Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 92 AUGUST-SEPTEMBER, 1956 No. 4

SECTION MEETING
SOUTHERN CALIFORNIA
Univeristy of Southern California June 7, 1956

Histidine Decarboxylase and Histamine Binding in Rabbit Platelets.* (22573)

RICHARD W. SCHAYER AND YUTAKA KOBAYASHI.
The Rheumatic Fever Research Institute, Chicago, Ill.

The rabbit is the only species known which has a high concentration of histamine in the platelets(1). The origin of this histamine has hitherto been unknown. In this paper we show that rabbit platelets, *in vitro,* have a pronounced ability to decarboxylate C^{14} L-histidine and to bind the resulting C^{14} histamine in stable condition. The enzyme histidine decarboxylase has been prepared from rabbit platelets in soluble form and a study of its properties reported.

Methods. In some of the first experiments the entire buffy coat of rabbit blood was used; later, platelet preparations, practically free of blood cells, were obtained by the method of McIntyre.† These two preparations will be referred to as "buffy coat," and "isolated platelets," respectively. Blood collection and platelet isolation were done in silicone-coated glassware. Platelet preparations were incubated with 10 γ C^{14} L-histidine(2) activity 2.4 x 10^7 cpm per mg base. Incubations were carried out under nitrogen, at 37°C in 0.5 to 1.0 ml isotonic phosphate buffer, pH 7.4, containing 0.2% glucose. The C^{14} histamine formed was determined by isotope dilution assay using a rigorous extraction procedure which eliminates contamination by the excess C^{14} L-histidine(3). The histamine was counted as the dipicrate at infinite thickness in flow counters‡ and corrected for background only (background 20-22 cpm). Under the experimental conditions described above there is no significant destruction of C^{14} histamine by rabbit platelet preparations.

Demonstration of binding of new C^{14} histamine by rabbit platelets. Isolated platelets from 50 ml blood were divided into 3 portions and incubated with C^{14} L-histidine as described. At the end of the incubation period the tubes were centrifuged and platelets and supernates analyzed separately for C^{14} histamine. Platelets gave values of 126, 127 and

* This investigation was supported by a research grant A-1087, from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Public Health Service.

† This unpublished method was kindly provided by Dr. Floyd McIntyre of the Abbott Laboratories. Rabbit blood is added to an equal volume of cold 2.5% sodium citrate solution and centrifuged in the cold for 5 minutes at 1200-1500 rpm. The cells are removed leaving a suspension of platelets.

‡ In one experiment (Fig. 2, upper curve) data were obtained by counting the samples in a Packard Tri-Carb Liquid Scintillation Spectrometer. For use in this counter it was necessary to prepare the dibenzenesulfonyl derivative of histamine(8) instead of the picrate.

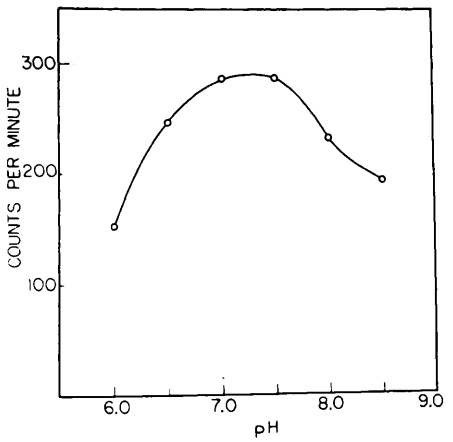

FIG. 1. Effect of pH on binding of histamine by rabbit platelets.

133 cpm averaging 129 cpm. Supernates gave values of 30, 32 and 33 cpm. Thus 80% of the newly formed histamine remains bound in the platelets.[5] The remaining 20% escapes into the supernate either by diffusion, or by damage to the platelets, during incubation and centrifugation. If all the C^{14} L-histidine present were decarboxylated, the activity of the histamine dipicrate would be 10,500 cpm.

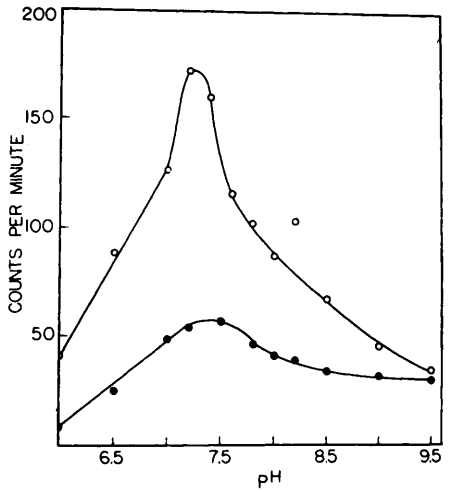

FIG. 2. Effect of pH on histidine decarboxylase activity. Upper curve, enzyme from isolated rabbit platelets; counts made with liquid scintillation counter. Lower curve, enzyme from rabbit buffy coat; counts made with flow counter.

[5] The buffy coats of human, rat and guinea pig blood were also tested for histidine decarboxylase activity; it was extremely low or absent in all cases.

Thus the values given above for the platelets correspond to about a 1.3% conversion of histidine to bound histamine.

Effect of pH on binding of C^{14} histamine. Aliquots of a suspension of rabbit buffy coat in saline were incubated with C^{14} L-histidine in phosphate buffers of various pH value. The effect of pH is shown in Fig. 1.

Effect of pH on histidine decarboxylase activity. Platelet preparations were suspended in saline, frozen and thawed 6 times, then centrifuged for 30 minutes at 25,000 x g. Aliquots of the supernate, free of particles, were added to buffers of various pH and incubated with C^{14} L-histidine as usual. Results are shown in Fig. 2.

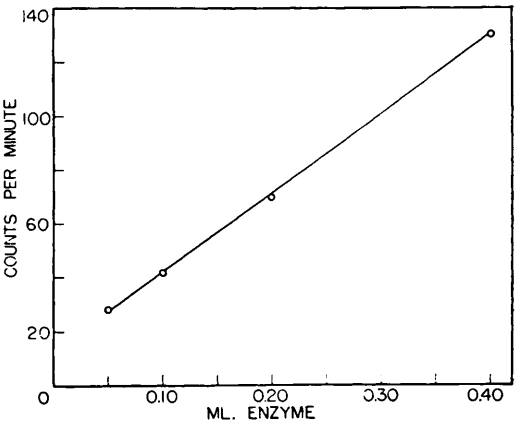

FIG. 3. Effect of concentration of histidine decarboxylase from isolated rabbit platelets on histamine formation.

Effect of concentration of histidine decarboxylase on rate of C^{14} histamine formation. Histidine decarboxylase from isolated platelets, prepared as described above, was used in varying concentrations for the incubation. Results are shown in Fig. 3.

Stability of histidine decarboxylase of rabbit platelets. Histidine decarboxylase was prepared from isolated platelets. The enzyme preparation was divided into 3 portions; one was assayed fresh while the other 2 were frozen, lyophilized and stored at —15°C. The latter were reconstituted by dissolving in distilled water and assayed at varying intervals of time. Triplicate assays of the fresh enzyme were 61, 64, and 68 cpm

TABLE I. Effect of Various Compounds on Activity of Histidine Decarboxylase of Isolated Rabbit Platelets.

Compound	Concentration, molar	Histidine decarboxylase activity*	Avg	% inhibition
Control		95, 98, 98, 100, 100, 104, 105	100	
Hydroxylamine	1×10^{-5}	105, 109	107	0
	1×10^{-4}	43, 48	46	54
β-2-thienylalanine	$.6 \times 10^{-3}$	89, 89	89	11
Potassium cyanide	$.6 \times 10^{-3}$	50, 65	58	42
Semicarbazide	$.6 \times 10^{-3}$	23, 23	23	77

* Data were obtained from 2 experiments. In order to make a comparison, avg counts per min. of controls were multiplied by a factor to convert to 100. Other values in this table were obtained by multiplying the observed count by the same factor.

averaging 64 cpm. A sample stored for 2 days assayed 71, 73 and 73 cpm averaging 72 cpm. A sample stored 3 weeks assayed 52, 56 and 58 cpm averaging 55 cpm. Thus the enzyme can be lyophilized and stored for a few days without loss of activity. There appears to be a slight loss of activity in samples several weeks old.

Effect of inhibitors on the activity of rabbit platelet histidine decarboxylase. Inhibition of histidine decarboxylase from various mammalian tissues, principally rabbit kidney, has been extensively studied(4). The effect of several of these inhibitors on the activity of rabbit platelet histidine decarboxylase is shown in Table I.

Discussion. We have demonstrated that isolated rabbit platelets have the ability to decarboxylate C^{14} L-histidine and to bind the resulting C^{14} histamine in stable form. Conversion of histidine to histamine by particle-free extracts of isolated rabbit platelets is shown to be enzymatic by measurements of the effects on activity of pH (Fig. 2), concentration (Fig. 3) and inhibitors (Table I). In studies on histidine decarboxylase from other laboratories the sources of enzyme were complex tissues like kidney and liver(5) in which an undetermined amount of the enzyme was from cells possessing a histamine-binding mechanism. It is believed that the present paper is the first to describe the properties of histidine decarboxylase from a relatively homogeneous source which possesses not only the ability to synthesize histamine, but also the ability to bind it in stable form. The inhibitors of histidine decarboxylase reported by Werle(4) are also effective on the enzyme from rabbit platelets. There are quantitative differences, however. Rabbit platelet histidine decarboxylase has a pH maximum at 7.2 to 7.4. This is markedly different from the higher values, about pH 9.0, reported by earlier workers for histidine decarboxylase from rabbit kidney(6). In a recent paper by Graham, *et al.*(7) a large number of tissues were tested for histidine decarboxylase activity at pH 9.2. On the basis of our finding of maximum activity at pH 7.2 to 7.4, and low activity at 9.2 it would seem necessary to find the pH maximum for each tissue before comparison of histidine decarboxylase activity is made.

Summary. Rabbit platelets decarboxylate C^{14} L-histidine and bind the resulting C^{14} histamine in stable condition. Soluble histidine decarboxylase has been prepared from rabbit platelets and a study of its properties reported.

1. Code, C. F., *Physiol. Rev.*, 1952, v32, 47.

2. Schayer, R. W., *J. Am. Chem. Soc.*, 1952, v74, 2440.

3. Schayer, R. W., Davis, K. J., and Smiley, R. L., *Am. J. Physiol.*, 1955, v182, 54.

4. Werle, E., *Biochem. Z.*, 1940, v304, 201; Holtz, P. and Heise, R., *Arch. exp. Path. Pharmak.*, 1937, v186, 269.

5. ———, *ibid.*, 1938, v296, 315.

6. Werle, E., and Herrmann, H., *ibid.*, 1937, v291, 105.

7. Graham, H. T., Hannegan, T. W., and Nourse, C. M., *Biochim. et Biophys. Acta*, 1956, v20, 243.

8. Schayer, R. W., *Am. J. Physiol.*, in press.

Received June 28, 1956. P.S.E.B.M., 1956, v92.