the blood at any given moment. The further study of blood plutonium levels in relation to administration of chelating agents would seem to offer much promise of enlightenment with regard to the mechanisms involved in the therapeutic effectiveness of these agents.

Summary. A single intravenous dose of diethylenetriaminepentaacetate (DTPA) one hour after plutonium administration resulted in excretion of approximately 90% of the plutonium. As compared with untreated controls, retention of plutonium in the skeleton was reduced by a factor of 10 and retention in the liver was reduced by a factor of 30. Two pigs treated with 5 consecutive daily injections of DTPA excreted 11 and 19% of the plutonium injected 60 days prior to treatment.

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Histidine Decarboxylase Activity of Basophils from Chronic Myelogenous Leukemic Patients. Origin of Blood Histamine.* (26554)

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It is well established that relatively large amounts of histamine are present in tissue mast cells(1-3), and leukocytes(4-6), especially the granulocytes and probably largely the basophils(4,7).† Eosinophils have also been claimed to contain histamine(7).

A polemic has existed since the first report of Code in 1936(4) regarding the origin of histamine in the body. Some argued that it originated solely from ingestion with food and from decarboxylation of histidine by intestinal flora, and others asserted that it also originated by the action of a tissue decarboxylase. The bulk of evidence supports the concept that tissue mast cells of mammalian organisms decarboxylate histidine to histamine(1-3,11) although some workers have denied this(8). Only a few investigators have ruled out bacterial decarboxylation in their *in vitro* studies. These are Werle, *et al.*(12) who used sterile precautions and tested for contamination after incubation; Gustafsson, Kahlson and Rosengren who worked with germ-free rats(9); and Schindler, Day and Fisher who worked with sterile

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† Whether or not the blood basophils are related to mast cells is not settled.

tissue cultures of malignant murine mast cells (11).

No one has demonstrated that a human tissue can synthesize histamine in the proved absence of bacteria, nor has the origin of blood histamine been elucidated. Schayer and Kobayashi(10) showed that unsterile rabbit blood platelets convert about 1.3% C^{14}-histidine to C^{14}-histamine, but the rabbit is the only species known which has a high concentration of histamine in the platelets, although histaminase may be present in the absence of histamine in the platelets of other species. For these reasons we attempted to demonstrate the ability of a sterile human cell, the blood basophil, to convert C^{14}-histidine to C^{14}-histamine.

Methods. The only practical way to obtain concentrated human blood basophils seemed to be to use the buffy layer from patients with chronic myelogenous leukemia and very high basophilia. Such cases are rare. The literature has been well documented recently(13). After a protracted search, 3 cases were found over a period of several years. Studies on the first were done with unlabeled histidine and the results were equivocal. The other 2 yielded satisfactory results with radioactive histidine.

To strengthen the evidence that the basophils alone synthesized histamine, buffy layers predominating in other types of leukocytes were studied as well. These included lymphocytes from a case of chronic lymphatic leukemia, myeloblasts from 2 acute myelogenous leukemic patients, and eosinophils from one case of parasitic infestation and 2 cases of non-specific drug reaction. Platelets from a case of polycythemia vera also were studied.

In each case, 25 ml of blood were drawn under sterile conditions, and immediately added to an equal volume of a solution containing 5% polyvinylpyrrolidone and 0.5% trisodium citrate(14). The erythrocytes were allowed to sediment for 30-60 minutes, and the supernatant plasma containing the leukocytes was drawn off with a syringe and centrifuged at 29.000 $\times$ G for 10 minutes. The plasma layer was removed and 0.1-0.2 ml of the wet packed cells washed once with isotonic Tyrode's solution, recentrifuged, transferred to a Potter-Elvejhem glass homogenizer (Kontes Conical Type) and homogenized in 1 ml ice cold distilled water.

The homogenates were incubated with 16 μg (in 100 λ) of L-histidine-2-(ring)-C^{14} (California Corp. for Biochemical Research, Los Angeles) containing 0.3 μC or 200,000 counts per minute (CPM) at infinite thinness in the Nuclear-Chicago low-background flow counter. Forty micrograms of pyridoxal-5-phosphate (Merck) in 2 ml of 0.1 M potassium phosphate buffer, pH 7.4, were added as cofactor. Aminoguanidine, 100 μg was added to inhibit diamine oxidase. The incubation was carried out in the Dubnoff Metabolic Incubator at 37°C for 3 hours. Controls were run in which the enzyme was inactivated with heat or 2 drops of concentrated perchloric acid. All solutions and glassware were sterile, and strictly sterile precautions were observed throughout, transfers being made under ultra-violet lamps. At the end of all incubations, aliquots of all flasks were tested for the absence of bacteria by streaking on conventional blood-agar plates. Occasional cultures were made in tubes of standard media. The results on the rare samples which showed contamination were discarded.

The labeled histamine formed was isolated as the picrate by the isotopic dilution method of Schayer, Davis and Smiley(15), plated out on planchets and counted at infinite thickness in a windowless counter on an automatic planchet changer (Tracerlab and Nuclear-Chicago).

Results are summarized in Table I. Platelets obtained from a case of polycythemia vera with a platelet count of 500,000, were isolated by a modification of the method of Klein, *et al.*(16), and similarly studied. No radioactive histamine was formed.

The results clearly show that only the buffy layers from the patients with basophilic leukemia formed histamine. In the second case, which gave 717 CPM (7170 at infinite thinness), the amount of histamine formed was estimated to be about 0.5 μg, representing about a 3% yield from the histidine added.

It would of course be desirable to obtain

TABLE I. Histidine Decarboxylase in Various Types of White Cells from Leukemic Patients.

Exp.	W.B.C.	Bas., %	Lympho., %	Eos., %	"Pro cells" and myeloblasts	Others, %	cpm*
N	15,100	77	5	5	0	10.0	415
S	71,370	49	3	7	2	39	717
B	301,000	0	98	0	0	2.0	0
Ba	54,000	0	0	0	99	1.0	2
R	30,000	0	—	43	—	57	1
O	13,000	0	49	28	0	23	1
M	15,000	0	—	70	—	30	0
Ch	15,400	0	22	0	78	0	0

* Counts after subtraction of cpm of identical acid killed blanks. The blanks were consistently about 20 cpm above a background of 2-5 cpm in the Nuclear-Chicago counter. The few samples run on the windowless Tracerlab counter also were 20-30 cpm above a background which usually averaged 25 cpm.

sterile basophils in pure form, possibly by modifications of the methods used for isolating mast cells, or by means of culturing them if possible. Nevertheless, the data offer an explanation for the origin of blood histamine other than that which might be absorbed from foods such as cheese, lobster, meat, etc., absorbed after decarboxylation of histidine by bacterial flora, or released from other sites in the body such as mast cells and gastric mucosa, although this is unlikely under most normal conditions.

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