Effect of Adrenalectomy on Rate of Metabolism of Histamine in the Mouse.* (19895)

RICHARD W. SCHAYER, ROSA L. SMILEY, AND JEAN KENNEDY.
(Introduced by Alvin F. Coburn.)
From the Rheumatic Fever Research Institute, Northwestern University Medical School, Chicago, Ill.

Rose and Browne(1) have shown that adrenalectomized rats have a markedly decreased ability to inactivate injected histamine as compared to intact animals. The levels of histamine employed were necessarily very large. 24 μg per g of body weight. This change could be restored essentially to normal by administration of adrenocortical extract(2).

Because of the association of histamine with certain inflammatory states, and the anti-inflammatory activity of cortisone and ACTH, the implications of these findings are of considerable importance. The availability of radioactive histamine made it possible to repeat these experiments using much lower histamine concentrations. The mouse was chosen as the experimental animal so that the entire carcass could be analyzed for histamine.

Materials and methods. The synthesis of histamine, labeled in the 2-position of the imidazole ring, has been published(3). Male Swiss albino mice weighing approximately 20 g were adrenalectomized† or sham-operated 3 days before injection. In one experiment mice were injected intraperitoneally with 0.05 μg C^{14}-histamine‡ per g of body weight (Table I); in a second experiment the level was 1.0 μg C^{14}-histamine per g of body weight. (Table II). At various intervals of time after injection the mice were killed; the urinary bladders were removed, excepting mice killed less than 10 minutes after injection; the carcasses were immediately frozen in dry ice-acetone. The mice were skinned and each homogenized (Waring blendor) in water containing 40 mg carrier histamine; the skins were then added and homogenization continued. Protein was precipitated with trichloroacetic acid and histamine extracted from the filtrate and isolated as the dipricrate in a manner similar to that previously described (4). The histamine dipicrate was readily

TABLE I. % of C^{14}-Histamine in Mouse Carcass at Various Time Intervals Following Intraperitoneal Injection. .05 μg histamine/g body wt.

	Min.										
	3	5	7	10	12	15	20	30	60	90	120
Adrenalectomized	73	65	62	35	42	40	19	13	4	4	2.5
Sham-operated	69	67	34	32	38	27	24	10	4.5	3.4	1.8

TABLE II. % of C^{14}-Histamine in Mouse Carcasses 15 and 30 Min. Following Intraperitoneal Injection. 1 μg histamine/g body wt.

	Adrenalectomized	Sham-operated
15 min.	42	43
	33	32
	50	31
30 min.	22	20
	25	27
	35	47
	37	48

* Supported by a contract with the U. S. Atomic Energy Commission.

† The authors are indebted to Dr. Georges Ungar and Miss E. Irene Pentz for demonstrating the proper technic of adrenalectomy.

‡ Histamine solutions were in 0.004 N hydrochloric acid. The previously suggested procedure(5) of neutralization with sodium bicarbonate is not recommended if an appreciable time elapses before use; significant deterioration can occur in very dilute solutions under these conditions.

In many previous assays using this procedure we have found that minute amounts of C^{14}-histamine can be readily separated from very large excesses of histidine, histidine metabolites, and histamine metabolites, all of comparable radioactivity. In some cases the histamine dipicrate was recrystallized many times, using Norit, and often employing two different solvents alternately. The radioactivity was invariably constant after the second crystallization. To add further assurance that the radio-activity being measured was due to histamine and not a contaminant, histamine dipicrate samples were converted to the free base. In each case, after sublimation, the free histamine showed the calculated increase in specific activity.

purified to constant radioactivity by recrystallization from hot water using Norit.‖ The original histamine solution was assayed by adding the same amount used for injecting the mice to the usual amount of carrier (40 mg) then preparing and counting the dipicrate. For example 1.00 μg C^{14}-histamine added to 40 mg carrier, after conversion to the dipicrate, produced 500 counts per minute when counted at infinite thickness and corrected for background only. When 1.00 μg C^{14}-histamine was injected into a mouse the isolated histamine dipicrate produced 200 counts per minute at infinite thickness and corrected for background only. Therefore, the percentage of histamine remaining in the entire mouse is $200/500 \times 100 = 40\%$. All counts were made on 4.5 square cm plates in flow counters with background about 20 c.p.m.

Results. The data of Tables I and II show that although there are often marked differences between individual mice treated identically, there is no significant difference in the rate of histamine destruction between adrenalectomized and sham-operated mice. This discrepancy with the findings of Rose and Browne may be due to differences in histamine concentrations administered, species differences, or variations in experimental procedure. In any event the results suggest that the participation of the adrenal cortex in histamine inactivation under physiological conditions is questionable and that the matter requires further clarification.

Summary. No significant difference was found between adrenalectomized and sham-operated mice in the ability to destroy minute quantities of injected C^{14}-histamine.

1. Rose, B., and Browne, J. S. L., *Am. J. Physiol.*, 1938, v124, 412.
2. Rose, B., *Am. J. Physiol.*, 1939, v127, 780.
3. Schayer, R. W., *J. Am. Chem. Soc.*, 1952, v74, 2440.
4. ———, *J. Biol. Chem.*, 1952, v199, 245.
5. ———, *J. Biol. Chem.*, 1952, v196, 469.

Received July 31, 1952. P.S.E.B.M., 1952, v81.

Effect of Epidermal Damage upon Serum Polysaccharides. (19896)

WILLIAM H. KNOBLOCH, JR., PATRICK NAGLE, C. L. SHETLAR, AND M. R. SHETLAR.
(Introduced by A. A. Hellbaum.)

*From the Departments of Biochemistry and Surgery, University of Oklahoma School of Medicine, the Research Laboratory of the Veterans Administration Hospital, and the Oklahoma Medical Research Foundation, Oklahoma City.**

Elevations of serum polysaccharides have previously been reported by Shetlar, *et al.*(1), to occur in dogs following production of sterile turpentine abscesses, of bacterial abscesses and of talc granulomas, the injection of turpentine intrapleurally, and experimental surgery. The cause and mechanism of these elevations is of considerable interest. Seibert *et al.*(2), have suggested that the elevation noted in malignancies, tuberculosis, and other pathological conditions is related to tissue destruction. Shetlar *et al.*(1) have suggested that this phenomenon may just as logically be correlated with tissue proliferation and repair. Since a number of carbohydrate rich protein fractions occur in serum, it also seems likely that they may vary differentially. Shetlar *et al.*(3) using a sodium sulfate fractionation procedure, noted that the distribution of polysaccharide among different serum protein fractions varied in different diseases. In the course of a study of treatment of thermal injury in dogs the opportunity to study this problem further presented itself. If the source of the serum polysaccharides, which rise after injury, is the injured tissue itself, one would expect the lymph polysaccharides to rise after injury at a more rapid rate than those of the serum. Consequently, the experiment was designed to allow polysaccharide studies of lymph samples from dogs at various times after burning.

Methods. In this study nonglucosamine

* Contribution No. 25 from the Oklahoma Medical Research Foundation.