.8	70.5	4.1	4.6	.50
	52	39.1	74.1	2.7	5.1	.56
C Bled	0	35.1	61.5	1.8	5.9	.42
	12	*	*	2.7	5.4	.63
	24	30.1	63.5	3.7	8.8	.82
	30	29.1	59.8	3.3	9.3	.51
	36	31.0	67.5	1.6	8.0	.57
	45	31.7	62.5	4.3	9.1	.61
	52	31.0	62.3	3.6	4.8	.66
D Bled	0	36.8	72.1	2.4	4.9	.68
	12	35.4	75.2	3.6	6.9	.61
	24	31.3	69.7	2.4	4.5	.51
	30	33.3	71.5	4.2	5.0	.62
	36	33.8	67.0	2.9	7.3	.60
	45	36.7	64.8	2.7	10.4	.46
	52	33.8	68.4	3.0	5.4	.75

* Inadequate autoradiographic preparations.

No such changes were observed in the present studies.

The percent of labeled normoblasts would be changed if the relation of DNA synthesis time to generation time were altered. A change in cellular distribution is also expected to result in a change of the percentage of labeled erythrocytic precursor cells, since the orthochromatic normoblasts do not incorporate H^3-thymidine. But the percentage of labeled cells remained essentially unchanged after bleeding (Table I).

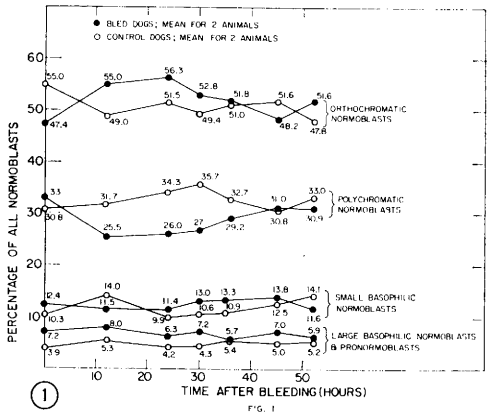
The mean grain counts in the different cell types (Fig. 2) fluctuated rather widely in control and in bled animals. Grain count data, because of wide limits of error, are considered to be semi-quantitative measurements. Since there was no consistent difference between the values obtained in control and bled animals, it is probable that the rate of DNA synthesis in the bled dogs was not changed. However, for both bled and control dogs, the mean grain counts for the pronormoblasts and large basophilic normoblasts showed a tendency to rise within the observation period. This finding is not understood and needs further evaluation.

Because of the subjective nature of morphological classification, nuclear diameters in micra were recorded for all observed cells in an attempt to provide a more objective basis for evaluating data. The results based on nuclear diameters led to the same conclusions as those based on overall cellular morphology.

The results suggest that either the experimental design and the methods used were not sensitive enough to detect an early response of erythrocytic precursors to acute anemia, or that a cellular response with regard to a change in the relation of DNA synthesis time to generation time was not manifest in the normoblasts up to 52 hours after bleeding. The latter conclusion agrees with that from similar studies by Alpen(12).

Summary. Dogs were bled 40% of their blood volume (32 ml/kg). Thereafter, at intervals to 52 hours, the *in vitro* uptake of tritiated thymidine by erythrocytic precursors

of the marrow did not differ from the controls as measured by percent labeling and grain counts in morphologically differentiated cells.



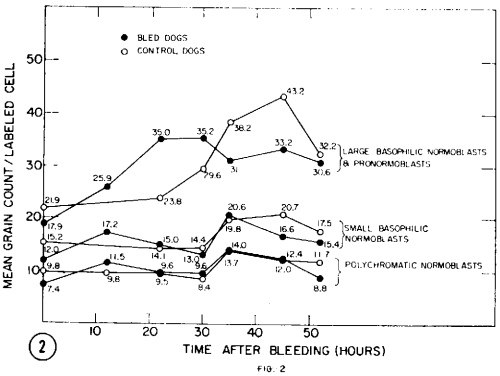


FIG. 1. Percentage of normoblasts in dog bone marrow as a function of time after bleeding.

FIG. 2. Mean grain count of labeled normoblasts in dog bone marrow as a function of time after bleeding. Each point represents mean of 2 animals.

Also, no alterations from control values were found with regard to morphological distribution, relative numbers of the marrow normoblasts, and mitotic index. The data suggest that the acute blood loss did not influence detectably the chosen parameters for the time intervals studied.

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Received February 13, 1964. P.S.E.B.M., 1964, v116.

Bioassay of Adrenocorticosteroids by the Dog Eosinophil Response. (29291)

G. TONELLI, J. HAYNES, E. HEYDER AND I. RINGLER

Department of Metabolic Chemotherapy and Statistical Design and Analysis Group, Lederle Laboratories, American Cyanamid Co., Pearl River, N. Y.

Numerous investigators have elaborated on the bioassay of adrenocorticoids in adrenalectomized mice, based on a decrease in circulating eosinophils(1-3). Tolksdorf(4,5) reported eosinopenic potencies of natural and synthetic corticoids in this species. The adrenalectomized dog also has been used for assessment of eosinopenia. Liddle *et al*(6) measured the decrease in eosinophils 4 hours following intravenous administration of graded doses of steroids. When assessed on an equimolar basis, 9α-fluorohydrocortisone acetate was 20 and 9α-chlorohydrocortisone acetate 8 times more active than the non-