PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 25–29 (1974)

Role of Histaminase and Corticosterone on the Depletion of Tissue Histamine in the Irradiated Rats (38036)

SHIGERU KOBAYASHI
(Introduced by T. Sado)

Division of Physiology and Pathology, National Institute of Radiological Sciences, Chiba, Japan 280

An exposure of animals to lethal or sublethal doses of radiation causes a significant loss of systemic histamine. Since the loss is generally accompanied by an increase in the histamine level of the blood as well as of the urine (see Bacq and Alexander (1) for review), investigators in this field have directed their attentions to the liberation of histamine from the damaged mast cells (2–4). Two other factors that must be considered in studying the loss of histamine from the irradiated tissues are: (a) the alteration of the activity of tissue histaminase and (b) the role of adrenocortical function on this enzyme activity. For example, the histaminase (diamine oxidase) has been known to be a major histaminolytic enzyme in a variety of animal species studied (5), and therefore, alteration in its activity must necessarily affect the content of tissue histamine. However, the role of this enzyme on the depletion of tissue histamine of the irradiated animals has not received adequate attention. On the other hand, it is also known that irradiation of animals causes an activation of the adrenocortical function (6, 7), and that corticoid might affect the tissue histamine level (8–11) through its enhancing effect on histaminase activity (8, 11). Thus, in the present study, attempts have been made to examine (a) the relationship between the histamine contents and the histaminase activities of a variety of tissues and organs of irradiated rats and (b) the role of corticoid on the histaminase activity of the lung of irradiated as well as nonirradiated, adrenalectomized rats. The results indicate that the histamine depletion of irradiated tissues could result not only from its liberation from the damaged mast cells (3) but also from the enhanced histaminase activity of the irradiated tissues due to the activation of adrenocortical function.

Materials and Methods. Animals. Male Wistar strain rats weighing 250–300 g were given 650 R of whole-body X-irradiation (Shimazu Shin'ai 250 X-ray generator operated at 200 kVp, 20 mA; with 0.5 mm Cu + 0.5 mm Al filters; focus-skin distance of 100 cm; dose-rate, 25 R/min in air). Two, four and seven days after the irradiation, the treated animals were sacrificed by cervical dislocation. After collection of blood by heart puncture with a heparinized syringe, the liver, kidney, lung and small intestine (a part of the duodenum) were taken out. The blood plasma was obtained by centrifugation of the heparinized blood at 4°. During the course of the tissue– and plasma–preparation, care was taken to avoid adsorption of histamine to the glasswares by the use of paraffin-coated pipettes and/or plastic tubes.

Assessment of histaminase activity. Based on a preliminary test of an assay method of tissue histaminase, the following procedure was used for the assessment of this enzyme activity. Fresh tissues were homogenized in 3 volumes of Krebs-Ringer bicarbonate buffer, pH 7.4 using a glass homogenizer placed in an ice bath. The homogenates were then assessed for histaminase activities. The reaction mixture for this assessment consisted of the following: 2 ml (in case of lung and small intestine)

or 4 ml (in case of liver, kidney and plasma) of tissue homogenates, 15 μmoles (for lung and small intestine) or 1.0 μmoles (for liver, kidney and plasma) of neutralized histamine·2HCl, 20,000 cpm amount of ^{14}C-histamine (ring-2-^{14}C-histamine·2HCl; sp act, 54.3 mCi/mmole; The Radiochemical Centre, Amersham), 30 μmoles of glucose, 5 μmoles of streptomycin, and Krebs-Ringer bicarbonate buffer, pH 7.4, to make a final volume of 5 ml. This reaction mixture was then incubated in a plastic tube for 2 hr at 37° under 5% CO_2 and 95% O_2 atmosphere. After the incubation, the reaction mixture was chilled in an ice bath and mixed with 60,000 cpm of carrier ^{3}H-histamine·2HCl (^{3}H-histamine·2HCl (G); sp act, 7.2 Ci/mmole; New England Nuclear Corp., Boston). Perchloric acid was then added to the reaction mixture to make a final concentration of 0.5 *N*. From this reaction mixture, the histamine was extracted into *n*-butanol according to the method of Shore *et al.* (12). The resulting butanol fraction was washed with salt-saturated 0.1 *N* NaOH, and, to remove the salt from the extract, the butanol fraction was mixed with 1.5 volumes of toluene and centrifuged for 20 min at 3,000 rpm. The organic phase was evaporated to dryness. The resulting residue was then chromatographed on a chromatogram sheet (6060 Silica gel, Eastman Kodak Company, Rochester) in the solvent system of *n*-butanol: conc. ammonia water:ethanol (30:8:5, v/v). After development of the chromatograph for 2.5 hr, the chromatogram sheet was scanned for radioactivities by a chromatogram scanner (TLC-1, Aloka, Tokyo). The radioactive histamine area corresponding to the reference histamine (0.45 R_f values) was cut off and put into a counting vial. After addition of 1.0 ml of ethanol, the vial was warmed at 37° for 15 min, followed by a further addition of 10 ml of toluene containing 0.4% PPO (2,4-diphenyloxazole) and 0.01% POPOP (β-bis-(2-phenyloxazole)-benzene) (13), and then the radioactivity was measured by a liquid scintillation counter (Mark I, Nuclear Chicago, Chicago) using a double-channel operation. In order to obtain net ^{3}H-counts, an appropriate correction was made. The rate of disappearance of histamine during the incubation period was calculated from the difference in the ^{14}C/^{3}H ratio between the zero time control and incubated sample. The histaminase activity was expressed as μmoles of substrate histamine decomposed/g tissue/hr.

Histamine determination. The histamine content was assessed by the method of Shore *et al.* (12) with a slight modification, which was an introduction of a tracer amount of ^{3}H-histamine for recovery correction. Briefly, 0.5–1.5 g of tissue or blood plasma was homogenized in a Teflon homogenizer containing 2–4 ml of 0.5 *N* perchloric acid and 10,000 cpm amount of ^{3}H-histamine. Subsequent procedure, i.e., extraction of histamine into *n*-butanol and return of the histamine to an aqueous solution (0.1 *N* HCl), were essentially the same as those described by these workers. Finally, an aliquot of the 0.1 *N* HCl phase was used for histamine determination by *o*-phthalaldehyde fluorescence method (12). Another aliquot was transferred to a counting vial and dried with a stream of N_2. The vial was then added with ethanol and toluene phosphor, and the radioactivity was measured with the use of the liquid scintillation counter. The result of this radioactivity measurement was used for recovery correction.

Treatment of adrenalectomized rats. Rats were adrenalectomized bilaterally by the dorsal route under pentobarbital sodium anesthesia and were divided into 3 groups. Three days after the operation, the first group of rats were exposed to a whole-body X-irradiation of 650 R; the second group received 8 hourly ip injections of corticosterone (1 mg/kg of body weight) suspended in 0.5 ml saline for 3 days; the third group was given 8 hourly ip injections of 0.5 ml saline for 3 days. All of the adrenalectomized rats were given 1% NaCl solution *ad lib* throughout the experimental period. They were sacrificed at 6 days after the operation. In order to elucidate the difference in the enzymic response to X-irradiation between adrenalectomized and nonadrenalectomized rats, two additional groups of unoperated rats, one irradiated and the other nonirradiated, were also used as controls.

Results. Evaluation of the assay method of histaminase. The assay method of histaminase was first evaluated by employing the rats lung homogenates. The results indicate the following: (a) Apparent rate of reaction was almost proportional to the amount of the probable enzyme activity present in the reaction mixture. (b) There was a nearly proportional increase in the amount of histamine metabolized as a function of the incubation time within a period of 2 hr. (c) Inhibitory effect of substrate histamine on the histaminase activity was observed over the range of substrate concentrations examined (up to 3 m*M*) as has been shown by Kapeller-Adler (14) and others.

Effect of X-irradiation on histaminase activity and histamine content. The results of the effect of irradiation on the histaminase activity and histamine content of various tissues and organs of the rats are shown in Figs. 1 and 2. In the blood plasma of 650 R irradiated rats, there was no significant change in the histamine level at all time intervals studied (Fig. 1). Furthermore, histaminase activity could not be detected in the plasma or in the heparinized whole blood of either irradiated and non-irradiated rats. The histamine content of the liver began to decrease on the second day and, on days 4 and 7, it was about 30% of the control level. However, the histaminase activity of the liver did not change significantly during the period of observation (Fig. 1). Histamine contents as well as histaminase activities in the lung and small intestine were higher than those observed in the liver and kidney. The shape of the curve of histamine content of the small intestine at various time intervals after irradiation was similar to that obtained with the liver (Fig. 2) but the enzyme activity of the small intestine decreased gradually until day 7. Thus, it appeared that there was no correlation between the histamine contents and histaminase activities in both liver and small intestine. In contrast, a negative correlation between these parameters was observed in the kidney and the lung; the histamine content of the kidney decreased to 60% of the control level

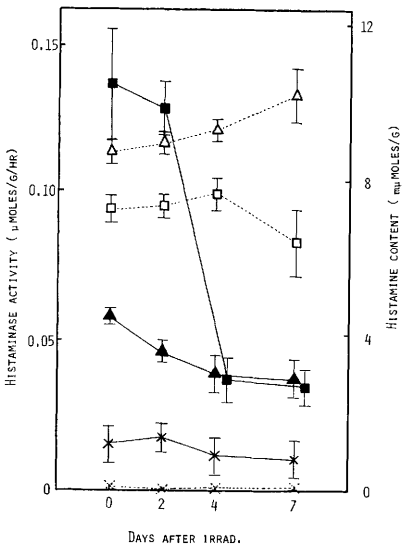

FIG. 1. Effect of whole-body X-irradiation of 650 R on the histaminase activities and the histamine contents in the kidney, liver, and blood plasma. Each value represents the mean ± S.E. from 4–8 determinations. △, Enzyme-kidney; ▲, content-kidney; □, enzyme-liver; ■, content-liver; ⁜, enzyme-blood plasma; ×, content-blood plasma.

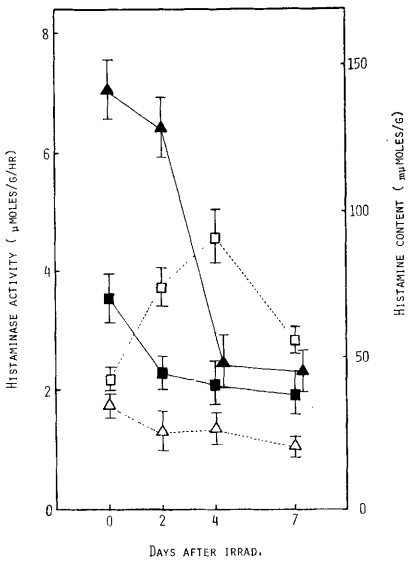

FIG. 2. Effect of whole-body X-irradiation of 650 R on the histaminase activities and the histamine contents in the lung and small intestine. Each value represents the mean ± S.E. from 4–8 determinations. □, Enzyme-lung; ■, content-lung; △, enzyme-small intestine; ▲, content-small intestine.

by day 7, while the histaminase activity of this organ increased gradually up to day 7 following irradiation (Fig. 1). Similarly, the histamine content of the lung decreased to 60% on day 2 and stayed on this level by day 7, while the histaminase activity of this organ increased significantly during the first 4 days after irradiation, reaching the maximum level of 200% of the control on day 4, and returned toward the original level by day 7 (Fig. 2).

Effect of corticosterone administration and X-irradiation on the histaminase activity of the lung of adrenalectomized rats. It has been known that an exposure of animals to lethal or sublethal doses of ionizing radiations elicits an activation of adrenocortical function and that corticoid affects the level of tissue histamine (8–10). Therefore, an experiment was designed to test a possibility that apparent enhancement of the lung histaminase activity following irradiation could have been due to an increased corticosterone production induced by X-irradiation. The results are summarized in Fig. 3. As has been shown previously, the histaminase activity of the lung of intact rats increased after irradiation. However, when adrenalectomized rats were exposed to the same total doses of radiation, no increase in the histaminase activity was observed. It was also shown that the histaminase activity of the lung of adrenalectomized rats increased significantly by administration of corticosterone for 3 days ($P < 0.05$).

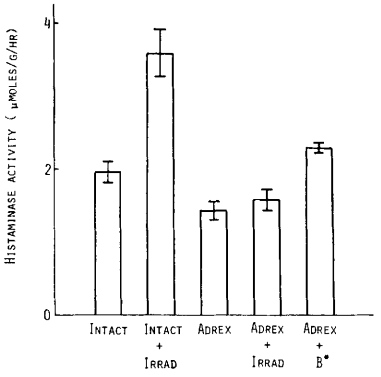

FIG. 3. Effect of whole-body X-irradiation of 650 R and corticosterone administration on the histaminase activity of the lung. Each value represents the mean ± S.E. from 5 determinations. * Compound B, corticosterone.

Discussion. The results presented in this paper show that an exposure of rats to 650 R of X-rays causes a reduction of histamine contents in various tissues and organs but this fall is not accompanied by a concomitant increase in the level of plasma histamine. These results are in conflict with a claim of Eisen *et al.* (3). These investigators related their findings on the depletion of tissue histamine of the irradiated rats with the increased histamine level in the blood reported by Weber and Steggerda (15). If the tissue histamine was released into the blood circulation, one should expect a concomitant rise in the histamine level of the blood. Indeed, Weber and Steggerda (15) reported that the histamine level of the blood plasma of rats increased once at 2 hr and then decreased to the control level and increased again at 5 days after irradiation with 600 R. In contrast, the results reported in this paper showed no significant change in the histamine content of the blood plasma at all time intervals studied (2, 4 and 7 days after irradiation). This discrepancy might be due to the difference in the assay method used. We consider that the present results appear more valid than those reported by these workers, since our data were based on a chemical assay method supplemented by a recovery correction.

It has been reported by others that a significant loss of systemic histamine was observed between the second and fifth day following irradiation (3). On the other hand, the rate of urinary excretion of histamine was reported to have increased only during the first 24 hr after irradiation (16). Furthermore, the data presented in this paper indicated that the histaminase activity of the whole blood as well as of the plasma was negligible in both irradiated and non-irradiated rats. These observations suggest an existence of a mechanism different from the histamine release for the disappearance of systemic histamine from the irradiated rats. In this connection, it is interesting to note that there was a negative correlation between the histamine content and the histaminase activity in some organs tested, i.e.,

the kidney and the lung (Figs. 1 and 2). This observation strongly suggests that the tissue histaminase has an important role in the depletion of histamine from certain tissues and organs of the irradiated rats.

Another important observation in this study was that X-irradiation had no effect on the histaminase activity of the lung of the adrenalectomized rats, while the activity of this enzyme increased significantly in similarly irradiated nonadrenalectomized rats. This result suggests that an enhancement of the tissue histaminase activity may be mediated through an activation of the adrenocortical function. The observation that treatment of adrenalectomized rats with corticosterone for 3 days resulted in a significant increase in the histaminase activities is consistent with this notion. Thus, it would appear that the elevation of plasma corticosterone level is also an important factor responsible for the depletion of tissue histamine in the irradiated rats. It may be concluded from these observations that the reduction of systemic histamine level in the irradiated rats results not only from the release of histamine from the destructed mast cells (3) but also from an acceleration of its destruction due to an increase in the histaminase activity in tissues and organs which is mediated through the activation of adrenocortical function.

Summary. The relationship between histamine contents and histaminase activities of the liver, small intestine, kidney, blood plasma and lung of rats exposed to 650 R of whole-body X-irradiation was examined. There was no correlation between these parameters in the liver and small intestine. In contrast, a negative correlation between these parameters was found in the kidney and the lung at 2, 4 and 7 days after irradiation. In particular, the histaminase activity of the lung increased significantly during the first few days after irradiation, reaching the maximum level of 200% of the control on day 4, and returned to the control level by day 7. Concomitantly, a gradual decrease in the histamine content in this organ was observed. Another important observation relates to the role of adrenocortical function on the activation of lung histaminase. Histaminase activity of the lung, which normally increased after irradiation of the intact rats, failed to increase when the animals had been adrenalectomized prior to the exposure to radiation. Data also showed that the activity of this enzyme elevated significantly when adrenalectomized rats were administered with corticosterone for 3 days. On the basis of these observations, significant roles of corticosterone and histaminase in the depletion of tissue histamine from irradiated rats were discussed.

The author gratefully acknowledges Dr. Toshihiko Sado for his help in the preparation of this manuscript, Drs. Masatoshi Seki, Nagasumi Yago, and Wataru Nakamura for helpful criticisms and Miss Hiromi Kurokawa for technical assistance.

1. Baqc, Z. M., and Alexander, P., "Fundamentals of Radiobiology" (2nd ed.), p. 411. Pergamon Press, New York (1961).

2. Smith, D. E., and Lewis, Y. S., Proc. Soc. Exp. Biol. Med. **82**, 208 (1953).

3. Eisen, V. D., Ellis, R. E., and Wilson, C. W. M., J. Physiol. **133**, 506 (1956).

4. Kiss, J., Strahlentherapie **104**, 624 (1957).

5. Schayer, R. W., Physiol. Rev. **39**, 116 (1959).

6. Hameed, J. M. A., and Haley, T. J., Rad. Res. **23**, 620 (1964).

7. Ichii, S., Kobayashi, S., and Omata, S., J. Rad. Res. **6**, 97 (1965).

8. Rose, B., and Browne, J. S. L., Amer. J. Physiol. **131**, 589 (1941).

9. Hicks, R., and West, G. B., Nature **182**, 401 (1958).

10. Telfold, J. M., Nature **197**, 600 (1963).

11. Karady, S., Rose, B., and Browne, J. S. L., Amer. J. Physiol. **130**, 539 (1940).

12. Shore, P. A., Burkhalter, A., and Cohn, V. H., Jr., J. Pharmacol. Exp. Therap. **127**, 182 (1959).

13. Snyder, S. H., Baldessarini, R. J., and Axelrod, J., J. Pharmacol. Exp. Therap. **153**, 544 (1966).

14. Kapeller-Adler, R., Int. Union Biochem. Symp. Ser. **30**, p. 429, 445 (1963).

15. Weber, R. P., and Steggerda, F. R., Proc. Soc. Exp. Biol. Med. **70**, 261 (1949).

16. Leitch, J. L., Debley, V. G., and Haley, T. J., Amer. J. Physiol. **187**, 307 (1956).

Received May 29, 1973. P.S.E.B.M., 1974, Vol. 146.