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THE EFFECT OF CUTANEOUS BURNS ON HISTAMINE IN MICE

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The effect of cutaneous burns on the histamine content of the tissues is interesting because it is possible that histamine plays some part in the causation of 'shock' after burns. This shock may be entirely caused by the local loss of plasma at the site of the burn (Rossiter, 1943); or, alternatively, the absorption of toxic substances into the general circulation may play some part in its causation (Moon, 1942). The formation, or liberation, of histamine might act either by increasing the permeability of the capillaries in the skin itself, or by modifying the circulation as a whole after absorption.

The possible significance of histamine in injuries was first suggested by the work of Dale & Richards (1918), and Dale & Laidlaw (1919) on the dramatic effects produced by the intravenous injection of small doses of histamine. It received support from Lewis & Grant (1924, 1926), Lewis & Harmer (1927), and Lewis (1927), who obtained evidence of the liberation of a histamine-like substance on irritation of the skin. When histamine was shown to be a normal constituent of the body (Best, Dale, Dudley & Thorpe, 1927; Dale & Dudley, 1929; Thorpe, 1928, 1930), it seemed highly probable that this very active substance played an important part in the response of the body to injury, but the evidence on this point is still inconclusive.

Histamine is not the only substance which may be liberated by injury; about twenty other different substances have been suggested as possible causes of shock, or toxaemia, following burns (Harkins, 1942). Histamine is, however, of special interest because it causes shock in much smaller doses than any other substance which has hitherto been isolated from tissues. It has the advantage, as an object for experiments, that, with suitable precautions, very low concentrations in tissues can be accurately measured by biological assay. This is only possible if the extracts are treated in such a way as to remove, or destroy, all other substances with an action on the pharmacological test used for the assay. The experiments may therefore miss some other toxic substance which plays a more important part than histamine in the causation of shock, but this limitation increases their precision.

It is generally agreed that extracts of skin normally contain histamine. The first experiments on the effects of burns on this histamine were carried out by Harris (1927), who used alcoholic extracts and compared them directly with histamine for their effects on the blood pressure of cats. He found that when the skin of an anaesthetized cat was burned with a hot flat-iron there was oedema, but no change occurred in the histamine equivalent of a given area of skin during the first hour. After this time, the histamine and the oedema fluid were both gradually absorbed.

Barsoum & Gaddum (1936) described a rise in blood histamine in human patients after burns, and this was confirmed by Code & Macdonald (1937), and by Rose & Browne (1940, 1942). The rise, however, does not occur at the same time as the shock. The significance of this phenomenon is thus obscure.

Various Japanese workers have studied this problem and the work of Kisima (1938) appears important, but is difficult to assess as the details are published in Japanese. He came to the conclusion that, when dog's skin was burned, there was a marked rise in the histamine content of the skin itself and also of the blood, urine, lungs, liver, kidney, spleen, pancreas, and intestine. He attributed these changes to the formation of histamine in the burned skin, and showed that, if the burned skin was removed, shock did not occur and the histamine content of the tissues did not rise. Lambert & Rosenthal (1943) have also come to the conclusion that burning causes the formation of histamine in dog's skin.

Methods

Mice were used in the experiments described here because it is comparatively easy to make an extract of the whole mouse and its excreta. It is thus possible to distinguish effects caused by the destruction or formation of histamine from those due to its transference from one part of the body to another. The technique was similar to that used by Alexander (1944) in this laboratory to study the disappearance of injected histamine. The mice were all male and white, weighing 20–30 g. and fed on oats, bread and bran. They were anaesthetized with ether and then plunged wholly, except for the head and neck, into hot water at 60°C. (thermostatically controlled) for 10 or 30 sec. Immediately after they had been removed from the water-bath they were carefully dried with filter paper. In some experiments they were killed within 10 min.; in others they were placed in metabolism cages in a warm room and given free access to water. The faeces and urine were collected together in the first two series of experiments. In the third series only urine was collected. After different periods they were killed by a blow on the head. Each carcass was divided into three fractions, the whole skin, the entire gastro-intestinal tract (with contents), and the rest of the body, and extracted by the methods described below. The excreta were either collected in trichloroacetic acid or preserved by the addition of chloroform to the collecting vessel.

Extraction of histamine. This was carried out in two distinct ways:

(*a*) *Chemical.* In some experiments, histamine was extracted by the method of Barsoum & Gaddum (1935) as modified by Code (1937). Minced tissues were ground in a mortar, and extracted for 1 hr. with 10% trichloroacetic acid, using 1·5 c.c. of acid to 1 g. tissue. Faeces and urine were treated likewise. After filtering by gravity, an aliquot of the filtrate was taken and heated with 10 c.c. of conc. HCl for 1·5 hr. on a boiling water-bath. The liquid was then evaporated to 5 c.c. *in vacuo* and the rest of the acid and water removed by washing the flask down

twice with absolute alcohol, and taking to dryness *in vacuo* each time. The rest of the procedure was as originally described.

(*b*) *Electrodialysis.* In some experiments the less drastic method of electrodialysis in a three-compartment cell, as described by MacGregor & Thorpe (1933), was used. A small cell, each compartment of which was of 15 c.c. capacity, with cellophane membranes separating the chambers, was assembled. The cathode was nickel sheet, and the anode was carbon. The cathode and anode compartments were filled with 10 or 15 c.c. of distilled water, and the middle compartment was filled with urine (0·2–3 c.c.) diluted with distilled water to 10 c.c., or with whole minced skin (4–7 g.) suspended in sufficient distilled water to give a final volume of 15 c.c. A current of 0·4 amp. was passed through the cell for 30 min. The temperature was kept below 40° C. by placing the apparatus in running water when necessary. The minced skin in the middle compartment was stirred with a glass rod from time to time to prevent any local aggregation of mince. The cathode liquid, water clear in the case of urine, and yellowish in the case of skin, was removed, and the cathode compartment washed out with distilled water. The combined liquid and washings were neutralized with 2N-HCl using Universal Indicator (B.D.H.), and then diluted, if necessary, to a suitable volume with Tyrode's solution in readiness for the biological assay. Removal of histamine was almost complete after 30 min. of electrodialysis, the content of histamine in extracts tested after 30, 40, 50 and 60 min. of dialysis being almost exactly the same. The active substance in the extracts was thermostable in N-HCl (boiling for 30 min.).

Estimation and identification of histamine. The extracts were first tested on a piece of guinea-pig's ileum suspended in 2 c.c. of Tyrode's solution containing atropine (0·1 μg./c.c.), in comparison with a standard solution of histamine phosphate. Concentrations are all calculated in terms of histamine base, on the assumption that this represents one-third of the weight of the phosphate.

This test is unspecific by itself. The conclusion that the active substance was histamine depends on the following facts:

(1) The extracts were so prepared as to include only substances soluble in trichloroacetic acid, and stable to boiling for 1·5 hr. in concentrated HCl. Histamine is the only known active substance in tissues with these properties. It was also shown that the active substance migrated towards the cathode through cellophane.

(2) In some cases, at the end of the experiment, a large excess (40–50 μg.) of histamine was added to the bath. After this treatment the preparation became temporarily insensitive to small doses of histamine, but remained sensitive to certain other stimulating substances such as carbachol. When the preparation was desensitized to histamine in this way it also became insensitive to the extracts.

(3) Most of the extracts, both before and after burning, were also tested in comparison with histamine by intravenous injection into atropinized cats anaesthetized with sodium pentobarbitone ('Veterinary Nembutal' given intravenously after induction with ether; 0·38 c.c./kg.). The depressor action of the extracts was indistinguishable from that of pure histamine phosphate, and the estimate of their histamine equivalent, obtained in this way, agreed in every case with that obtained by means of the guinea-pig's ileum with a maximum error of 25%. This quantitative agreement provides strong evidence for identifying the active substance as histamine.

Body temperature. In some experiments the rectal temperature was measured with a thermocouple.

RESULTS

In the first series of experiments the conditions resembled those chosen by Leach, Peters & Rossiter (1943); a temperature of 60° C. was applied for 30 sec. This was found to kill most mice in 7–10 hr. with marked oedema of the subcutaneous tissue. The mice were almost motionless and paid no attention to their surroundings. They made no spontaneous movements, their limbs

appeared toneless, and they responded to stimuli with very brief and limited movements of the head. The rectal temperature was very low.

In the second series of experiments the time of exposure to 60° C. was reduced to 10 sec. in order that the later changes might be followed over a longer period. In each prolonged experiment of this second series there was included a control unburnt mouse. The histamine content of its tissues was invariably lower than that of the burnt mice. The average values for the control mice give an unbiased estimate of the normal values among these

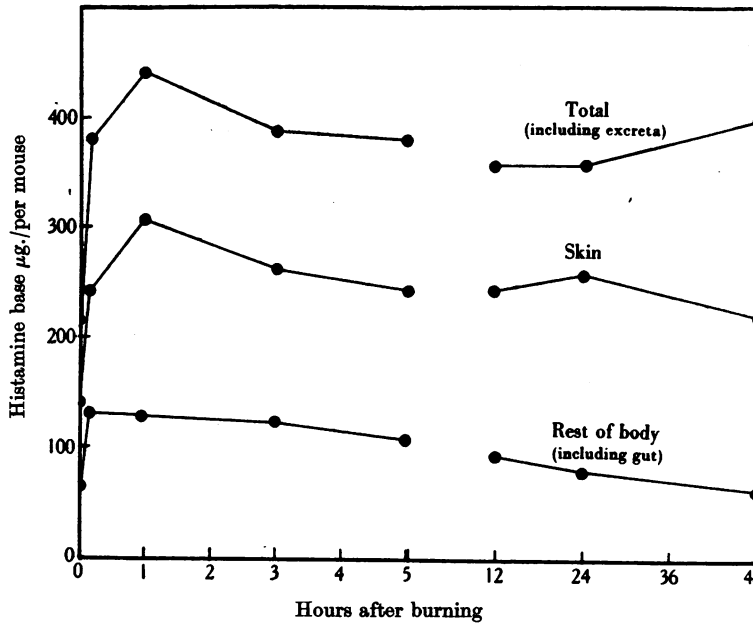

Fig. 1. Histamine content at different times after burning.

mice. In order to assess the factors involved, the control mice were anaesthetized and then plunged in water at room temperature. This did not appear to affect the histamine content. Heat, therefore, was the only known factor which could account for the differences in histamine content between the control mice and the experimental mice.

The individual results of these first and second series of experiments with extracts prepared chemically are given in Tables 1 and 2, from the average results of which Fig. 1 has been constructed.

The average total amount of histamine in the mouse's body rose during the first hour after the burn from 216 to 440 μg. There was no evidence of an increase in the histamine in the gut, but the proportional increase was about the same in the skin and in the rest of the body. Since 60–70% of the histamine in a mouse is in the skin, the total increase was mainly due to an

TABLE 1. Individual results of series 1. Histamine equivalents (μg.) for normal (*N*) mice and mice exposed to 60°C. for 30 sec. Extracts prepared chemically

Time after burning	Skin	Stomach and intestines	Rest of body	Excreta*	Total (including excreta when obtained)
(*N*)	110	10	72	—	192
	146	11	81	—	238
	172	6	45	—	223
	137	13	62	—	212
1–4 min.	164	10	70	—	244
	164	8	72	—	244
	140	7	82	—	229
	205	7	61	—	273
5–10 min.	230	17	185	—	432
	282	14	91	—	387
	260	17	125	—	402
	223	11	113	—	347
	227	13	90	—	330
1 hr.	282	13	78	0·11	373
	294	15	154	—	463
	288	10	125	—	423
	378	9	115	0·08	502
3 hr.	253	11	88	—	352
	302	6	157	0·15	465
	264	18	125	—	407
	231	14	76	0·25	321
5 hr.	281	10	73	0·8	364
	225	3	182	0·2	410
	230	14	166	1·2	411
	233	16	100	1·1	350

* Faeces only, no urine excreted.
(*N*) = neither anaesthetized, nor immersed in water, nor burned.

TABLE 2. Individual results of series 2. Histamine equivalents (μg.) for control (*C*) mice and mice exposed to 60°C. for 10 sec. Extracts prepared chemically

Time after burning or immersion hr.	Skin	Stomach and intestines	Rest of body	Excreta	Total (including excreta)
5	242	7	72	—	321
	215	12	90	17	334
	305	8	78	0·9*	392
	215	13	85	75	388
12	267	10	86	2*	365
	245	9	105	2·8*	362
	230	12	74	20	336
	228	13	70	40	357
(*C*)	*132*	*8*	*45*	*1*	*181*
24	264	7	62	20	353
	268	9	54	20	351
	281	6	75	1*	362
	220	8	95	40	363
(*C*)	*110*	*5*	*35*	*1·3*	*157*
48	210	10	50	60	330
	176	6	50	100	332
	267	8	40	180	495
	226	8	60	144	437
(*C*)	*146*	*5*	*34*	*3*	*188*

* Faeces only, no urine excreted.
(*C*) = anaesthetized and immersed in water at room temperature.

increase in the skin where the concentration rose from an average value of 24·4 to 47·8 μg./g. of wet tissue. A marked increase was observed within 10 min., and the maximum value was reached within an hour or less. The high concentration was maintained for 24 hr. There was some evidence of a fall after this time, but the concentration was still high after 48 hr.

Abnormal amounts of histamine were found in the first samples of urine collected after the burn, and the excretion of large amounts continued for at least 48 hr. The average amount recovered from the excreta in 48 hr. was 121 μg. per mouse, and it seems likely that this is the main route by which the excess histamine disappears from the body. There is, in fact, no evidence of any destruction of histamine in these mice. The figures for the total recovery of histamine from the mice and their excreta in the second series of experiments are remarkably constant between 5 and 48 hr. after the burn.

Electrodialysis. The first two series of experiments showed that the total amount of histamine extracted by a drastic process was increased by burning, and that the excess histamine was excreted in the urine. The histamine estimated in this way is, of course, not necessarily all present in an active form in the body. It may be largely formed from inactive precursors by the acid hydrolysis. It was also possible that the observed effects of burning were due to the liberation of preformed histamine from a compound which was not extracted by trichloroacetic acid. A third series of experiments was

TABLE 3. Histamine equivalents (μg.) for normal (*N*) mice, control (*C*) mice, and mice exposed to 60°C. for 30 sec. (skin experiments) and 10 sec. (urine experiments). Extracts obtained by electrodialysis. All tests on guinea-pig's ileum only, unless stated to the contrary

Time after burning		Skin	
		μg.	μg./g.
	(*N*)	107·2	15·3
		62·9	15·7
		100	18·1
		80	17·7*
	Mean	87·1	16·7
5–10 min.		214·5	35·7
		214·4	33
		140·7	28·1
		147	29·5*
	Mean	179·1	32·1
1 hr.		187	28·8
		148	29·6
		127·3	32·3
		133	33*
	Mean	148·8	31

Time after burning or immersion hr.		Urine	
		μg.	μg./c.c.
	(*C*)	*0·12*	*0·24*
12		13	65
		10·1	50·5
		13	43·3
	Mean	12	52·9
	(*C*)	*0·067*	*0·08*
24		21·3	42·6
		44·8	89·6
		21·4	23·7
		39·2	39·2
	Mean	31·7	39
	(*C*)	*0·26*	*0·13*
48		80	26·7
		60	30
		46·2	23·1
		150	50
	Mean	84	32·5

* Tested on cat's blood pressure.
(*N*) = neither anaesthetized, nor immersed in water, nor burned.
(*C*) = anaesthetized and immersed in water at room temperature.

therefore undertaken in which the most important parts of the earlier experiments were repeated, using electrodialysis to prepare the extracts. The results are recorded in Table 3.

The estimates of histamine obtained by electrodialysis were uniformly less than those obtained in the earlier experiments. This discrepancy may have been caused by the different method of extraction. The earlier estimates would include both free histamine and the combined histamine detected by Anrep, Ayadi, Barsoum, Smith & Talaat (1944). The extracts obtained by electrodialysis would presumably include only free histamine. Alternatively, the difference may be due to some unknown difference in the mice. The important point is that all experiments show that there is a large increase in the histamine

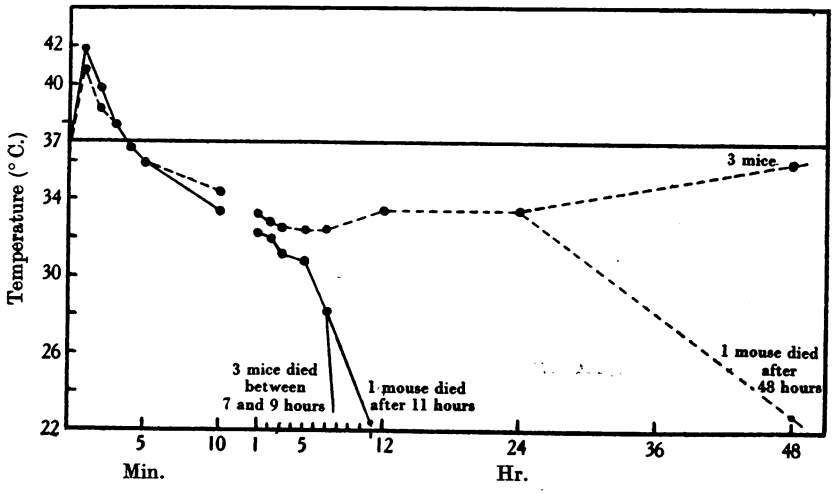

Fig. 2. Average rectal temperature in mice burned at 60° C. for 30 sec. (——) and for 10 sec. (-----).

content of the skin during the first hour after the burn, and that there is also an excess of histamine in the urine during the first 48 hr. The fact that these changes were detected by such a mild method of extraction as electrodialysis suggests that the increase is in the free histamine. On the other hand, if the apparent increase of histamine is due to the break up of a compound not extracted by trichloroacetic acid, this hypothetical compound must also fail to be carried through cellophane to the cathode. There is no reason to suggest the existence of such a compound.

Body temperature. Measurements were made of the body temperature, partly to determine whether the observed changes should be attributed to a local burning of the skin or to a rise in the general temperature of the body, and partly as a means of assessing the clinical condition of the mice. The results are shown graphically in Fig. 2, in which each point, unless shown to the contrary, represents the mean of four observations.

The mean rectal temperature rose during burning from a normal value of 37° C. to 40·8° after exposure for 10 sec., and to 41·8° C. after exposure for 30 sec. At the end of 10 min. it had fallen to about 34° C. When it fell below 32° C. the mice invariably died. In the mice which recovered, the temperature began to rise as the histamine disappeared on the second day.

It is unlikely that the slight hyperthermia shown by these results was the cause of the observed formation of histamine, though this possibility cannot be definitely excluded. It is more likely that the effect was due to the local burning of the skin. The ensuing hypothermia was presumably due to shock, and showed that this occurred at the same time that there was an increased amount of histamine in the mouse.

Discussion

The results show that histamine was formed in the skin within 10 min. of the application of heat, and this confirms the results of Kisima (1938) and Lambert & Rosenthal (1943), but not those of Harris (1927). The reasons for this discrepancy are under investigation.

This formation of histamine is particularly interesting because other forms of injury, such as anaphylaxis (Feldberg, 1941), are associated with the liberation of preformed histamine rather than with the formation of new histamine. It seems likely that this phenomenon plays some part in the circulatory response of the body to burns, but its full significance is not yet clear. The mice used in these experiments were all severely injured, but it is improbable that any significant part of the injury was due to the action of histamine which had been absorbed from the skin into the general circulation. The quantity formed was too small to have much effect on mice, which are remarkably insensitive to histamine, and appear to be scarcely inconvenienced by the intravenous injection of quantities 10 times larger. In other species, which are more sensitive to histamine, the absorption of this substance from burned tissues may have an effect, but it cannot have much effect on a mouse.

On the other hand, it is possible that the new formation of histamine in the skin played a significant part in the local response to injury, causing dilatation and increased permeability of the capillaries and dilatation of the arterioles by axon reflexes, and the consequent local accumulation of plasma at the site of the burn. It was observed that, within 1 hr., the subcutaneous tissues of these mice were distended with oedematous fluid which was liable to set into a jelly even during life. This may have been due to the escape of plasma through capillaries whose permeability had been increased by histamine. This local and prolonged action of the histamine may be more dangerous than the alarming but short action of histamine injected intravenously.

Rose & Browne (1940, 1942) studied clinically the blood histamine in burn shock and reported that although it was increased immediately after burning

it was actually decreased in active shock as compared with control values and those after recovery. In patients who die, the blood histamine is very low just before death. This may perhaps be because in shocked patients the rate of histamine absorption from the tissues is decreased and may even cease during severe circulatory collapse, as is known to be the case with other drugs injected subcutaneously.

Until recently, it has generally been assumed that histamine is not excreted in appreciable quantities in the urine. Recent work has shown that this assumption was untrue (Kapeller-Adler, 1941; Alexander, 1944). The evidence has been reviewed by Anrep *et al.* (1944), who obtained evidence that the normal urine usually contains some histamine and that this may be free, or combined in a form which is pharmacologically inactive. It is probable that both forms of histamine were estimated together by the method used in most of the present experiments.

The traces of histamine estimated in normal urine might be due to experimental error, as it is difficult to separate urine and faeces completely, and to avoid contamination with desquamated epithelium. There can, however, be no doubt that histamine was excreted in the urine of burned animals. The fact that all the excess histamine was excreted, and that little or none of it was destroyed in the mouse, is interesting, but cannot usefully be discussed until more is known about the fate of histamine injected in comparable doses in mice. The data of Alexander (1944) were obtained with much larger doses (3 mg. per mouse).

SUMMARY

1. Extensive cutaneous burns in mice caused the new formation of histamine, mainly in the skin, so that the total amount of histamine in the mouse was almost doubled in 10 min.

2. This excess histamine was mostly excreted in the urine during the next 48 hr., if the mice survived.

3. The relation of this phenomenon to shock following burns is discussed.

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