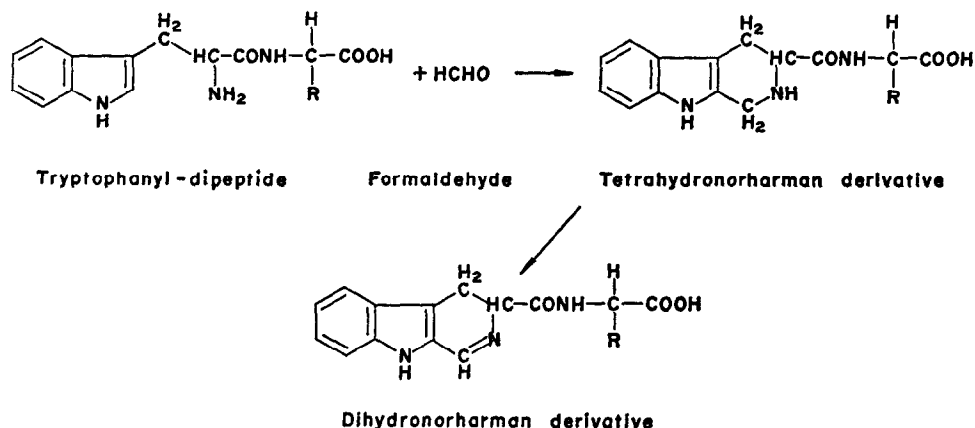sequence of reactions takes place with the tryptophanyl-peptides according to the following reaction scheme:

Tryptophanyl-dipeptide   Formaldehyde   Tetrahydronorharman derivative

Dihydronorharman derivative

Conceivably, all peptides and proteins having an N-terminal tryptophan residue will form highly fluorescent conjugates with formaldehyde under the reaction conditions specified. The method has been successfully adapted both for the chromatographic[6] and fluorescence histochemical detection[7] of such peptides.

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Changes in histaminase content following experimental thermal injury

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HISTAMINASE catalyses the oxidative deamination of histamine and occurs widely in many tissues. Plasma histaminase levels are remarkably increased in pregnancy.[1,2] Histaminase levels have been found, in this laboratory and others, to be raised in myocardial infarction,[3] bronchial asthma[4,5] and anaphylaxis.[6-9] Furthermore, histamine is known to be involved in many tissue challenge situations

like anaphylaxis and burn and as such probably contributes to peripheral tissue manifestations. The level of histamine in tissue at any given moment would be influenced not only by its storage level and rate of formation, but also by its catabolism. Hence, any possible relationship between tissue challenge and histaminase activity is of interest. The present investigation was undertaken to examine the influence of thermal injury on histaminase in experimental animals, some preliminary findings having been presented in an earlier study.[10]

Although the common identity of histaminase with diamine oxidase has not been established in all aspects, for the purposes of the present communications, the two enzymes would be assumed to be identical.

Methods and materials

Thermal injury *in vivo* was inflicted on urethane-anaesthetized rats and guinea-pigs by partial immersion in hot water at 52° and 60° respectively. Heparin was not administered to these animals since heparin has been shown to release histaminase *in vivo*.[11,12] Blood was collected immediately before, and 5, 15, 30 and 60 min after thermal injury, via polythene cannulae tied into the common carotid artery. Plasma histaminase was estimated according to the method of Aarsen and Kemp.[13] For estimation of histaminase content in tissue, the material was homogenized in 8 vol. of chilled phosphate buffer (pH 7·2) and centrifuged. The supernatant was treated in the same way as plasma. Results were expressed in terms of Provisional Units, one Provisional Unit being taken to be that required to produce an increase of 0·01 in optical density at 470 mμ after incubation for 4 hr according to the method of Aarsen and Kemp.[13]

Thermal injury *in vitro* was inflicted upon rat intestine utilizing a preparation previously described by the present workers.[14] In this procedure, two adjacent pieces of rat jejunum were slit along the mesenteric border, gently washed, and equilibrated in oxygenated Tyrode at 37° for 15 min. The test piece was then exposed for 5 sec to oxygenated Tyrode at 52°, after which it was again incubated in oxygenated Tyrode at 37° for any designated period after which it was transferred to ice-cold Tyrode for subsequent assay. The other piece (control) was treated in the same way as the test piece except that thermal injury was not inflicted.

Rats were depleted of histamine by subcutaneous injection of Polymyxin B twice daily for 5 days, the dose being gradually increased from 0·75 to 7·5 mg/kg. Extent of histamine depletion was judged from skin histamine content, estimated according to the method of Parratt and West.[15] The histamine-depleted group in the present experiment had 10–20 per cent of that of the control group.

Results

For thermal injury experiments *in vivo* in rats and guinea pigs, a control series was run in which thermal injury was omitted. In these control animals there was no significant difference between plasma histaminase content of samples collected over a period of 1 hr. However, thermal injury was followed by enhanced plasma histaminase levels both in rats and guinea-pigs. In rats, the rise was marked; of the samples examined, plasma collected 5 min after thermal injury contained the highest amounts of histaminase; plasma histaminase levels gradually declined thereafter.

In guinea pigs also there was a rise in the plasma histaminase level following thermal injury; however, the rise was not as marked as in the case of rats. Among the samples estimated, plasma collected 5 min after injury contained the highest amounts of histaminase. Levels returned to normal within 1 hr.

TABLE 1. PLASMA HISTAMINASE LEVEL CHANGES IN RATS AFTER THERMAL INJURY; FIGURES IN PARENTHESES INDICATE NUMBER OF EXPERIMENTS

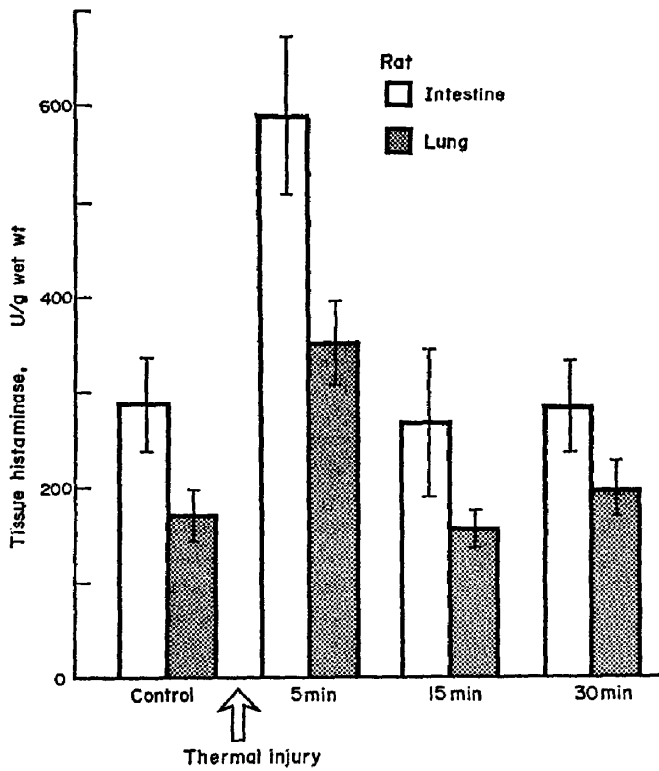|	Histaminase, Pr. units/ml plasma				
		Time (min)			
	Control	5	15	30	60
Control	7·3 ± 2·2 (10)	8·8 ± 2·8 (10)	8·9 ± 2·6 (10)	7·1 ± 2·0 (12)	8·2 ± 1·3 (14)
Thermal injury	7·9 ± 2·0 (10)	54·3 ± 11·8 (10)	26·2 ± 7·1 (10)	18·9 ± 5·0 (12)	17·3 ± 2·9 (14)

FIG. 1. Rat intestine and lung histaminase levels after thermal injury *in vivo*. Vertical bars represent standard error.
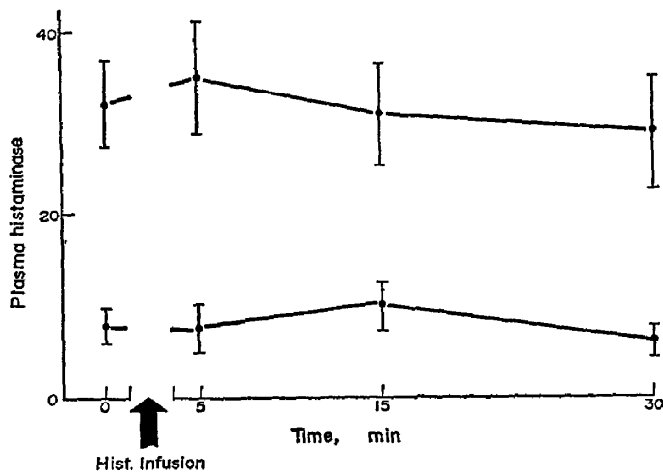

FIG. 2. Plasma histaminase levels in guinea-pig (upper curve) and rat (lower curve) before and after histamine infusion. Vertical bars represent standard error.

TABLE 2. PLASMA HISTAMINASE LEVEL IN GUINEA PIG AFTER THERMAL INJURY; FIGURES IN PARENTHESES DENOTE NUMBER OF EXPERIMENTS

	Histaminase Pr. units/ml plasma				
	Time (min)				
	Control	5	15	30	60
Control	35·8 ± 7·3 (8)	38·3 ± 5·2 (9)	39·1 ± 8·1 (9)	32·1 ± 6·1 (8)	36·4 ± 5·8 (8)
Thermal injury	31·6 ± 5·8 (8)	49·2 ± 9·8 (9)	58·8 ± 12·3 (9)	37·3 ± 6·7 (8)	35·2 ± 5·5 (8)

TABLE 3. CHANGES IN PLASMA HISTAMINASE LEVEL FOLLOWING THERMAL INJURY IN HISTAMINE-DEPLETED RATS

	Rat plasma histaminase, Pr. units/ml		
	Time after thermal injury (min)		
	Control	5	15
Mean ± S.E.	6·9 ± 1·3	47·6 ± 8·5	23·8 ± 3·9
Number of animals	(7)	(7)	(5)
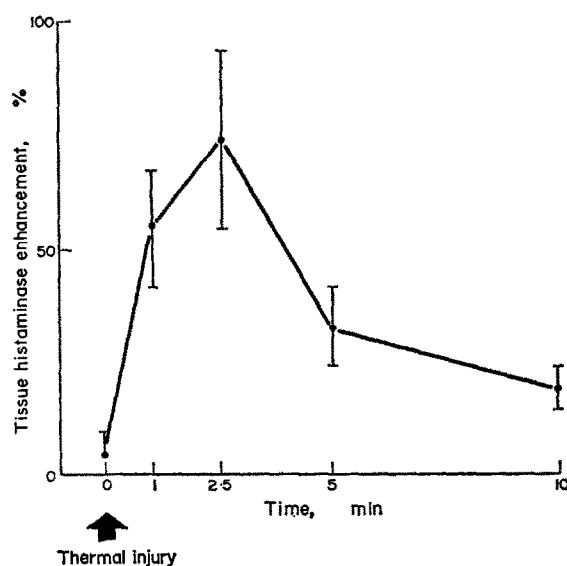
FIG. 3. Rat intestine: tissue histaminase content after thermal injury *in vitro*. The enhancement is shown in terms of percentage increase over control, taking the control value as 100 per cent.

In order to examine the tissue histaminase levels after thermal injury, rats were sacrificed before and at predetermined intervals after injury. The histaminase content of intestine and lung, two comparatively histaminase-rich tissues, were examined. In rat intestine and lung there was a rise in the tissue histaminase content.

The highest levels were obtained in rat 5 min after injury—i.e. at the same time as the highest levels of plasma histaminase.

The effect of altered histamine level *in vivo* on plasma histaminase content was also examined. To increase histamine level, histamine was administered by slow infusion to artificially ventilated rats (1 mg/kg) and guinea-pigs (0·25 mg/kg). This was not followed by any significant alteration of plasma histaminase level.

In histamine-depleted rats, there was no significant difference of pre-injury plasma histaminase content from that of control rats; furthermore, histaminase level was also enhanced after thermal injury.

The effect of thermal injury *in vitro* was examined on adjacent matched segments of slit rat jejunum mentioned above. The histaminase content of the two segments of the pair, when neither of them were exposed to thermal injury, differed very little, as can be seen from Fig. 3. However, as compared with the control tissue, the histaminase content of the injured tissue was considerably enhanced, which indicates that enhancement occurs after injury *in vitro* also. The time course involved in the enhancement of tissue histaminase content after thermal injury was examined by employing different incubation periods (1, 2·5, 5 and 10 min) following thermal injury. The findings have been shown in Fig. 3, and indicate that of the incubation periods examined the highest levels were found 2·5 min after injury. This indicates that the tissue enhancement occurs very quickly.

Discussion

Thermal injury thus appears to be followed by an enhanced plasma histaminase level. In the earlier study[6] by the present workers heparinized animals had been used; plasma histaminase levels were enhanced, though heparinized control levels were higher than in nonheparinized ones, particularly in the guinea-pig. In order to avoid this effect, heparin was omitted in the present series of experiments so as to eliminate its effect in view of the recent findings[7,8] which indicate that it raises plasma histaminase levels. The enhanced plasma histaminase levels after thermal injury is accompanied by an increased tissue histaminase content. Possibly the primary increase occurs in tissue which is reflected in the enhanced plasma levels. The increased histaminase levels appear more likely to be due to formation *de novo* rather than by depletion from preformed storage sites. The sequence of events leading to enhancement is not yet clear and requires further investigation. The steps involved could possibly be, in view of the time-course involved, formation from a precursor or by activation of inactive substance; induced enzyme synthesis also has to be considered. *Per se*, histamine released by tissue injury does not seem to be the trigger event since histamine infusion failed to enhance histaminase levels in the present series of experiments. This is in accord with the findings of Southren *et al.*[16] who did not find any increase in diamine oxidase activity of the plasma of women who had been injected histamine.

The enhancement of histaminase levels after thermal injury to histamine-depleted rats also supports the likelihood that enhancement of histaminase level is not changed directly and causally by altered histamine levels.

Preliminary experiments done in this laboratory to explore the possible relationship between histamine release and histaminase enhancement suggest some degree of correlation (Lahiri, unpublished observations, 1971). This does not necessarily indicate a causal relationship; both may be the effect of thermal injury, in which case the magnitude of both these effects would reflect the severity of injury.

The enhancement of histaminase activity may be of significance, since this will tend to lower histamine content which may affect the peripheral tissue responses contributed by histamine in thermal injury. Another feature is also worthy of note; apart from histamine, polyamines such as spermine are also histaminase substrates.[17,18] The biological significance of polyamines in diverse areas such as tissue regeneration and inter-relationship with nucleic acid metabolism is now being realized.[19] The present findings acquire further interest in this context.

In view of the present findings and the anaphylactic enhancement of histaminolytic activity, one may speculate that histaminase enhancement is one of the general responses of tissue challenge. Examination of other models of injury are needed before further conclusions can be reached. Some of the aspects mentioned above are now under investigation in this laboratory.

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Influence of trimedoxime and atropine on acetylcholinesterase activity in some parts of the brain of mice poisoned by isopropylmethyl phosphonofluoridate

THE COMMONLY accepted principles of treatment of acute organophosphate poisoning comprise the use of parasympaticolytic drugs and cholinesterase-reactivating substances, atropine[1,2] being the preferred parasympaticolytic. There is less agreement on the choice of cholinesterase-reactivating substances for the treatment of organophosphate poisoning. From many investigated reactivators pralidoxime (1-methyl-2-hydroxyiminomethylpyridinium iodide),[3–5] obidoxime [1,3-bis(4-hydroxy-iminomethylpyridinium)-2-oxapropane dichloride][6,7] and trimedoxime [1,1′trimethylenebis(4-hydroxyiminomethylpyridinium) dibromide][7–10] are considered as the most effective ones.

However, there exist some doubts about the use of cholinesterase reactivators in organophosphate poisoning: According to Oettel[11] reactivation of inhibited blood cholinesterase by obidoxime is very doubtful. Prinz[12] has established that through the action of oximes it is possible to obtain reactivation of cholinesterases *in vitro* but not *in vivo*. Nevertheless, the therapeutic effect of a combination of