in doses up to 20 mg dissolved in Locke-Ringer solution and injected into the coronary inflow cannula produced only slight, transient depression of contractions and some reduction of rate.

Concentrations of pangamic acid up to 6 $\times$ 10^{-4} M did not alter spontaneous contractions of the isolated rabbit ileum, nor did this concentration of drug modify contractions produced by acetylcholine.

Our data demonstrate that pangamic acid is not without pharmacologic effect. We have found the LD-50 in mice after intraperitoneal administration to be much lower than the value previously reported for this compound. It has been reported that thiamine hydrochloride possesses neuromuscular blocking activity and that this effect is antagonized by neostigmine methylsulfate(4,5, 6). It has also been demonstrated that intravenous administration of thiamine hydrochloride produced a transient but marked hypotension with concomitant bradycardia in anesthetized dogs. In these respects, pangamic acid produces pharmacological effects not unlike those of thiamine hydrochloride. Circulatory collapse has been reported clinically from intravenous and intramuscular injections of thiamine hydrochloride(10). We are not aware of any clinical reports of respiratory depression or severe hypotension resulting from administration of pangamic acid. However, it is conceivable that pangamic acid could produce undesirable clinical effects under circumstances of overdosage.

Summary. Pangamic acid (Vit. B-15) possesses definite pharmacologic properties. These include neuromuscular blocking activity in the rabbit and chicken as well as production of hypotension in anesthetized dogs. Neostigmine methylsulfate is an effective antagonist to the neuromuscular blockade produced in the rabbit. These effects appear to be qualitatively similar to those reported for thiamine hydrochloride.

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Leukokinetic Studies V. Uptake of Tritiated Diisopropylfluorophosphate by Leukocytes.* (26645)

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The use of radioactive diisopropylfluorophosphate (DFP^{32}) as a label for leukocytes has been described in previous reports from this laboratory(1-3). Leukocytes have been labeled *in vitro* and returned to the circulation of the donor(2), or they have been labeled *in vivo* by administering the DFP^{32} intravenously(1,3). During these studies it was demonstrated that polymorphonuclear (PMN) neutrophils but not lymphocytes are

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labeled by DFP^{32}. It was not possible to determine whether eosinophils, basophils and monocytes are labeled.

When leukocytes were labeled *in vivo* following intravenous administration of DFP^{32}, the blood leukocyte radioactivity curve obtained was complex(1,3). The shape of the curve suggested that granulocytes in the bone marrow as well as in the circulation were labeled, but that the circulating granulocytes, due to exposure to a higher initial concentration of DFP^{32}, were labeled about 3 times more heavily than those in the bone marrow.

The purpose of the present communication is to present data on uptake of tritiated DFP (H^3-DFP) by various morphologic types of leukocytes under both *in vitro* and *in vivo* labeling conditions. The binding of H^3-DFP by leukocytes obtained from both normal and leukemic subjects has been investigated and the relative degree of labeling of both blood and marrow leukocytes has been determined following intravenous administration of H^3-DFP.

Materials and methods. Tritiated DFP (H^3-DFP) was purchased as a solution in anhydrous propylene glycol from New England Nuclear Co., Boston, Mass. By special arrangement high specific activity H^3-DFP (2.2 mc/mg) was synthesized from isopropyl-2-H^3 alcohol. The DFP content of the material was measured within a week before use by the colorimetric method of Marsh and Neale(4).

Blood was labeled *in vitro* in 2 different ways. In the first, venous blood was drawn into a plastic bag containing ACD-A (Pliapak, Abbott Laboratories) and sufficient H^3-DFP was injected into the bag to give a final concentration of 0.3 μg DFP/ml of blood. In the second method, 4 volumes of venous blood were added to siliconized test tubes (Dow Corning Z-4141) containing one volume of solution consisting of dextran, 5.0%; glucose, 2.5%; disodium ethylenediamine tetraacetate, 0.5%; sodium chloride, 0.7%; and H^3-DFP to give a final concentration of 0.3 μg/ml. In both methods the blood was mixed and incubated for one hour at room temperature. An aliquot of the leukocyte-rich supernate was removed and centrifuged at 230 g for 10 minutes. The supernate was discarded and the cells were washed once in 3 ml of the individual's own plasma. The cell button was then resuspended in 0.5 ml of plasma and thin smears were prepared on #1 coverslips. The results obtained with both labeling methods were identical.

Leukocytes were labeled *in vivo* by injecting 2 mg of H^3-DFP diluted in 20 ml of isotonic saline intravenously. Simultaneous samples of blood and marrow were obtained 1 and 3 hours later and direct smears were made on #1 coverslips.

The blood and marrow smears were fixed in methanol, mounted on glass slides and covered with AR-10 stripping film (Kodak Ltd, London). Autoradiographs were prepared according to the technic of Pelc(5) as modified by Bond *et al.*(6). The film was developed and the slides stained after time periods of from 23-70 days. The number of silver grains overlying each of approximately 1000 cells of each morphologic type, or all the cells in each smear, whichever came first, was recorded. A similar number of areas of the same size lying between the cells was examined to determine the background grain count of each preparation. Mean grain count for each cell type was calculated and mean background count for areas of corresponding size was subtracted to give mean net grain count of each cell type.

Results. Uptake of DFP by normal blood leukocytes. The distribution of silver grains in the photographic emulsion overlying lymphocytes, monocytes, eosinophils and PMN neutrophils was compared with the grains over background areas of similar size. Blood from 3 normal subjects was examined. The results obtained on one of the 3 subjects are presented in Fig. 1. Mean net grain count for each cell type in each of the 3 blood samples is given in Table I.

PMN neutrophils were labeled while eosinophils and basophils were not labeled. In one of the specimens (Fig. 1B and Table I, Subject A) the monocytes were not labeled. In the blood from the other 2 subjects (B and C) the monocytes were labeled lightly but in each specimen less than 20% of the

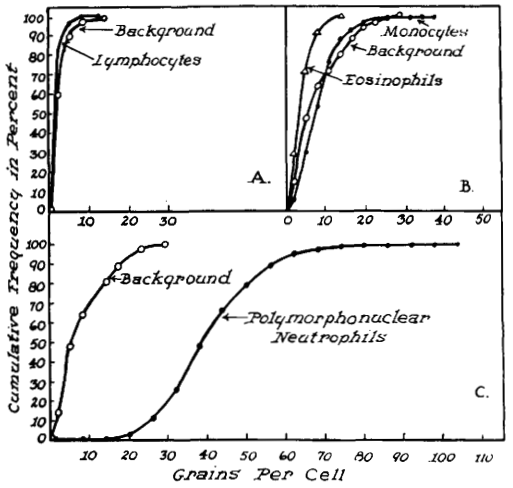

FIG. 1. Comparison of degree of labeling of normal blood leukocytes incubated *in vitro* with H^3-DFP. Distribution of grains over lymphocytes, monocytes and eosinophils is indistinguishable from background (A and B). In contrast, 85% of PMN neutrophils have more grains/cell than the most heavily labeled background areas (C).

monocytes had grain counts greater than the most heavily labeled background areas.

Typical examples of the autoradiographs obtained on normal blood are shown in Fig. 2.

Uptake of DFP by leukocytes in leukemic blood. Blood from subjects with chronic myelocytic leukemia (CML), acute myeloblastic leukemia (AML), chronic lymphocytic leukemia (CLL) and acute lymphoblastic leukemia (ALL) was incubated *in vitro* with H^3-DFP and the amount of label bound by the various morphologic cell types was determined. The results are shown in Table II.

Myeloblasts from AML and CML did not bind DFP; myelocytes contained approximately twice as much label per cell as metamyelocytes and PMN neutrophils exposed to the same concentration of H^3-DFP. Because the cytoplasmic granules were not visible in these autoradiographs it was difficult to identify promyelocytes, hence the significance of the promyelocyte grain count given in Table II may be questioned.

Lymphocytes and lymphoblasts from patients with acute or chronic leukemia did not bind DFP but those granulocytes which were present retained this property.

Since concentration of H^3-DFP and exposure time varied slightly in these studies, quantitative comparisons between samples cannot be made.

Binding of DFP by bone marrow as compared to blood leukocytes after labeling in vivo. To evaluate the initial level of cell labeling in the blood and marrow leukocytes, a hematologically normal subject with carcinoma of the mouth was given 2 mg of H^3-DFP intravenously. One and 3 hours later blood and bone marrow samples were obtained simultaneously. Autoradiographs were prepared in an identical manner so that quantitative comparison of marrow and blood labeling would be possible. The results are summarized in Fig. 3.

TABLE I. Mean Net Grain Count of Normal Blood Leukocytes Exposed to H^3-DFP.

Cell type	Subjects A	B	C
Polymorphonuclear			
Neutrophils	32.0	24.4	13.7
Eosinophils	-3.8	.2	.8
Lymphocytes	- .3	.5	0
Monocytes	.6	4.5	4.0

Blood from subjects A and B was labeled *in vitro*. Blood from subject C was labeled *in vivo*.

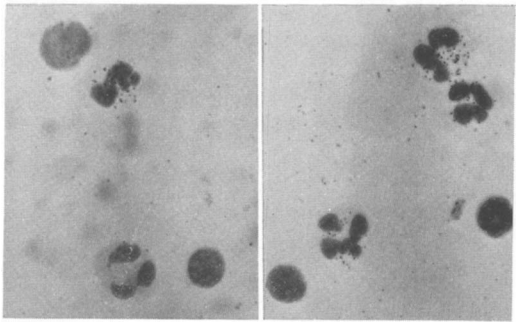

FIG. 2. Representative autoradiographs made from normal blood. Normal blood was incubated for 1 hr with 0.3 μg of H^3-DFP/ml of blood. Magnification 610 ×. (A) A monocyte and PMN neutrophil are in the left upper corner. An eosinophil and lymphocyte are in the lower right corner. Only the PMN neutrophil is labeled. (B) Three PMN neutrophils and 2 lymphocytes are seen. Again only the PMN neutrophils are labeled.

Blood PMN neutrophils contained 3 to 5 times as much label per cell as the marrow metamyelocytes and PMN neutrophils. The slightly higher grain count over the marrow PMN neutrophils as compared to the meta-

TABLE II. Mean Net Grain Count of Leukemia Blood Leukocytes Exposed to H^3-DFP.

Cell type	Subjects CML	AML	CLL	ALL
Myeloblast	.7	.1		
Promyelocyte	61.5			
Myelocyte	65.3			
Metamyelocyte	34.4			
Polymorphonuclear				
Neutrophil	27.2	28.9		29.8
Eosinophil	.2			
Basophil	.8			
Lymphoblast				.7
Lymphocyte			−.6	−.6

CML, AML, CLL and ALL refer to chronic myelocytic leukemia, acute myeloblastic leukemia, chronic lymphocytic leukemia, and acute lymphoblastic leukemia.

myelocytes was probably due to a number of more heavily labeled blood PMN neutrophils in the marrow preparations. The marked skewing of the marrow PMN neutrophil, grain-count distribution curve as compared to the other marrow granulocyte distribution curves supports this assumption.

The marrow myelocytes contained twice as much label per cell as the metamyelocytes and PMN neutrophils, a finding similar to that noted in chronic myelocytic leukemia blood (Table II).

Uptake of DFP by formed elements other than leukocytes. In the above studies label was not detectable in the erythrocytes, platelets, normoblasts or megakaryocytes.

Discussion. Myelocytes were the most heavily labeled cells in the bone marrow, while in blood only PMN neutrophils and perhaps a few monocytes were labeled.

Wachstein and Wolf(7) have demonstrated non-specific esterase in all of the formed elements of the blood and bone marrow. Furthermore, DFP^{32} has been used to label erythrocytes and platelets(8,9). However, in our studies, H^3-DFP was not demonstrated in marrow erythroblasts, myeloblasts, and megakaryocytes or in blood erythrocytes, platelets and lymphocytes. An explanation for the failure to detect H^3-DFP in these elements may be that the autoradiographic technic is relatively insensitive. It is also possible that suppression of the latent image by intracellular compounds(10) may have decreased autoradiographic efficiency over some of the cell types and thus interfered with detection of low levels of H^3-DFP binding. In any case it has been demonstrated in the present studies that concentration of DFP per granulocytic leukocyte is much greater than concentration per cell in any of the other formed elements in blood and bone marrow.

After intravenous injection of H^3-DFP, granulocytes in both the blood and bone marrow were labeled. In previous studies in which DFP^{32} was given intravenously the shape of the granulocyte disappearance curve suggested that as the heavily-labeled blood granulocytes left the circulation, they were replaced by cells from the bone marrow that contained less label. It was suggested that the bone marrow contained a store of granulocytes with about 30% as much label per cell as was initially present in the blood granulocytes(3). In an attempt to evaluate this postulate we have made quantitative estimates of degree of labeling of granulocytes in the blood and bone marrow after intravenous injection of H^3-DFP. Though we fully appreciate the difficulties in quantitative interpretations from autoradiographs, the only comparisons made here involve relative levels of labeling of different cells in the same or identically prepared autoradiographs. The present demonstration that marrow PMN neutrophils and metamyelocytes contained

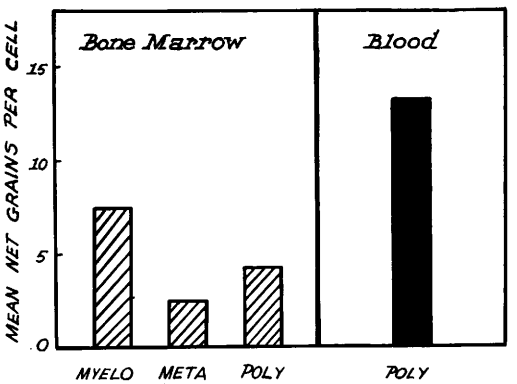

FIG. 3. Comparison of mean net grain counts in autoradiographs of marrow and blood granulocytes after intravenous injection of H^3-DFP. Mean net grain counts 3 hr after H^3-DFP injection are given.

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)

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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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