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# SKIN HISTAMINE IN CATS: ITS DEPLETION AND SUBSEQUENT RECOVERY AFTER INJECTION OF COMPOUND 48/80

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SUMMARY

1. The histamine content of the skin has been determined in untreated cats, in cats injected with the histamine liberator, compound 48/80, and in cats injected subcutaneously with an oily suspension of histamine or histidine after treatment with compound 48/80.

2. Skin histamine in cats varied from 2·2 to 37·5 $\mu$g/g. Apart from regional variations in the histamine content of the abdominal skin, lower values were obtained from cats kept on a laboratory diet which included aureomycin (mean 8·5 $\mu$g/g, range 2·2–13·1) than from cats on a high protein diet without aureomycin (mean 17·5 $\mu$g/g, range 5·2–37·5).

3. Compound 48/80 caused a depletion of skin histamine which was never complete, and a residue of 3–7 % remained; in cats which originally had a low histamine content of skin, depletion was even less and up to 20 % of the original histamine remained.

4. Recovery of skin histamine in cats after injections of compound 48/80 was slow, at least 110 days being required for full restoration. The rate of recovery was not altered by daily subcutaneous injections of histidine, but an effect was found with daily subcutaneous injections of histamine. Histamine had no effect for the first 33 days, but at 55 days the percentage histamine content of the skin was greater than in the cats treated with histidine or the oily suspending vehicle, and at 110 days the histamine content was not only much greater than the other two groups but was greater than that before injection of compound 48/80.

5. No *in vitro* histamine formation was detected in the skin samples either before compound 48/80 treatment or at any time during the subsequent recovery of the histamine to control levels.

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6. The results support the theory that the histamine found in skin tissues of the cat is not formed there but is taken up from the blood stream.

## INTRODUCTION

In mammals, histamine in the tissues and urine may arise by decarboxylation of histidine in the tissues, or from histamine present in the blood after its absorption from the intestine where it is probably formed by bacterial action on histidine in the food. These two sources of tissue histamine appear to vary in different species. Many rodent tissues are readily able to form histamine *in vitro* when incubated with histidine; however, the same tissues from cat and dog do not appear to be able to do so. When histidine is given orally to dogs, the free histamine in the urine and the acid gastric secretion from a Heidenhain pouch are greatly increased but these increases do not occur when the intestinal tract has been sterilized first with antibiotics (Irvine, Duthie & Waton, 1959). In the guinea-pig, histidine given orally also leads to an increase of free histamine in the urine but this increase is unaffected by sterilization of the intestinal tract (Waton, 1963). These findings suggest that in guinea-pigs, in contrast to dogs, bacterial decarboxylation of histidine in the intestine and absorption of the histamine so formed is not an important source of tissue histamine.

In cats, the origin of histamine found in both tissue and urine is uncertain; attempts to show decarboxylation of histidine by the tissues have been mainly unsuccessful, and it is believed that the origin of histamine is similar to that in dogs (Waton, 1964).

The effect of daily subcutaneous injections of histamine or histidine on the levels of histamine in cat skin, depleted of its histamine by treatment with the histamine releaser compound 48/80, was studied. The histamine or histidine was injected in an oily suspension so that they were slowly released into the blood stream (Smith, 1953).

## METHODS

Adult cats of both sexes weighing 2–4 kg were used. Biopsy skin samples, either squares of about 2 × 2 cm or vertical strips of about 1 cm wide and 7–12 cm long, were removed under fluothane–nitrous oxide anaesthesia, after shaving the selected area with electric clippers. Crystalline penicillin and polybactrin were applied to the wounds which were then closed with Michelle clips. Each sample was freed of subcutaneous fat and most samples were divided into three parts for the estimations of total histamine, of releasable histamine, and of histidine decarboxylase activity. Each part was weighed and finely chopped with scissors and then used as follows:

*Histamine content.* The skin samples were dropped into 5 vol. trichloroacetic acid solution (10 %, w/v) and left for at least 12 hr before they were centrifuged. The supernatants were then extracted four times with 4 vol. anaesthetic ether to remove the acid. Excess ether was removed by gentle heating *in vacuo*. After final neutralization with sodium bicarbonate, the

extracts were tested for their histamine content on the isolated guinea-pig's ileum suspended in a 5 ml. organ-bath containing oxygenated Tyrode solution at 37° C with atropine ($10^{-7}$ g/l.). Mepyramine maleate was used according to the procedure of Reuse (1948) to establish that histamine was the active substance present in the extracts.

*Histamine release in vitro.* The method used has been described by Westerholm (1960). The skin samples were dropped into 5 ml. of 0·9 % NaCl solution and incubated for 1 hr at 37° C with 1 ml. of compound 48/80 in a concentration of 10, 100 or 200 $\mu$g/ml. After incubation, the mixtures were centrifuged and the supernatants removed. Trichloroacetic acid (5 vol.) was added to each of the supernatants and the histamine content of the extracts determined.

*Histidine decarboxylase activity in vitro.* The skin samples were incubated for 3 hr at 37° C in 10 ml. 0·05M phosphate buffer (pH 7·4) containing L-histidine monohydrochloride (1 mg/ml.) and pyridoxal-5-phosphate (10 $\mu$g/ml.) (Waton, 1956). After incubation, the pH was brought to 5·5 with N-HCl, and the mixtures were boiled and then filtered. After neutralization with sodium bicarbonate, the filtrates were tested for their histamine content on the atropinized ileum of a guinea-pig.

*Histamine depletion in vivo.* Intraperitoneal injections of compound 48/80 (dissolved in 0·9 % NaCl solution) were given on four successive days, as follows: day 1, 0·5 mg/kg; day 2, 1·0 mg/kg; day 3, 2·0 mg/kg, and day 4, 2·0 mg/kg. The skin histamine was determined in a sample removed 3 days before the first injection as well as in a sample removed 24 hr after the last dose of compound 48/80.

*Histamine restoration in vivo.* On the day after the last injection of compound 48/80, three groups of five cats were each given daily subcutaneous injections. The doses of histamine and of histidine were administered in a mixture of beeswax and paraffin oil (Smith, 1953). Group A received histamine dihydrochloride (60 mg/ml.), group B histidine monohydrochloride (60 mg/ml.), group C received the oily mixture only. The injections were made into the subcutaneous tissue covering the thigh muscles and behind the position of the most posterior skin sampling area. This avoided contamination of the skin samples which were later removed for the histamine assay. Six vertical strips of skin, three from each side, about 3 cm wide, starting 5 cm below the spine and finishing 2 cm above the line of the nipples, were removed.

## RESULTS

### *Histamine content of cat skin*

The histamine content of skin samples, squares of about 2 × 2 cm, from the same area of healthy cats varied widely. This variation was probably linked with the source of the cats. For example, the mean value for twenty-five cats from the closed colony at Mill Hill was 8·5 $\mu$g/g (range 2·2–13·1 $\mu$g/g), whereas it was 17·5 $\mu$g/g (range 5·2–37·5 $\mu$g/g) for twenty-nine cats supplied by local dealers. This variation may be related to the diet, for the cats at Mill Hill received a diet containing more cereal and other vegetable protein than that fed to the cats supplied by the outside dealers. Further, the cats from the closed colony regularly received an antibiotic, aureomycin, in their diet. In a few preliminary experiments, a group of closed-colony cats were given raw liver and more meat protein, and the aureomycin was withdrawn. This procedure doubled the skin histamine values, a result which suggests that tissue histamine in cats originated from the meat in their diet, possibly by bacterial decarboxylation of histidine.

Apart from these differences in skin histamine, there was some variation depending on the area from which the skin was taken, as shown diagrammatically in Fig. 1*A*. Around the nipples, the histamine content was high (over 30 $\mu$g/g), less along the mid line and somewhat lower still along the sides of the abdomen. A similar increase in the concentration of skin histamine around the nipples has previously been reported for the guinea-pig (Feldberg & Miles, 1953). In ten vertical strips taken from the sides of the abdomen, five from each side, the values varied between 12·5 and 18·8 $\mu$g/g (mean 14·4 $\mu$g/g), as shown in Fig. 1*B*.

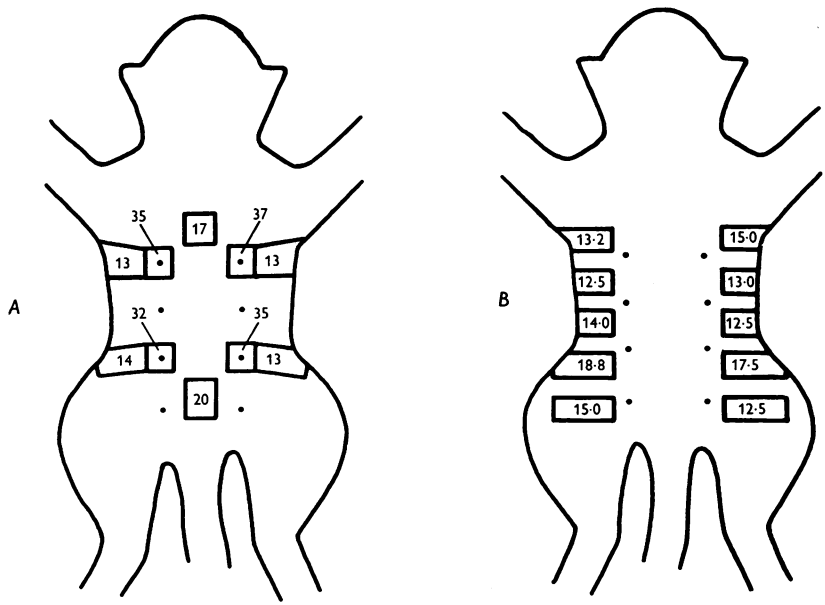


Fig. 1. Diagrams of the ventral body surface of two cats to show regional differences in the histamine content ($\mu$g/g fresh skin). *A*, localized areas from a black female weighing 2·5 kg; *B*, vertical strips from a ginger male weighing 2 kg, starting 2 cm above the line of the nipples and extending out and up the sides for 4 cm.

Kahlson, Nilsson, Rosengren & Zederfeldt (1960) reported that regenerating skin tissue of rats after wounding had an increased histamine-forming capacity. To test whether the removal of one strip of cat skin influences the histamine content of another strip removed at a later date, strips were taken from three cats on days 0, 5, 18, 37 and 59, and the histamine content of each strip determined. The results are given in Table 1. The value of the first strip has been taken as 100 % and subsequent values are given as percentages of this value. The histamine content increased slightly and was highest by the time the fourth strip was removed on day 37 but was almost back to the control value by day 59.

*Histamine depletion and subsequent recovery.* For experiments in which depletion and subsequent recovery of skin histamine after compound 48/80 were studied, vertical strips of skin were removed, starting 2 cm above the line of the nipples and extending out and up the sides for 4 cm. The number of strips was limited to three per side, leaving a width of about 5 cm of intact skin between each strip. The strips were removed in the order shown in Fig. 2.

TABLE 1. Histamine content of vertical strips of cat skin removed at various times after the first incision. Each value is expressed as a percentage of that of the first strip of skin removed on day 0, and is the mean from three cats

| Time of removal of strip of skin (days) | Histamine content (% of first strip) |
|---|---|
| 0 | 100 |
| 5 | 124 |
| 18 | 126 |
| 37 | 135 |
| 59 | 105 |

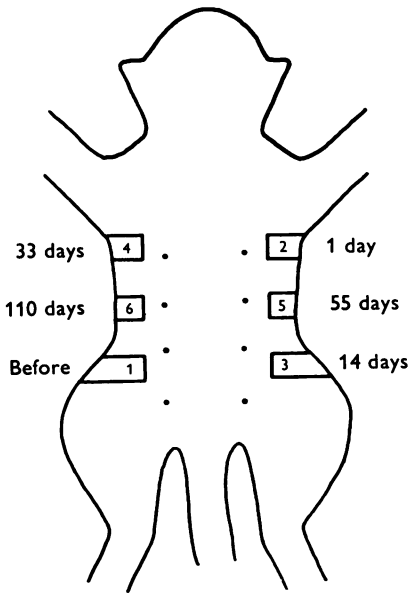


Fig. 2. The six locations from which skin samples were removed for assay of their histamine content. Strip 1 was used before depletion of the skin histamine with compound 48/80, and strips 2–6 were used at the various times shown after depletion.

*Depletion of skin histamine.* Injection of compound 48/80 produced vomiting, defaecation, vasodilatation and itching during the 4-day treatment starting with 0·5 mg/kg and ending with 2·0 mg/kg. In these

cats, as well as in four out of seven cats surviving double the doses of compound 48/80, there was always a residue of skin histamine, usually 3–7 % of the pre-injection level. Typical results from fifteen cats are shown in Table 2. The depletion can be seen by comparing the results in the fourth column (day 1 after compound 48/80) with those in the third column (3 days before compound 48/80). In some cats in which the skin histamine values were low, the histamine depletion was not large. For example, the skin of one cat (not shown in Table 2) contained only 3·7 μg/g histamine before injection and 0·8 μg/g after injection of compound 48/80—that is, 21 % of the pre-injection level. These cats were not used in the recovery experiments.

*Recovery of skin histamine.* The results of the last five cats of Table 2 which received daily injections of the oily suspending vehicle (group C)

TABLE 2. Effect of compound 48/80 on the histamine content of cat skin (μg/g), and the subsequent repletion of skin histamine when these animals were given daily subcutaneous injections either of histamine in oil (group A) or of histidine in oil (group B), or of the oil alone (group C)

| | | Histamine content | | | | | |
|---|---|---|---|---|---|---|---|
| | | | Days after compound 48/80 | | | | |
| Group | Cat no. | 3 days before compound 48/80 | 1 | 14 | 33 | 55 | 110 |
| A | 1 | 11·0 | 0·8 | 4·8 | 5·3 | 7·0 | 10·0 |
| | 2 | 9·0 | 1·2 | 3·5 | 7·0 | 8·8 | 15·2 |
| | 3 | 13·1 | 0·6 | 5·5 | 6·0 | 6·7 | 20·6 |
| | 4 | 10·5 | 0·6 | 3·8 | 5·0 | 8·0 | 12·9 |
| | 5 | 10·1 | 0·5 | 2·5 | 7·5 | 9·5 | — |
| B | 6 | 11·5 | 0·4 | 5·5 | 7·3 | 8·0 | 10·7 |
| | 7 | 10·5 | 0·5 | 5·0 | 7·3 | 6·7 | 7·5 |
| | 8 | 8·5 | 0·4 | 5·0 | 3·0 | 6·3 | 9·0 |
| | 9 | 15·0 | 0·3 | 1·5 | 7·0 | 7·5 | — |
| | 10 | 12·0 | 0·9 | 1·7 | 7·0 | 6·0 | — |
| C | 11 | 10·5 | 0·5 | 4·0 | 6·0 | 5·3 | 7·5 |
| | 12 | 6·0 | 0·4 | 3·8 | 3·0 | 4·3 | 7·5 |
| | 13 | 11·3 | 0·6 | 5·8 | 4·7 | 6·3 | 8·4 |
| | 14 | 10·0 | 0·5 | 3·7 | 5·3 | 6·0 | 10·4 |
| | 15 | 12·6 | 0·4 | 3·0 | 6·5 | 9·0 | — |

show that skin histamine after depletion by compound 48/80 recovered slowly. In all five cats it was incomplete at day 55, and in two of the four in which the skin histamine was determined on day 110 it was still incomplete. The time course of their recovery is plotted graphically in Fig. 3, by the continuous line joining the open circles. Each open circle represents the mean value obtained from the cats of group C and expressed as a percentage of the histamine value obtained before the injections of compound 48/80.

Daily injections of histidine did not affect the histamine recovery as shown by the results obtained from the five cats of group B (Table 2).

These results are plotted graphically as mean percentage values by the interrupted line in Fig. 3. On the other hand, daily injections of histamine had some effect on the later stages of histamine recovery. This is seen from the results of the five cats of group A given by the line joining the triangles in Fig. 3. No effect was apparent for the first 33 days but at day 55, the mean percentage recovery was 75 % as compared with 62 % and 60 % of groups C and B respectively. A much greater difference was apparent at day 110, the mean value (145 %) for group A being significantly greater

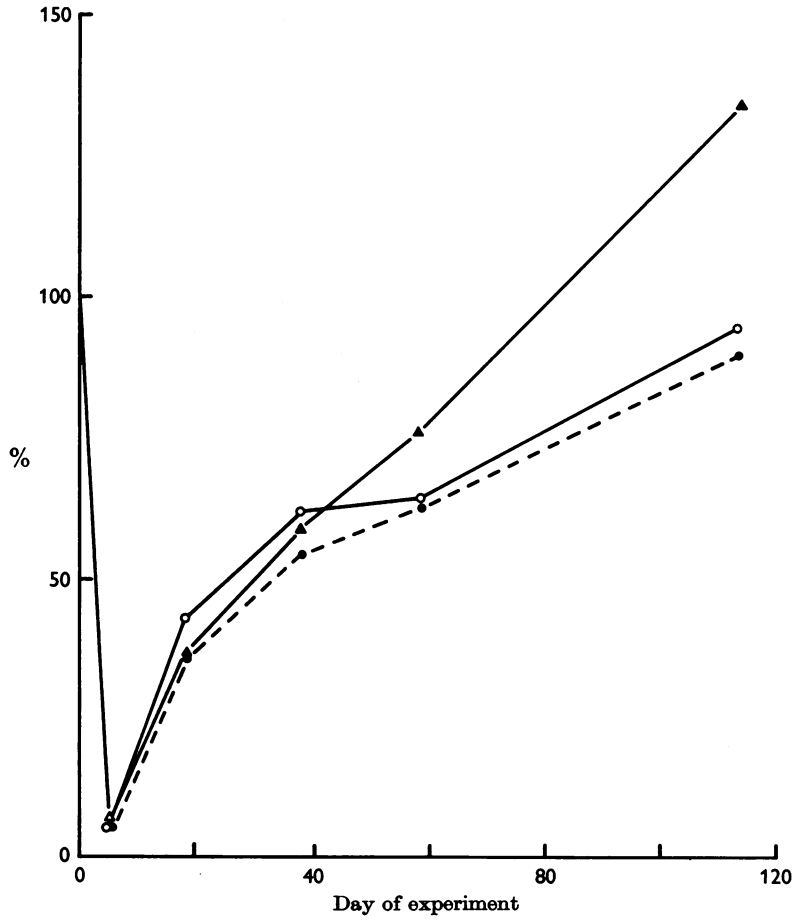


Fig. 3. Effect of compound 48/80 on the histamine content of cat skin, and the subsequent repletion of skin histamine when groups of five cats were given daily subcutaneous injections either of histamine in oil (▲—▲) or of histidine in oil (●—●) or of the oil alone (○—○). The result on each cat has been expressed as a percentage of the histamine present in the skin before treatment with compound 48/80, and the points shown are the means of these percentages.

($P < 0{\cdot}01$) than those of other groups. Thus the histamine injection affected not only the late stages of histamine recovery but produced skin histamine values higher than those present before depletion by compound 48/80.

During administration of histidine and of the vehicle alone, the cats continued to eat normally and their body weight steadily increased, whereas during the administration of histamine, the cats did not eat much and appeared ill during the first weeks, showed signs of histamine intoxication, and lost weight. Later, they appeared to become more resistant to histamine, started to eat more and gained weight. Figure 4 shows the mean changes in body weight for all three groups of cats. No pathological signs

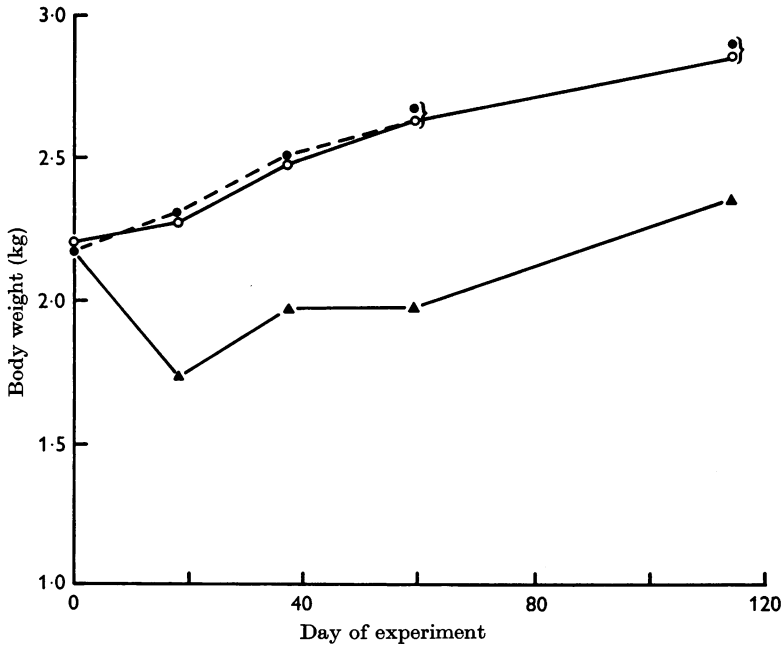


Fig. 4. Effect of compound 48/80 (given at time 0) and of subsequent daily doses of histamine (▲—▲), histidine (●—●) or oil (○—○) on the body weight (kg) of cats. Each point represents the mean value of three cats.

of histamine intoxication were found post mortem when the histamine-treated cats were killed after day 110. Complete absorption of the histamine was confirmed by extracting the injection sites and assaying the extracts for histamine.

*In vitro release of histamine*

Skin samples from cats not treated with compound 48/80, when incubated with compound 48/80, lost histamine, the release depending on

the concentration of compound 48/80 in the incubation mixture; with 10 $\mu$g/ml. about 13 %, with 100 $\mu$g/ml. about 30 %, and with 200 $\mu$g/ml. about 40 %, of the skin histamine was released. In contrast, no release of histamine was observed, even with a concentration of 200 $\mu$g/ml. of compound 48/80, in samples of histamine-depleted skin from cats previously treated with this histamine liberator. Further, a release of not more than 10 % occurred from skin after full recovery of its histamine at day 110, i.e. in skin samples removed at that time from cats 8, 12 and 14 of Table 2. In skin samples removed before histamine recovery was complete, the release on incubation with 200 $\mu$g/ml. compound 48/80 was never more than 7 %.

### *Formation of histamine in vitro*

With the non-isotopic method used in the present experiments, no histidine decarboxylase activity was detected at any time in the skin samples. This applied also to skin samples taken from cats treated with compound 48/80. In rats, treatment with compound 48/80 results in activation of the enzyme which is present in the skin (Schayer, Rothschild & Bizony, 1959).

## DISCUSSION

The present results show that there are regional variations in the skin histamine of cats, similar to those already reported for guinea-pigs (Feldberg & Miles, 1953), but it has been possible to obtain several pieces of skin from one cat with approximately the same histamine content, by taking vertical strips from its side. As long as these strips are not too close, the removal of one piece does not influence the histamine content of an adjacent strip when this is subsequently removed; this might have been expected from the results of Kahlson *et al.* (1960), who observed in rats that regenerating tissue around skin wounds had a greatly increased histamine-forming capacity.

The finding of a higher histamine content in the skin of untreated animals which had been fed on a diet rich in animal protein, but containing no antibiotic, supports the theory that absorption of histamine from the intestine is at least partly the source of the skin histamine.

After intraperitoneal injections of compound 48/80, there was a profound release of histamine from the skin, but this was never complete. Even when doses which killed three out of seven cats were given, it was found that a residue of histamine of 3–7 % generally remained, thus confirming in cats an observation made by Feldberg & Talesnik (1953) in rats and dogs.

It is possible that, in the few cats which had a low initial skin histamine content and in which depletion was not so great, some degree of natural

release had occurred before the experiment, and the value measured before injection was not the normal histamine content. This is supported by the fact that the values after depletion were within the range of those found in other cats.

Histamine which had been replenished in the skin after treatment with compound 48/80 was found to be more resistant to the releasing action of compound 48/80 than that in skin of untreated cats. This is probably owing to the fact that new mast cells are refractory to the disruptive action of this histamine liberator. This explanation was given by Riley & West (1955) who reported a similar result in rats.

The results also show that the recovery of skin histamine in cats given injections of the histamine releaser compound 48/80 is slow like that in dogs, and slower than in rats in which full recovery takes about 45 days (Feldberg & Talesnik, 1953). Injected subcutaneously in an oily suspension, histidine had no effect on the rate of recovery, whereas histamine, while it had no effect during the first 30 days, later speeded up recovery and led in fact to a rise above the original control values in the same time taken for the untreated cats to return to pre-injection levels. The delay of 30 days suggests that histamine storage is dependent on the presence of mast cells and has to await the re-formation of these cells. Only when these have been formed can the histamine be taken up and held in the tissues. Further, the later speeding up of the recovery suggests that at least a great part of the skin histamine is taken up from the circulation rather than derived from decarboxylation of histidine in the tissues. The fact that it was not possible to show, at any stage of recovery, the presence of histamine formation in the skin by the *in vitro* non-isotopic method is in accord with this view. Thus, the cat differs from rodents, in which tissue histamine stores are apparently derived from histidine through the action of the histidine decarboxylating enzymes present in the tissues. In the cat, on the other hand, histamine may not be formed in each tissue in which it is found but only in one or more specialized tissues, or it may always be derived from the histamine formed in the intestine and taken up by the tissues after its absorption into the blood stream (Waton, 1963).

Control cats, as well as the cats injected with histamine or histidine, were receiving a normal diet, and it is possible that sufficient histamine as well as histidine was always available to the tissues and that their supply by injection was merely an unwanted excess. One possibility of showing perhaps even more conclusively whether systemic histidine or histamine is responsible for the stores of tissue histamine would be to repeat the experiments on cats fed with a histidine-free diet or after sterilization of intestine.

## REFERENCES

FELDBERG, W. & MILES, A. A. (1953). Regional variations of increased permeability of skin capillaries induced by a histamine liberator and their relation to the histamine content of the skin. *J. Physiol.* **120**, 205–213.

FELDBERG, W. & TALESNIK, J. (1953). Reduction of tissue histamine by Compound 48/80. *J. Physiol.* **120**, 550–568.

IRVINE, W. T., DUTHIE, H. L. & WATON, N. G. (1959). Urinary output of free histamine after a meat meal. *Lancet*, i, 1061–1064.

KAHLSON, G., NILSSON, K., ROSENGREN, E. & ZEDERFELDT, B. (1960). Wound healing as dependent on rate of histamine formation. *Lancet*, ii, 230–234.

REUSE, J. J. (1948). Comparison of various histamine antagonists. *Br. J. Pharmac. Chemother.* **3**, 174–180.

RILEY, J. F. & WEST, G. B. (1955). Tissue mast cells: Studies with a histamine-liberator of low toxicity (Compound 48/80). *J. Path. Bact.* **69**, 269–282.

SCHAYER, R. W., ROTHSCHILD, Z. & BIZONY, P. (1959). Increase in histidine decarboxylase activity of rat skin following treatment with Compound 48/80. *Am. J. Physiol.* **196**, 295–298.

SMITH, A. N. (1953). Release of histamine by histamine liberator Compound 48/80 in cats. *J. Physiol.* **121**, 517–538.

WATON, N. G. (1956). Studies on mammalian histidine decarboxylase. *Br. J. Pharmac. Chemother.* **11**, 119–127.

WATON, N. G. (1963). Urinary excretion of free histamine in guinea-pigs after oral histidine. *J. Physiol.* **165**, 174–178.

WATON, N. G. (1964). Is tissue histamine formation necessary in cat, dog and man? *Int. Arch. Allergy appl. Immun.* **22**, 87–101.

WESTERHOLM, B. (1960). *In vitro* release of histamine from cat skin by Compound 48/80. *Acta physiol. scand.* **50**, 41–48.