ON THE CONCENTRATION OF HISTAMINE IN DIFFERENT PARTS OF THE BRAIN AND HYPOPHYSIS OF THE CAT AND ITS MODIFICATION BY DRUGS

By
Humayun K. M. A. Hye

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INTRODUCTION

The object of the present investigation was two-fold: (a) to obtain values for the concentration of histamine extractable from different parts of the cat's brain and hypophysis and (b) to see whether or not the concentration of histamine in the hypophysis and adjoining parts of the brain could be modified by drugs. The drugs were chosen mainly from those which are known to affect the metabolism of histamine or of other amines known to be present in the brain.

The material for this thesis has therefore been divided into two parts: the first, contains the results on the concentration of histamine in different parts of the brain, together with an account of the methods employed for its estimation, and a review of the relevant literature; the second part contains the results obtained with drugs and discussion of their actions in terms of what is known about the origin and metabolism of histamine in brain.

Part of the information contained in this thesis has already been published in the Proceedings of the Physiological Society (Adam and Hye, 1964).
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PART I

ON THE CONCENTRATION OF HISTAMINE IN DIFFERENT PARTS OF THE BRAIN AND HYPOPHYSIS OF THE CAT
HISTAMINE IN THE BRAIN

Interest in histamine and the nervous system grew out of experiments on the vascular responses of the skin in animals and in man. Histamine was seen as intervening in two ways: first, as a substance which mediated the vasodilator response when sensory nerves were stimulated antidromically (Lewis and Marvin, 1927; Ungar, 1935) and secondly, as a substance which, when liberated in the skin, elicited an axon reflex by its action on sensory nerves (Lewis, 1927). The chemical isolation of histamine from mammalian tissues (Best, Dale, Dudley and Thorpe, 1927) provided the essential basis for these views of the role of histamine. The observation that antidromic stimulation of sensory nerves in the dog was followed by a sharp rise in the volume of gastric juice (Ungar, 1936), was taken as further evidence for the presence of nerve fibres which acted by release of histamine or a substance like it. Ungar (1936) applied the term 'histaminergic' to these nerves. Although the gastric
excitant in the plasma was never identified (Ungar and Parrot, 1939), the finding nevertheless raised the question whether or not sensory nerves contained histamine which could have been released on stimulation.

Kwiatkowski, in 1943, provided the first clear evidence for the presence of histamine in the mammalian nervous system. He showed that most of the histamine resided in the peripheral nerves and that in the central nervous system, the quantity was small and often not detectable. He also showed that sensory nerves contained more histamine than motor nerves and that the concentration of histamine in whole blood rose after antidromic stimulation of posterior nerve roots. Kwiatkowski's findings on the occurrence of histamine in the nervous system were later confirmed and extended (Euler, 1949; Werle and Weicken, 1949; Werle and Palm, 1950, 1952).

The paucity of histamine in the central nervous system in contrast with the high concentration in the peripheral nerves, may have prompted the investigation undertaken by Harris, Jacobsohn and Kahlson (1952). These authors demonstrated by reliable methods that
parts of the brain adjoining the hypophysis were rich in histamine. They studied material obtained from dog, cat and pig and found a high concentration of histamine in the hypothalamus, particularly in the eminentia media. Elsewhere in the brain, namely, in the thalamus, caudate nucleus and cerebellum, the concentration was less than could be detected by their method. They were unable to confirm the high values previously reported by Kwiatkowski (1943) for the cerebellum.

Other workers have since reported on the histamine concentration in brain tissue of various species (Cicardo and Stoppani, 1949; Strengers and Maas, 1956; Clouet, Gaitonde and Richter, 1957). Shore, Burkhalter and Cohn (1959) estimated histamine by a spectrofluorimetric method and concluded that the concentration of histamine for the whole brain was about the same in the rabbit, dog and rat. They also found that the concentration did not vary much in different parts of the dog and rabbit brain, including the cerebellum.

More recently Adam (1961), who used a sensitive biological method, studied the distribution of histamine in dog's brain.
Adam's results agreed in general with those of Harris et al. (1952), in that the highest concentrations in the brain occurred in the hypothalamus. He showed further that the concentration fell gradually in passing from the hypothalamus to the thalamus and brain stem. The concentration was low in the pons, medulla and cerebral cortex; none was detected in the cerebellum or in the white matter of the brain.

Adam (1961) also examined the hypothalamus for mast cells but found none except in the hypophysial stalk where they occurred entirely in the pars tuberalis of the gland.

Carlini and Green (1963) estimated the histamine concentration in the rat's brain by a method similar to Adam's and obtained a mean value of 54 ng/g. They also estimated the histamine in the rat's brain by the spectrofluorimetric method of Shore et al. (1959) and concluded that the high value (mean 246 ng/g) obtained by this method was erroneous and probably due to the presence of interfering substances. McGeer (1964) in a recent communication described the regional distribution of histamine in the cat's brain, as estimated by a modification of the
### Table 1

Regional distribution of histamine in brain

Estimated concentrations (ng/g) of histamine as reported by various authors

<table>
<thead>
<tr>
<th></th>
<th>Whole brain</th>
<th>Hypothalamus</th>
<th>Thalamus</th>
<th>Midbrain</th>
<th>Medulla and pons</th>
<th>Cerebral cortex</th>
<th>Cerebellum</th>
<th>Caudate nucleus</th>
<th>Brainstem</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cat</strong></td>
<td>&lt;60 (5)</td>
<td>700-8300 (5)</td>
<td>&lt;400 (5)</td>
<td>280 (1)</td>
<td>100 (1)</td>
<td>200 (1)</td>
<td>1500-2000 (1)</td>
<td>&lt;50 (3)</td>
<td>&lt;600 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1250 (12)</td>
<td>500 (12)</td>
<td>600 (12)</td>
<td>400-500 (12)</td>
<td>300-400 (12)</td>
<td></td>
<td>200 (12)</td>
<td></td>
</tr>
<tr>
<td><strong>Dog</strong></td>
<td>9800-17300 (2)</td>
<td>200-6200 (3)</td>
<td>&lt;300 (5)</td>
<td>200-220 (10)</td>
<td>&lt;30-60 (10)</td>
<td>100-160 (1)</td>
<td>1500 (1)</td>
<td>&lt;200 (5)</td>
<td>&lt;200 (9)</td>
</tr>
<tr>
<td></td>
<td>200-400 (8)</td>
<td>240-300 (10)</td>
<td>140-270 (10)</td>
<td>70 (10)</td>
<td>44-50 (1)</td>
<td>190-240 (8)</td>
<td></td>
<td>140 (10)</td>
<td></td>
</tr>
<tr>
<td><strong>Rabbit</strong></td>
<td>200-400 (8)</td>
<td>500-900 (13)</td>
<td>170-390 (13)</td>
<td>300-350 (8)</td>
<td>160-370 (13)</td>
<td>200-250 (8)</td>
<td>60-160 (13)</td>
<td>&lt;30-70 (13)</td>
<td>300-350 (8)</td>
</tr>
<tr>
<td><strong>Guinea-pig</strong></td>
<td>&lt;100 (5)</td>
<td>200-400 (6)</td>
<td>mean 690</td>
<td>300-350 (8)</td>
<td>200-250 (8)</td>
<td>44-50 (1)</td>
<td>190-240 (8)</td>
<td>&lt;30-70 (13)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>200-300 (8)</td>
<td>mean 270</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cattle</strong></td>
<td>100-700+ (3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2000-3000+ (4)</td>
<td>20 (5)</td>
<td>1000+ (3)</td>
<td></td>
</tr>
<tr>
<td><strong>Rat</strong></td>
<td>4300±1000±(3.5, SD) (7)</td>
<td>200-400 (8)</td>
<td>54±3 (11)</td>
<td>4300±300 (9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

( ) Reference

*Range of concentrations in different parts

*Figures given in terms of histamine dihydrochloride

References:

1. Kristakowski, 1943
2. Cicciro and Stoppini, 1949
3. Von Euler, 1949
4. Werle and Wecken, 1949
5. Harris et al., 1952
6. Strengers and Mass, 1956
7. Cloquet, Gaitonde and Richter, 1957
8. Shore et al., 1959
9. Szecserény and Kovács, 1959
10. Adam, 1961
11. Carlini and Green, 1963
12. McGeer, 1964
13. Adam, unpublished (based on 11 rabbits)
spectrofluorimetric method of Shore et al. (1959). The lowest concentration, which was found in the cerebellum, was 200 ng/g. Elsewhere in the brain he reported higher values. But the method was not sufficiently investigated to exclude the possible presence of interfering substances, as observed by Carlini and Green (1963). Values for the concentration of histamine in the brain of different species have been reported by various authors and are shown in Table 1. Although the histamine concentration in whole brain is low, it nevertheless shows an uneven distribution when examined on a regional basis. As pointed out by Adam (1961), the concentration in different parts of the dog's brain is roughly similar to that reported for noradrenaline by Vogt (1954) and 5-hydroxytryptamine (5-HT) by Amin, Crawford and Gaddum (1954). It would seem that histamine is one among several biologically active amines which are present in high concentration in the hypothalamus.

1/2 HISTAMINE IN THE HYPOPHYSIS

Histamine in the hypophysis has so far received little attention. It was first isolated by Abel and Kubota (1919) from
### Table 2

Estimates of histamine concentration in the hypophysis

Expressed as histamine base μg/g of tissue

<table>
<thead>
<tr>
<th></th>
<th>Anterior lobe</th>
<th>Posterior lobe</th>
<th>Hypophyseal stalk (median eminence)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>1.0 - 19.0</td>
<td>0.4 - 26.5</td>
<td>5.7 - 30.0</td>
<td>Harris et al., 1952</td>
</tr>
<tr>
<td></td>
<td>2.3 - 15.2 (mean 7.7)</td>
<td>2.0 - 23.0 (11.4)</td>
<td>6.0 - 27.0 (14.3)</td>
<td>Adam, 1961</td>
</tr>
<tr>
<td>Cat</td>
<td>0.26 - 43.0</td>
<td>&lt;1.0 - 76.0</td>
<td>3.6 - 25.5</td>
<td>Harris et al., 1952</td>
</tr>
<tr>
<td>Pig</td>
<td>0.1 - 1.0</td>
<td>1.2 - 7.0</td>
<td>2.3 - 11.0</td>
<td>Harris et al., 1952</td>
</tr>
<tr>
<td>Rabbit</td>
<td>0.34 - 0.95 (mean 0.67)</td>
<td>0.2 - 0.64 (0.39)</td>
<td>-</td>
<td>Adam (unpublished)</td>
</tr>
</tbody>
</table>

(based on 11 rabbits)
from bovine glands. Harris et al. (1952) were the first to estimate its concentration in different parts of the hypophysis. The values they obtained in the dog and cat were not only high but extremely variable (Table 2).

Adam (1961) confirmed these findings in the dog and it was clear from his results that values for the concentration of histamine in different parts of the hypophysis varied over a wider range than those for different parts of the brain. He suggested that the greater variation in the gland was attributable to the presence of mast cells.

MAST CELLS AND HISTAMINE IN THE BRAIN AND HYPOPHYSIS

Mast cells are common in many tissues in the higher vertebrates. They are not only rich in histamine but account for most of the histamine present in different tissues of the body. The subject has been reviewed in the monograph by Riley (1959). Their presence in the peripheral nerves and apparent absence in the central nervous system may explain the difference in the concentration of histamine in these two parts of the nervous system. Nevertheless the extent to which mast cells
contribute to histamine in the peripheral nerves still remains to be clarified. In the rabbit and in cattle, only part of the histamine extractable from peripheral nerves is localized in mast cells (Werle and Schauer, 1956). Riley and West (1955) failed to release most of the histamine from peripheral nerves in rats, even after prolonged treatment with the histamine liberator compound 48/80. In contrast with mast cell histamine, which is present mostly in dense particles (Hagen, Barrnett and Lee, 1959), histamine in adrenergic nerve fibres does not sediment with large particles (Euler, 1958).

Mast cells have not been found in the brain substance of most mammals (Zimmermann, 1908; Riley and West, 1955; Padawer, 1957; Nepryakhin, 1960). But they have been observed in the meninges of rat (Waldeyer, 1875; Wislocki and Leduc, 1952) and also in dog's choroid plexus (Tsusaki, Eriguchi and Kojo, 1951; Adam, 1961). In the cat, Gray (1935) found mast cells in the hypophysial stalk but none in the hypothalamus. Cammermayer (1947) encountered mast cells in the area postrema of man but this finding has not so far been confirmed. According to Krabbe (discussion to Quensel, 1928), the hedgehog
alone among the mammals has mast cells within the brain substance, the cells being particularly common in the habenular region.

But mast cells are present in the hypophysis of the dog (Arvy and Quivy, 1955; Adam, 1961) and cat (Gray, 1935). In the dog, Adam found them in all parts of the hypophysis but mostly in the pars tuberalis underlying the eminencia media; none were seen in the hypothalamus. In the cat, Gray observed mast cells in the posterior lobe and in the infundibulum but not in the anterior lobe or in the hypothalamus.

Since histamine in brain is not associated with mast cells, it must be present in some other type(s) of cell. Studies on the subcellular distribution of histamine in the brain of guinea-pig, dog and rat have revealed that most of the amine in the brain is located in particles which are much smaller than mast cell granules (Michaelson and Whittaker, 1962; Michaelson and Dowe, 1963; Carlini and Green, 1963). Michaelson and Dowe (1963) reported that most of the 'bound' histamine in dog's hypothalamus sedimented with the crude mitochondrial and the microsomal fractions, whereas most of the hypophysial histamine sedimented, like the mast cell granules, in the
low speed, nuclear fraction. The subcellular
distribution of histamine in the brain is more
like that of acetylcholine (Hebb and Whittaker,
1958; Whittaker, 1959) and 5-HT (Whittaker,
1959; Michaelson and Whittaker, 1963) and is
quite unlike that of histamine in the mast cells
(Hagen et al., 1959).

FORMATION OF HISTAMINE IN BRAIN

Various tissues of different mammalian
species contain the enzyme histidine decarboxy-
lase (for references see Waton, 1956). The
enzyme has been demonstrated in peripheral
nerves (Werle and Palm, 1952), in the brain
and spinal cord of cattle (Holz and Westermann,
1956) and in the brain of rat (Schayer, 1956a),
cat, pig and dog (White, 1959). In the cat,
White (1959) found that decarboxylation was
greatest in the hypothalamus, where the rate of
turnover of $^{14}$C histidine to $^{14}$C histamine was
10 to 15 times greater than in the cerebral
cortex. The cerebellum had the least activity
among the areas examined. Formation of $^{14}$C
histamine in the brain has also been demonstrated
in vivo, in the cat, after intraventricular
administration of $^{14}$C histidine (White, 1960).
Here again, the hypothalamus showed a higher
activity than elsewhere in the brain.

In the rat, $^{14}$C histamine injected into the blood is not taken up by the brain (Halpern, Neveu and Wilson, 1959). In the cat, $^{14}$C histamine is taken up by the hypophysis from the circulation, but not by the hypothalamus (Adam, Hye and Watson, 1964). This would imply that histamine which is present in brain is also formed there. The hypothalamus not only contains the highest concentration of histamine in the brain (Harris et al., 1952; Adam, 1961), but it also forms histamine more actively than any other part of the brain studied so far.

Udenfriend, Lovenberg and Weissbach (1960) reported that several animal tissues, including the brain, contain an aromatic amino acid decarboxylase which acts non-specifically on a group of aromatic L-amino acids, such as 5-HTP, DOPA, tyrosine, tryptophan, phenylalanine, as well as on histidine. This enzyme differs in pH optima and in other respects from the decarboxylase specific for L-histidine (Weissbach, Lovenberg and Udenfriend, 1961). It appears that animal tissues contain at least two enzymes which can decarboxylate histidine. The relative importance, distribution and the role of these two enzymes in the formation of
histamine is not yet known.

Histidine decarboxylase activity of various mouse tissues increases several fold after stress, or after the injection of adrenaline or of endotoxins (Schayer and Ganley, 1959; Schayer, 1960). This increased synthesis of histamine is independent of major endocrine and nervous regulatory mechanisms and is not affected by antihistamines, anti 5-HT compounds or atropine (Schayer, 1962). Schayer (1960) postulated that this increased synthesis of histamine is adaptive and so maintains a balance between histamine and catecholamines, which in turn helps to maintain circulatory homeostasis. Whether the histamine forming activity in brain bears any such relationship to the catecholamines present there, is not yet known.

CATABOLISM OF HISTAMINE IN BRAIN

At first it was thought that diamine oxidase was the only enzyme which inactivated histamine (Zeller, 1951; Tabor, 1954). The main product of histamine oxidation by diamine oxidase is imidazole-4(5)-acetic acid (ImAA) (Tabor, 1951). A conjugate of ribose and ImAA
is excreted in the urine of rats and mice following large doses of histamine or ImAA (Karjala, 1955; Tabor and Hayaishi, 1955). The formation of this conjugate is inhibited by diamine oxidase inhibitors (Schayer, 1953; Karjala, Turnquest and Schayer, 1956).

Schayer has since shown that methylation in the ring nitrogen remote from the side chain is an important pathway for the degradation of histamine in the body. The catabolism of histamine in the body has been reviewed by Schayer (1959).

Properties of the methylating enzyme (imidazole N-methyl transferase) have been studied in vitro (Lindahl, 1958, 1960; Brown, Tomchick and Axelrod, 1959; Gustafsson and Forshell, 1963). This enzyme transforms histamine to l-methyl-4-(β-aminoethyl)imidazole (l,4-methylhistamine). In the transmethylation process, the methyl group is supplied by S-adenosyl methionine.

In vivo, ring methylation is followed by subsequent oxidation of the methylated product to give l-methylimidazole-4-acetic acid (MeImAA). The oxidation of l,4-methylhistamine to MeImAA in the body is by an enzyme resembling monoamine oxidase (Schayer and Karjala, 1956;
However, it has been reported that 1,4-methylhistamine may also be oxidised in vitro by histaminase (considered as a separate enzyme from diamine oxidase) which had been purified from hog's kidney (Kapeller-Adler and Iggo, 1957; Kapeller-Adler and Macfarlane, 1963).

Methylation in the ring is the major pathway of histamine catabolism in the brain. Brown et al. (1959) purified imidazole N-methyltransferase from guinea-pig brain, where it is highly active. The enzyme activity was found only in the soluble supernatant fraction. Axelrod, MacLean and Albers (1961) studied the regional distribution of the enzyme in the brain of monkeys. The activity was found in all parts examined but within the brain, about a ten-fold range of activity was noted. The highest activity was in the neurohypophysis and the lowest, in the pons and cerebellum. The hypothalamus showed considerable methylating activity.

Brain tissue, in vitro, transformed ^14C histamine to 1,4-methylhistamine and MeImAA, but hardly any ImAA was formed (White, 1959). After intraventricular administration of ^14C histamine in the cat, it was mainly
1,4-methylhistamine and MeImAA that were found and not ImAA (White, 1960). The formation of MeImAA in the brain, under such conditions was found to be inhibited when the animal was pretreated with a monoamine oxidase inhibitor (White, 1961a).

The question arises whether methylation in the ring converts histamine into a substance which acts on specific receptors in the brain or whether it represents only the first step in the inactivation of histamine. Not much is known about the effects of 1,4-methylhistamine on the brain. On the basis of tests on plain muscle, 1,4-methylhistamine is much less active than histamine. On the guinea-pig ileum it has only 0.6 per cent and on the cat's arterial blood pressure, only 0.2 per cent of the activity of histamine (Lee and Jones, 1949). It is 200 times less active than histamine on bronchial resistance in the guinea-pig (Westling, 1957).

THE POSSIBLE OCCURRENCE OF OTHER PHARMACOLOGICALLY ACTIVE IMIDAZOLES IN THE BRAIN AND HYPOPHYSIS

Two other imidazoles, namely, β-N-methylhistamine (MH) and β-N-dimethylhistamine (DMH),
which are both methylated in the amino group of the side chain, are known for their similarity to histamine (Hofmann, 1953). Biological methods of assay do not distinguish between histamine and these derivatives. Both MH and DMH have been reported to occur in human urine (Kapeller-Adler and Iggo, 1957). Theoretically these compounds may be involved in the metabolism of histamine (Schayer, 1959).

Werle and Palm (1952) were unable to detect MH and DMH in the sciatic nerve or in the stellate ganglion of cattle. According to Vartiainen (1935), MH had twice, and DMH had about one third, of the activity of histamine on the guinea-pig ileum; on human skin all three compounds had similar activity. Schild (1947) observed that MH was 2.5 times as active as histamine on the guinea-pig ileum and that equiactive doses of both were equally inhibited by mepyramine maleate. Werle and Palm (1952) confirmed that histamine antagonists could not distinguish between histamine, MH and DMH, and showed that diamine oxidase inactivated these methylated derivatives (side chain) as well as histamine. Recently, it was reported that MH and DMH are both even more potent than histamine in producing gastric secretion.
Paper chromatography has been used to separate MH and DMH from histamine (Werle and Palm, 1952; Ames and Mitchell, 1952; Kapeller-Adler and Iggo, 1957). Like histamine, MH and DMH are detectable on paper by the diazo reaction (Pauly, 1904; Hofmann, 1953). Shifrine and Zweig (1964) have reported a chromatographic method for the separation of histamine and its important metabolites by cationic exchange on cellulose phosphate paper.

The presence of MH and DMH in the brain has not been previously investigated.

PHYSIOLOGICAL SIGNIFICANCE OF HISTAMINE IN BRAIN

The possible existence of nerve fibres which act by liberating histamine has already been mentioned. After the introduction of the antihistamines attempts were made to obtain further evidence for the release of histamine in the antidromic vasodilator response. Parrot and Lefebvre (1943) and Holton and Perry (1951) found that antihistamines had no effect on the response. According to Ibrahim, Stella and Talaat (1951) the response was reduced. The evidence has since been reviewed by various authors (Gaddum, 1952; Parrot and Reuse, 1954;
Euler, 1956). There is so far no direct evidence that antidromic stimulation of sensory nerves liberates histamine from the nerve endings and, on the whole, the evidence is against the existence of 'histaminergic' nerves. Nevertheless, the evidence does not exclude the participation of histamine in the antidromic vasodilator response. The problem is further discussed in the recent monograph by Keele and Armstrong (1964).

Virtually nothing is known about the role of histamine in the central nervous system. Histamine is unevenly distributed in the brain, which also contains enzymes for its formation and destruction. This would suggest that it may have a role to play in the specialized functions of those regions where it is present in high concentration.

**Effects of histamine on the brain.** Some indications, however, might be obtained from a study of the actions of histamine on the brain. The pronounced peripheral actions of histamine and its inability to pass from the blood into the brain make it difficult to study any direct actions which it might have on the central nervous system. Moreover, the
Peripheral effects could themselves initiate changes in the central nervous system (Sokoloff, 1959).

In the whole animal, an apparent narcotic effect of histamine in the cat was observed by Dale and Laidlaw (1911). De Jong (1945) described a 'catatonic' effect in mice. Histamine has been reported to change the EEG pattern in rats (Bovet, Kohn, Marotta and Silvestrini, 1958) and to stimulate the vasomotor centre in the cat and dog (Feldberg and Kwiatkowski, 1933). The findings by Häusler (1953) that histamine stimulates motoneurones in the frog could not be confirmed by Angelucci (1956).

Crossland and Mitchell (1956) reported that intracarotid injection of histamine, increases electrical activity of the cerebellum. Similar increased activity was observed after the injection of an extract of cerebellum. The active substance in the extract has since been identified as ergothioneine (Crossland, Mitchell and Woodruff, 1964).

When histamine is perfused through the cerebral ventricles of the cat, it penetrates into the brain tissue and is taken up more by the grey than the white matter (Draškoci, Feldberg, Fleischhauer and Haranath, 1960;
White, 1960). In the conscious cat, intraventricular injection of histamine causes marked changes in the behaviour of the animal: sedation, salivation, increased respiration and muscular weakness (Feldberg and Sherwood, 1954; White, 1961a). Similar effects are also obtained in conscious mice (Haley, 1957), where large doses produce convulsions (Kohn and Millichap, 1958). In anaesthetised cats, intraventricular injections of histamine cause a rise of the arterial blood pressure which is central in origin (Trendelenburg, 1957a; White, 1961a). But this pressor response is not produced in the dog (Bhargava and Tangri, 1959).

Trendelenburg (1957a) suggested that histamine stimulated sympathetic ganglion cells in the brain, since, by analogy, small doses of histamine facilitate transmission of impulses through the superior cervical ganglion of cat (Trendelenburg, 1954, 1956, 1957b). However, Feldberg and Vartiainen (1935) did not find any such action of histamine on ganglionic transmission, and it has also been reported that histamine depresses ganglionic transmission (Gertner and Kohn, 1959).

The discovery of the action of reserpine
on the stores of 5-HT (Brodie, Pletcher and Shore, 1955), noradrenaline (Holzbauer and Vogt, 1956) and of dopamine (Carlsson, Lindqvist, Magnusson and Waldeck, 1958) in the brain, has inspired a large number of investigations on the metabolism of these amines (for references see Shore, 1962). Although it is still not possible to ascribe a definite function to any of these amines in the brain, it is clear that histamine should also be included among the biologically active substances which may be of physiological importance in the brain (Erspamer, 1961).

METHODS OF ESTIMATING HISTAMINE IN THE BRAIN

The quantitative estimation of histamine extractable from tissues generally involves two steps: first, purification of the histamine and secondly, its estimation by a biological or chemical method. Various methods have been used for the estimation of histamine in tissues. Discussion will be confined to those methods which have been used for brain tissue.

Biological method. Barsoum and Gaddum (1935) developed a sensitive method for the
bioassay of histamine on the guinea-pig ileum. Until now this preparation, in different forms, has remained the most reliable biological method for estimating small quantities of histamine. Code (1937) modified and to some extent simplified the method of Barsoum and Gaddum (see also Code and McIntire, 1956). This method though reliable, is not sufficiently sensitive for the brain where the concentrations of histamine are generally low (for example, see Harris *et al.*, 1952).

When the quantity of histamine present in the test solution is less than can be estimated by the conventional method of bioassay in a bath, the sensitivity of the assay can be increased by applying the undiluted test solution directly to the strip of guinea-pig ileum. The gut can be placed in a minute bath (Mongar and Schild, 1950) or suspended in air for superfusion, as described by Gaddum (1953). Such a method is only applicable if the test solution has been sufficiently purified and if it has a composition similar to the solution bathing the ileum between the doses. Adam, Hardwick and Spencer (1954) described a semi-automatic device for bioassay of histamine by
superfusion, where quantities as low as 1.0 to 2.5 ng/ml of histamine in the test solution could be estimated with reasonable precision. Adam (1961) used this method of bioassay for estimating histamine in different parts of dog's brain. The extract of brain tissue was purified using columns prepared by mixing the cationic exchanger Amberlite CG 50 with cellulose powder (Adam, Hardwick and Spencer, 1957; Adam, 1961). Histamine was fully adsorbed on such columns and could be easily eluted with a small volume of 0.25 N HCl.

Chemical methods. Three micro-chemical methods have been described for the estimation of histamine in tissues. These are: (1) colorimetric, based on the diazo reaction (Rosenthal and Tabor, 1948); spectrophotometric, based on a reaction with dinitrofluorobenzene (McIntire, White and Sproull, 1950; Lowry, Graham, Harris, Priebat, Marks and Bregman, 1954), and (3) spectrofluorimetric (Shore et al., 1959). Only the fluorimetric method has been used for estimation of histamine in brain tissue. The fluorimetric method depends on extraction of the histamine into alkaline-butanol and on condensation of the amine with o-phthalaldehyde to yield a product with strong fluorescence (Shore et al., 1959).
Several authors have reported disagreement between values for histamine obtained by fluorimetric and other methods (Graham, 1961; Noah and Brand, 1961; Erspamer, 1961). Carlini and Green (1963) estimated the concentration of histamine in rat’s brain and compared the results obtained by the biological (Adam, 1961) and the fluorimetric method (Shore et al., 1959). They concluded that the high value obtained by the fluorimetric method was due to other substances combining with o-phthalaldehyde. On chromatography several such fluorescent compounds were detected. Some of the conflicting results reported on the concentration of histamine in the brain (in the same species, see Table 1) are probably due to the difference in the method of estimating histamine in brain tissue.
SECTION 2
EXPERIMENTAL

Choice of animals. Adult cats of either sex were used in the experiments. The body weights ranged from 2.5 to 4.5 kg.

2/1 REMOVAL OF THE BRAIN AND HYPOPHYSIS

Under light ether anaesthesia the left carotid was cannulated and the animal was bled to death. Approximately 90 to 110 ml of blood could be collected. The skull was exposed by a mid-line incision and an opening was made with a trephine. The upper part of the skull was removed with bone nibblers, leaving the dura mostly intact. The ossified tentorium of the cerebellum was carefully removed.

The dura was cut and turned over to the sides. The exposed brain was then gently lifted from the front, and its attachment to the base of the skull severed while keeping its connection with the hypophysis intact. By cutting the dura behind and at the sides of the hypophysis, it was always possible to remove the brain and the hypophysis together with their attachment intact. The cord was divided at the level of the second vertebra. The time taken from death of the animal to the
removal of the brain was about 15 min. The brain was placed in a cold flask immediately after its removal. During the dissection which was commenced immediately, the brain was placed on a cold plate surrounded by a mixture of ice and solid CO₂.

**DISSECTION OF THE BRAIN**

The pia-arachnoid on the ventral surface of the tuber cinereum was stripped off. The hypophysis was cleared from the surrounding vascular tissues and separated by dividing at the junction of the gland with the hypophysial stalk. The greater part of the stalk was left still attached to the brain. The **anterior lobe** was detached from the **posterior lobe**. The **hypophysial stalk** was removed by cutting round the median eminence of the tuber cinereum (Figs. 1 and 2). The stalk has a pinkish colour in contrast with the pale grey appearance of the surrounding tuber cinereum.

---

*The anatomy of the hypophysis and the tuber cinereum in the cat has been described in detail by several authors (De Beer, 1926; Wislocki, 1937; Nowakowski, 1951; Metuzals, 1959). Rioch, Wislocki and O'Leary (1940) have defined and given a nomenclature for the different parts in this region in mammals. For the identification of other parts of the cat's brain, several publications were consulted (Winkler and Potter, 1914; Gurewitsch and Chatschaturian, 1928; Rioch, 1929; Ingram, Hennett and Ranson, 1932; Jasper and Ajmone-Marsan, 1960).*
The brain was then divided transversely along a line passing between the pons and the inferior colliculi. The anterior part was further divided into right and left halves by a mid-sagittal section. The hypothalamus (excluding the infundibulum) on both sides of the brain was divided into four parts (Fig. 1) and the corresponding parts from each side were pooled. The samples were cut in rectangular blocks (Fig. 1) to a depth of about 3 mm.

Other samples (except the area postrema) were usually collected only from the right half of the brain. The samples are described as follows:

**Anterior lobe.** Contained mainly the pars distalis of the adenohypophysis.

**Posterior lobe.** Contained mainly the infundibular process and the pars intermedia of the adenohypophysis.

**Hypophysial stalk.** Contained the greater part of the infundibular stem, the median eminence of the tuber cinereum and the glandular tissue of the pars tuberalis (of the adenohypophysis). This was confirmed by histological examination (S. 3/3).

**Corpora mammillaria.** Removed by making three clear cuts round the mammillary body on each side (Fig. 1).

*Samples collected from both sides (right + left) of the brain and pooled.*
Fig. 1. Medial sagittal section of cat’s brain.
Demarcation of samples: (1) anterior lobe; (2) posterior lobe; (3) hypophyseal stalk; (4) corpora mammillaria; (5) preoptic region; (6) ventral hypothalamus; (7) dorsal hypothalamus; (8) medial thalamus; (9) central grey matter; (10) region of red nucleus; (11) superior colliculus; (12) inferior colliculus.
Fig. 2. Transverse section of cat's diencephalon at the level of median eminence of the tuber cinereum. Configuration according to Jasper and Ajmone-Marsan (1960). Demarcation of samples: (3) hypophysial stalk; (6) ventral hypothalamus; (7) dorsal hypothalamus; (8) medial thalamus; (13) dorsolateral thalamus; (14) ventrolateral thalamus.

For nomenclature and key to abbreviations see next page.
**Key to abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>CF</td>
<td>Columna fimbriae</td>
</tr>
<tr>
<td>CL</td>
<td>Nc. centralis lateralis</td>
</tr>
<tr>
<td>Em</td>
<td>Median eminence</td>
</tr>
<tr>
<td>HpD</td>
<td>Hypothalamus posterior (dorsal part)</td>
</tr>
<tr>
<td>HpL</td>
<td>Hypothalamus lateralis</td>
</tr>
<tr>
<td>HpV</td>
<td>Hypothalamus posterior (ventral part)</td>
</tr>
<tr>
<td>LD</td>
<td>Nc. lateralis dorsalis</td>
</tr>
<tr>
<td>MD</td>
<td>Nc. medialis dorsalis</td>
</tr>
<tr>
<td>NCM</td>
<td>Nc. centralis medialis</td>
</tr>
<tr>
<td>RE</td>
<td>Nc. reuniens</td>
</tr>
<tr>
<td>Sm</td>
<td>Nc. submedicus</td>
</tr>
<tr>
<td>TMT</td>
<td>Tractus mammillo-thalamicus</td>
</tr>
<tr>
<td>V</td>
<td>Ventricle</td>
</tr>
<tr>
<td>VL</td>
<td>Nc. ventralis lateralis</td>
</tr>
<tr>
<td>VM</td>
<td>Nc. ventralis medialis</td>
</tr>
<tr>
<td>VPM</td>
<td>Nc. ventralis postero-medialis</td>
</tr>
</tbody>
</table>
Preoptic region. Taken from the anterior part of the hypothalamus in front of a line joining a point between the anterior commissure and the massa intermedia with the posterior border of the optic chiasma (Fig. 1). The sample corresponded with the area preoptica medialis of the hypothalamus.

Ventral hypothalamus and dorsal hypothalamus. The larger posterior part of the hypothalamus (excluding the corpora mammillaria) was divided in the middle in two parts of about equal size (Fig. 1). The ventral hypothalamus was the ventromedial part and the dorsal hypothalamus was the dorsomedial part of the (posterior) hypothalamus. The lateral boundary of these two samples was the fornix (columna fornicis) and the mammillothalamic tract (Fig. 2). This tract (Tr. mammillothalamicus) radiates into the mammillary body and was useful as a guide for the dissection.

Medial thalamus. Taken from the region of the massa intermedia. A rectangular block of tissue was cut out to a depth of about 3 mm (Figs. 1 and 2).

*Samples collected from both sides (right + left) of the brain and pooled.*
Dorsolateral thalamus. Corresponded closely to Nc. lateralis dorsalis of the thalamus and was collected as a triangular block of tissue from the dorsolateral surface of the thalamus (Fig. 2).

Ventrolateral thalamus. Part of the Nc. ventralis lateralis and Nc. ventralis posteromedialis of the thalamus (Fig. 2).

Medial geniculate body and lateral geniculate body. Taken by cutting at their bases along the surface of the thalamus.

Central grey matter. Corresponded to the substantia grisa centralis, which is sharply separated from the massa intermedia and is the mass of grey matter which surrounds the aqueduct in concentric layers. A block of tissue was cut on both sides of the aqueduct (Fig. 1) to a depth of about 2mm.

Region of red nucleus. The area immediately ventral to the central grey matter (Fig. 1).

Superior colliculus and inferior colliculus. Removed by cutting along their bases.

Cerebral cortex. Different areas of the telencephalic cortex were sampled. According to the cytoarchitectonic map of cat's brain
produced by Gurewitsch and Chatschaturian (1928), the samples were from areas 2, 4b, 52, 50, 20 and 22 of the sensory cortex and from areas 24 and 30 of the cingulate gyrus.

**Cerebellum.** Taken from the vermis.

**Caudate nucleus.** Taken from the middle part of the nucleus.

**Area postrema.** Dissected out from both sides of the floor of the 4th ventricle (posterior part).

**Corpus callosum and optic nerve.** Taken as samples of white matter. The perineural connective tissue was stripped off from the optic chiasma and the optic nerve before the collection of the samples.

**Choroid plexus.** Taken from the lateral ventricle.

Other miscellaneous samples were:

**Hippocampus.** Taken as a transverse segment in the inferior horn.

**Floor of the 4th ventricle.** Was the grey matter cut to a depth of about 2mm.

**Region of the reticular formation.** Was the region in the pons and medulla or tegmentum of the midbrain.

**Brachium pontis**
Nc. cuneatus
Nc. gracialis
Pineal body

In all 31 different areas were sampled, including those of the hypophysis. The samples were obtained from a total of 25 cats.
2/2/1 Weighing of samples

The sample was weighed without delay on a torsion balance of 100 mg capacity using a wire basket. The time required for the collection of samples was about 30 min. The weight of any particular sample varied from cat to cat (Table 5), partly because of differences between cats but also because of the error of dissection. In order to reduce this error, the dissection of the brain was always done by the same person.

2/2/2 Storage of samples

Most of the samples were extracted immediately after their collection. Samples from the hypophysis, hypothalamus, medial thalamus and the area postrema were always extracted fresh. Samples from the rest of the brain were sometimes frozen at -17°C for 24 hours, and at other times also extracted fresh. For storage, the tissue was immediately frozen by placing in a small bottle which had been previously cooled on solid CO₂.

The effect of storage on histamine extractable from paired tissue samples of dog's brain was examined by Adam (1961). Similar experiments were carried out in the present work and
are described in Appendix I.a. The differences between the fresh and stored samples (24 hours) were usually small.

2/3 EXTRATION AND PURIFICATION OF HISTAMINE

Adam, Hardwick and Spencer (1957) developed a method of estimating histamine in plasma. Adam (1961) modified this method for the estimation of histamine in different parts of dog's brain. This method with minor modifications was used in the present work.

2/3/1 Apparatus

Tissue grinders. These were made of 15 ml heat-resistant conical centrifuge tubes (MSE Cat. No. 69353). The lower part was ground and fitted with a corresponding pestle, made of soft glass. The tube was calibrated to contain 5 ml.

Glass tubes for column chromatography. Columns were made in glass tubes (Fig. 3) which were 13 - 14 cm in length and 8.0 mm internal diameter. The tube was surmounted by a bulb of 25 ml capacity and closed by a glass tap. The overall length was 27 - 28 cm. The dead space below the column (tap and capillary below
Fig. 3. Glass tube for column. For details see text.
it) was about 0.3 ml. A plastic cap covered the top of the bulb.

Nine such tubes were held by spring clips on a Dexion frame.

Refrigerator centrifuge. Type MSE "Medium".

pH Meter. Marconi, Type TF 511 D with 'serum electrode' Type TM 1922C-1.

2/3/2 Chemicals and Solutions

Chemicals used in the experiments were of analytical grade unless stated otherwise. Fresh, glass distilled water was used for solutions and elsewhere in the procedure.

Ion exchange resin. Amberlite, Type CG 50, 100 - 200 mesh (Rohm and Haas Co., Philadelphia). The resin is a weak cation exchanger with carboxylic (-COOH) groups. It is supplied in the acidic (H) form.

Cellulose powder. Whatman Standard Grade for chromatography.

Trichloroacetic acid (BDH). 6 per cent w/v solution in water (TCA). The solution was filtered.

Hydrochloric acid. 0.1 N and 0.25 N HCl prepared from concentrated volumetric solution (BDH). 6.0 N and 4.0 N HCl prepared from
concentrated HCl (36 per cent HCl, BDH).

**Sodium hydroxide.** 0.1 NaOH prepared from sodium hydroxide pellets (BDH) and titrated against standard 0.1 N HCl, using phenolphthalein as internal indicator.

**Neutral red (BDH).** 0.01 per cent (w/v) in water.

**Phosphate buffer:** 0.2 molar: Na\(^+\) concentration 390 mEq/litre. 15.6 g NaH\(_2\)PO\(_4\).2H\(_2\)O dissolved and made up to 500 ml with water

= Solution A

28.4 g Na\(_2\)HPO\(_4\) dissolved and made up to 1 litre with water

= Solution B

26.5 ml of Solution A was mixed with 473.5 ml of Solution B to make 500 ml of buffer solution. The pH of this solution was 7.92 and the total Na\(^+\) concentration was calculated as 390 mEq/litre.

**Phosphate buffer:** 0.05 molar: Na\(^+\) concentration 100 mEq/litre. 256.0 ml of the 0.2 molar buffer (390 mEq/l Na\(^+\)) was made up to 1 litre with water. The pH of this solution was 8.0 and the total Na\(^+\) concentration was calculated as 100 mEq/litre.

**Chromic-sulphuric cleaning solution.** 70.0 g/l of sodium dichromate in concentrated
sulphuric acid (commercial grade).

2/3/3 Procedure

Cleaning of glassware. Glassware was left in cleaning solution for at least 2 hours. Later it was rinsed thoroughly, first in warm, running tap water and then six times with distilled water. Apparatus in contact with concentrations of histamine above 100 ng/ml was soaked in 4N HCl overnight and later rinsed with 2 N NaOH before being finally cleaned with chromic-sulphuric solution.

Protection of glassware. Much of the clean, dried glassware was stored in boxes to protect it from dust in the air. Pipettes used for measuring volumes of reagents or solutions in the procedure were stored individually in stoppered glass tubes, thereby reducing the risk of cross contamination.

Preparation of resin. 30 g of resin was suspended in 1.0 litre of distilled water in a beaker and allowed to sediment for 10 min. The water was decanted. This procedure was repeated 6 times. The final deposit was transferred to a porcelain dish and dried at 45°C for 48 hours. The cake was broken up with a small pestle.
The resin prepared in this way was free from very fine particles.

**Preparation of the columns.** 50.0 mg of the prepared resin was mixed with 300 mg of cellulose powder in a 25 ml conical flask. 4.0 ml of 0.1 N NaOH was added to the flask and the contents were mixed by gentle shaking. The resin-cellulose mixture was left overnight at 4°C for use on the next day. The resin was thereby partly converted into the sodium form.

The tube was cleaned with chromic-sulfuric solution immediately before use. A pad of glass wool was placed at the bottom of the tube and the tap was lightly greased with white vaseline. The tube was rinsed from inside, first with distilled water and then with the buffer solution (0.05 molar, 100 mEq/1 Na⁺).

5 ml buffer solution (0.05 molar, 100 mEq/1 Na⁺) was placed in the flask containing the resin-cellulose suspension. The contents of the flask were well mixed and poured into the tube. The tap was fully opened and the resin-cellulose mixture was allowed to form a column. When the liquid level reached the top of the column, the tap was closed. The height of the column was about 42 mm. The volume occupied
by the column was about 2 cm$^3$.

**Equilibration of column.** 20 ml of buffer solution (0.05 molar, 100 mEq/l Na$^+$, pH 8.0) was applied to the column, and care was taken to wash down the inner side of the tube. The buffer solution was allowed to flow at a rate of about 1 ml in every 3 min, by adjusting the tap. The column was equilibrated in this way for 1 hour, at the end of which it was ready for use.

Nine columns were usually prepared. Eight of these were for the tissue extracts and one was used as a control.

**Extraction of the tissue sample.** The sample was removed from the weighing basket with the aid of a fine glass rod and lowered into a measured amount of TCA in the tissue grinder. The volume of TCA used was 5 $\mu$l/mg of tissue. Stored, frozen samples were placed directly in the TCA. The tissue was immediately ground and the process continued with the addition of a small volume (1 ml) of water. The final volume in the tube was made up to 5 ml with water. The suspension was thoroughly mixed and the tube was sealed with parafilm. Usually eight samples were extracted at a time.

The efficiency of the extraction was tested.
in a separate experiment which is described in Appendix I.b.

**Centrifugation of the extract.** The tubes were centrifuged at 2000 r.p.m. at 4°C for 30 min.

**Collection of aliquot.** An aliquot of 4.6 ml was removed from the clear supernatant using a Pasteur pipette. The aliquot was placed in a stoppered, graduated 10 ml tube. The precipitate was discarded.

**Preparation of the solution for adsorption**

**Neutralization of the aliquot.** The aliquot of the supernatant was neutralized with 0.1 N NaOH delivered from a 1 ml burette. One drop (~0.04 ml) of neutral red solution was used as internal indicator. Approximately 0.02 ml of alkali was required for each mg of tissue. After the point of neutralization had been reached, one drop (0.02 ml) of 0.1 HCl was added from another burette to make the solution just acidic.

**Adjustment of pH and Na⁺ concentration in the solution.** The neutralized aliquot was brought to pH 8.0 and to a total Na⁺ concentration of approximately 100 mEq/l by the addition
of phosphate buffer (0.2 molar, 390 mEq/1 Na⁺, pH 7.92). Water was then added to a final volume of 8.0 ml. The volume of buffer depended on the quantity of Na⁺ added in the neutralization (Na⁺ derived from the tissue was neglected).

In the hypothetical case where the aliquot does not require the addition of 0.1 N NaOH (i.e. the solution is already neutral), the volume of buffer (0.2 molar, 390 mEq/1 Na⁺) for the adjustment is calculated as follows:

\[
\frac{8 \text{ ml} \times 100 \text{ mEq/1}}{390 \text{ mEq/1}} = 2.05 \text{ ml}
\]

When alkali was added, the volume of buffer required was less than 2 ml. The exact volume of buffer was calculated as follows:

Na⁺ present in 1.0 ml of buffer (390 mEq/1 Na⁺)

\[
= \frac{390 \times 23}{1000} = 8.97 \text{ mg}
\]

Na⁺ present in volume (ml) of 0.1 N NaOH added

\[
= (2.3 \times \text{ ml of 0.1 N NaOH added}) \text{ mg}
\]

Volume of buffer (390 mEq/1 Na⁺) to be added

\[
= (8.97 \times 2.05) - (2.3 \times \text{ ml of 0.1 N NaOH added})
\]

\[
= 8.97
\]

A calibration curve was drawn on the basis of this calculation. For a given volume of 0.1 N NaOH added, the required volume of buffer could
be read off directly from the graph. After addition of the buffer the volume in the tube was made up to 8.0 ml with water. The solution was mixed and was ready for transfer to the column.

Adsorption and elution

Adsorption. 8.0 ml of the buffered aliquot was applied to the equilibrated column. The graduated tube was washed with 2.0 ml of buffer solution (100 mEq/l Na+, pH 8.0) and this was placed on the column. The control column received 10.0 ml of buffer solution (100 mEq/l Na+, pH 8.0).

The flow was controlled at the rate of 1 ml in 3 min. The effluent was collected and its pH, which was determined electrometrically as a routine, was compared with the pH of the effluent from the control column. The pH of the effluent was 8.0 ± 0.1 and seldom varied beyond these limits.\(^x\)

Wash. After the adsorption step, the column was washed with 5.0 ml water, which was allowed to flow at the rate of about 1.0 ml in 3 min. The effluent was discarded.

\(^x\)Historamine has two pK\(_a\) values: one due to the imino nitrogen in the imidazole ring is pK\(_a\) 5.90 and the other due to the amino group is pK\(_a\) 9.70 (Levy, 1935). At pH 8.0 nearly all the histamine (98.04 per cent) present in the solution would be ionized. Adsorption of histamine by the column at pH 8.0 is complete (Adam et al., 1957).
Elution. The elution was carried out with 2.0 ml of 0.25 N HCl, followed by 3.0 ml water. The eluate was collected in a stoppered glass tube (Quickfit, MF 24/3, B 24/29, capacity 50 ml). The flow rate during the elution was reduced to about 1 ml in every 4 min. The neutral red remained adsorbed at the top of the column.

A detailed study of the elution was carried out in a separate experiment, where the eluate was collected in fractions of 1 ml. The fractions were analysed for the pH values, and for the amount of Na+ and histamine present. The results are given in Appendix I.e.

Evaporation of the eluate. The eluate was dried at 55°C under reduced pressure (20 to 30 mm Hg). The evaporation was done in a thermostatically controlled water bath, the tube containing the solution being connected to a high pressure water pump through an adaptor. The vacuum was gradually reduced when the residue was seen to be still slightly moist, to prevent loss from flaking. The residue contained mainly NaCl derived from the column.

Heating with strong HCl. 1 ml of 6 N HCl was added to the tube and the tube heated for 30 min in an oil bath at 100°C (Adam, 1961).
The tube was immersed in the oil only up to the liquid inside. The stopper was loosened and left in situ. Under these conditions the HCl refluxed in the tube without detectable loss of vapour.

The acid was evaporated at 60°C under reduced pressure (20 to 30 mm Hg). Flaking was not observed at this stage and complete drying was possible. The neck and the stopper of the tube were dried with a stream of hot air to remove traces of HCl. (At this stage the tubes were generally stored overnight).

**Final drying of the residue.** The last traces of acid were removed from the residue by heating the tube in a water bath at 80°C for 10 min and under reduced pressure (20 to 30 mm Hg). The neck of the tube and the stopper were again dried with hot air. The contents of the tube, which will be referred to as the dried residue, were then ready for bioassay.

The dried residue contained about 24 mg of NaCl (Adam, 1961). This value was confirmed by estimating the total Na present in the eluate and in the dried residue by Flame Photometry. The details of the estimations are given in Appendix I.c. The mean value of NaCl present
in the dried residue was calculated as 23.64 mg (range 22.8 to 24.75) from 10 different observations.

Total potassium in dried residue obtained from brain tissue extracts was also investigated, since a small excess of K⁺ in the test solution as compared with Tyrode's solution could interfere with the assay (Adam et al., 1954). The results of this investigation are given and discussed in Appendix I.d.
A flow sheet of the procedure for extraction and purification of histamine for bioassay

Extract with TCA (5 μl/mg)

Centrifuge (5 ml)

ppt (discard)

Remove aliquot of SN (4.6 ml + 0.1 N NaOH (to pH 7) + Buffer, 0.2 molar (to pH 8 and 100 mEq/l Na+)) (Volume 10 ml)

Adsorption (pH 8)

effluent (discard)

Wash

Elution (2 ml 0.25 N HCl + 3 ml water)

Evaporation of the eluate

Residue + 1 ml 6 N HCl (30 min at 100°C)

Evaporate HCl

(Dried residue)
2/4 BIOLOGICAL ASSAY

The assay was carried out by superfusion on the guinea-pig ileum (Gaddum, 1953), using a semi-automatic apparatus as described by Adam et al. (1954).

2/4/1 Solutions

Chemicals of analytical grade were used.

*Atropinized Tyrode's solution.* The composition of the solution is given below. This solution is referred to in the text as Tyrode's solution.

<table>
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<tr>
<th>Stock solution</th>
<th>Tyrode's solution</th>
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<tbody>
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<td></td>
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<td>NaCl</td>
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<tr>
<td>MgCl₂</td>
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<tr>
<td>NaHCO₃</td>
<td>36.0</td>
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<tr>
<td>Glucose</td>
<td>-</td>
</tr>
<tr>
<td>Atropine sulphate (10⁻⁴)</td>
<td>(10⁻⁷)</td>
</tr>
</tbody>
</table>
The concentration of CaCl$_2$ and MgCl$_2$ in the stock solutions was adjusted by titration against 0.1 N AgNO$_3$, using 0.04 ml of potassium chromate (5 per cent w/v) as internal indicator.

**Histamine standard solutions.** The material used was histamine acid phosphate (BDH, m.w. 307.15). Values for histamine in the text refer to the base (m.w. 111.15), unless stated otherwise.

**Stock histamine standard, 5 μg/ml in water.** Except when in use this solution was kept frozen at -17°C and used from time to time for periods of 2 to 3 months. Solutions kept in this way showed no loss of activity when compared with solutions prepared from salt on the day of the assay. The following dilutions were prepared in Tyrode's solution at the time of the assay.
a) **100 ng/ml.** 2.0 ml of the 5 \( \mu g/ml \) solution made up to 100 ml with Tyrode's solution.

b) **10 ng/ml.** 20.0 ml of the 100 ng/ml solution made up to 200 ml with Tyrode's solution.

c) **3 ng/ml.** 6.0 ml of the 100 ng/ml solution made up to 200 ml with Tyrode's solution.

d) **Ten Assay Standards. 0.25 to 2.5 ng/ml.** Prepared from the 10 ng/ml solution in the following way.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>From 10 ng/ml Made up with</th>
<th>To 50.0 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 ng/ml</td>
<td>1.25 ml Tyrode's solution in volumetric flasks</td>
<td>0.25 ng/ml</td>
</tr>
<tr>
<td>0.50 ng/ml</td>
<td>2.50 ml</td>
<td></td>
</tr>
<tr>
<td>0.75 ng/ml</td>
<td>3.75 ml</td>
<td></td>
</tr>
<tr>
<td>1.00 ng/ml</td>
<td>5.00 ml</td>
<td></td>
</tr>
<tr>
<td>1.25 ng/ml</td>
<td>6.25 ml</td>
<td></td>
</tr>
<tr>
<td>1.50 ng/ml</td>
<td>7.50 ml</td>
<td></td>
</tr>
<tr>
<td>1.75 ng/ml</td>
<td>8.75 ml</td>
<td></td>
</tr>
<tr>
<td>2.00 ng/ml</td>
<td>10.00 ml</td>
<td></td>
</tr>
<tr>
<td>2.25 ng/ml</td>
<td>11.25 ml</td>
<td></td>
</tr>
<tr>
<td>2.50 ng/ml</td>
<td>12.50 ml</td>
<td></td>
</tr>
</tbody>
</table>

**Modified Tyrode's solution.** This was for reconstitution of the dried residue. The
concentration of NaCl in this solution was only 3.65 mg/ml. The solution was made up from a 'Stock Solution A' in the following way.

**Tyrode stock**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>To make 200 ml</strong></td>
<td><strong>KCl</strong></td>
<td>(8.0 g/l) 50.0 ml</td>
</tr>
<tr>
<td><strong>'Stock Solution A'</strong></td>
<td><strong>CaCl₂</strong></td>
<td>(5.5 g/l) 50.0 ml</td>
</tr>
<tr>
<td>from Tyrode stocks</td>
<td><strong>MgCl₂</strong></td>
<td>(4.0 g/l) 50.0 ml</td>
</tr>
<tr>
<td></td>
<td><strong>NaH₂PO₄·2H₂O</strong></td>
<td>(2.0 g/l) 50.0 ml</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>200.0 ml</strong></td>
</tr>
</tbody>
</table>

5.0 ml of the 'Stock Solution A' was diluted with water and 0.5 ml of NaHCO₃ solution (Tyrode stock, 36.0 g/l) added to it. The volume was made up to 50.0 ml with water. Finally the volume was made up to 92.0 ml by adding 42.0 ml Tyrode's solution.

The dried residue in the tube was dissolved in 5.0 ml of this solution. Any further dilution of the test solution was carried out with the Tyrode's solution only.

---

This was to compensate for the presence of about 24 mg of NaCl in the dried residue. When the dried residue was dissolved in 5 ml of this solution, the composition of the final solution (test solution) became close to that of Tyrode's solution. With the concentrations used, the test solution could be slightly hypertonic compared with the Tyrode's solution. If the extreme values of NaCl present in the dried residue were 22 and 25 mg (Appendix I.c.), on reconstitution the test solution would not differ by more than +5.0 or -3.0 per cent, as compared with the Tyrode's solution for the total NaCl. Such differences would not affect the bioassay by superfusion (Adam et al., 1954).
Mepyramine-Tyrode's solution. A stock solution of mepyramine maleate, 10 μg/ml (base) in water, was kept frozen at -17°C. When required 1.0 ml of this solution was made up to 100 ml in Tyrode's solution to make 100 ng/ml solution of mepyramine.

15.0 ml of the 100 ng/ml mepyramine solution was made up to 500 ml with Tyrode's solution to make Mepyramine-Tyrode's solution containing 3 ng/ml (3 x 10^{-9}) of mepyramine.

2/4/2 Superfusion Apparatus

The apparatus used was similar to that described by Adam et al. (1954) as shown in the diagram (Fig. 4). The various parts were mounted vertically on a strip of Dexion. In the present apparatus the 'doser' (F) was operated by a solenoid relay (R.A. Webber Ltd., Bristol, type SCM for 24 volts D.C.) which was placed immediately above the plunger, to which the solenoid core was fixed by a coupling device. The plunger was made of a steel wire, enclosed in a polythene tube (2 mm o.d.) which was drawn out to a closed fine point. This served as a valve at the opening of the 'doser' which was a 1 ml glass tuberculin syringe. The 'doser'
Fig. 4. Superfusion apparatus: (A) Tyrode's solution; (B) Tyrode + histamine; (C) Tyrode + mepyramine; (D) capillary resistance; (E) solenoid relay; (F) 'doser' with valve; (G) warming tube; (H) superfusion chamber; (J) lower end of 'doser' with valve; (K) lower end of warming tube with glass hook and thread in position. The timing unit and thermostatically controlled water reservoir are not shown in the figure.
was calibrated to contain 0.6 ml. The weight of the solenoid core was sufficient to keep the valve closed when the solenoid was inactive. The height to which the plunger would be lifted on activation of the solenoid was adjustable. This adjustment made it possible to widen or narrow the valve and thereby to control the flow-rate of the dose. The connections to the solenoid were through a variable potentiometer (100 Ohms) which served to control the energy supplied to the relay.

The dose (0.6 ml) flowed over the gut in 8 to 9 sec. The flow of Tyrode's solution returned 1 to 2 sec later. The gap between these two flows was kept as short as possible to avoid spontaneous contractions of the gut. The point of maximal contraction after a dose of histamine was reached before the flow of Tyrode's solution had returned.

The pre-heating tube (G) was flared at its inlet and at the outlet to prevent retention of fluid by capillary action. The rim at the outlet carried a short glass arm which ended in a hook (K). Warm solution flowed along this arm: drops formed on the hook, fell along the enclosed thread and broke on the gut to enclose it in a
film of liquid. The drops re-formed at the end of the gut and went to waste.

Relays 1, 2 and 3 were P.O. type relays (100 Ohms). A Mariotte bottle (A) of 2 litre capacity served as the reservoir for Tyrode's solution. The rate of flow from the bottle was controlled by a capillary resistance (D) which allowed a flow of 4.6 ml/min. This amounted to 60 drops per minute on the gut. The flow stopped with the action of Relay 1.

A Mariotte bottle (B) of 500 ml capacity contained the histamine solution (3 ng/ml), which flowed through a capillary resistance at the same rate as the Tyrode's solution, but only when Relay 2 was active. This dose was delivered automatically and served to sensitize the gut to histamine before starting the assay. When either the solenoid relay (E) of the 'doser' or Relay 2 was active, the flow of Tyrode's solution was automatically stopped by the simultaneous action of Relay 1. The solenoid relay and Relay 2 were connected by a two-way switch so that when one of the two was active, the other was disconnected from the circuit.

A Mariotte bottle (C) of 500 ml capacity contained Tyrode's solution with mepyramine (3 ng/ml)
when this was used. The flow of this solution through a capillary resistance was at the same rate as that of Tyrode's solution (i.e. 4.6 ml/min). Relay 3 served the same purpose as Relay 1 and so stopped the flow of mepyramine-Tyrode's solution while a dose was flowing over the gut.

The control of the dose cycle was through a Sequential Timing Unit as described by Austin (see Adam et al., 1954).

The conditions of the assay can be summarized as follows:

**Dose cycle** ............................................. 72 sec
Time for the dose to flow over gut .......................... 8 sec
Solennoid relay of doser activated for ...................... 10 sec

**Flow rate**
Tyrode's solution .................................... 4.6 ml/min (= 60 drops on gut)
Dose (0.6 ml) ........................................ 8-9 sec (variable)

**Temperature**
Water bath reservoir .................................... 34°C
Inside superfusion chamber ............................... 33°C
Flow at the outlet of warming tube ....................... 32°C-33°C

**Lever:** light balsa wood

Total length .............................................. 27.5 cm
Magnification ............................................ 8.4 times
Fixed tension ............................................ 500 mg
Variable load ............................................ 210 mg
Writing point: frontal, made of capillary glass tube.
**Guinea-pig ileum**

A strip of the terminal part of ileum, 3 to 4 cm long when slightly extended, was taken from young guinea-pigs weighing 150 to 250 g. As compared with that of heavier animals, the gut was usually thinner and showed less spontaneous activity but became more readily sensitive to small doses of histamine.

**Procedure of assay**

**Sensitization.** The gut was left under superfusion with only Tyrode's solution flowing over it for at least 30 min. The histamine standard solutions were prepared and sensitization of the gut with 3 ng/ml histamine solution was started. The Timing Unit was switched on with connections to Relays 1 and 2. The dose cycle started with doses of 3 ng/ml histamine once every 72 sec. The drum was kept at a low speed (2 mm/min). The paper was very lightly smoked. With the first few doses the gut usually showed increasing sensitivity but soon the contractions became of similar magnitude and an equilibrium was reached. The base line normally remained stable and straight but varied to some extent with very sensitive guts. When
the gut was found to be poorly sensitive, it usually improved with the passage of time and it was seldom necessary to change it for another strip.

Dose-response curve. When the gut was sufficiently sensitive and stable, a dose-response curve was recorded with histamine standard solutions from 0.25 to 2.5 ng/ml. The doses were placed in the 'doser' by hand using separate Pasteur pipettes. In the range 0.25 to 2.5 ng/ml of histamine, the dose-response curve was approximately linear and the gut usually detected differences of 0.25 ng/ml in this range (Fig. 5). Often both an ascending and a descending dose-response curve was recorded. A lower dose followed by a much higher one (or the reverse) sometimes affected the response, owing to residual solution in the 'doser'. Interference from this cause within a sequence of doses seldom occurred because it was always possible to perform the assay within a narrow range of the dose-response curve. When Tyrode's solution was applied from the 'doser' (in place of a dose), there was usually a small contraction of the gut.

*These were special long Pasteur pipettes (about 30 cm) which were drawn out and bent at the tips. About 2 ml of solution could be withdrawn conveniently from a 50 ml flask. Similar pipettes were used for applying the test solution on the 'doser'. 
Fig. 5. Responses of superfused guinea-pig's ileum to doses of histamine standard solutions, Tyrode's solution and a purified extract of ventral hypothalamus. The first part of the tracing shows a dose-response curve and the second part a typical assay. The numerals give the doses of histamine standard solutions in ng/ml. Other doses are labelled as follows: TYR, Tyrode's solution; TF, a fractional dose of the test solution; T, test solution diluted 1 in 4 and estimated to contain 1.12 ng/ml histamine.
This was always less than the contraction with 0.25 ng/ml histamine standard and was regarded as a 'zero effect'.

**Test solution.** The dried residue was dissolved in 5.0 ml of the modified Tyrode's solution (see page 53). Since many of the test solutions required further dilution, the first dose was a trial fractional dose of the test solution. 0.2 to 0.3 ml of the test solution was placed in the 'doser'. and Tyrode's solution from a wash bottle was added in the 'doser' up to the mark at 0.6 ml. The proper dilution of the test solution, required for the assay could be inferred from the contraction with the trial dose. When required, a dilution of the test solution was made in a 10 ml graduated cylinder.

**The assay.** This was done by bracketing. An arithmetical scale for the standard doses was chosen (Adam et al., 1957). Usually 5 or 6 doses of the test were interposed between doses of the standards. A dose of Tyrode's solution was given between two sets of assays.

The estimate of concentration of histamine in a tissue sample was calculated as follows:
Estimate of concentration (ng/ml) X dilution factor X 5 X 5/4.6 in the test solution

= Estimate as nanogram (ng) in whole tissue sample

\[ \text{ng of histamine in sample} \times 1000 \]
\[ \text{weight of sample in mg} \]

= Estimated concentration of histamine ng/g of the tissue sampled

The factor 5/4.6 was used to correct for the volume of aliquot (4.6 ml) collected from the supernatant, assuming that the histamine present in the extract was equally distributed in the supernatant and in the much smaller volume occupied by the precipitate.

2/4/5 Mepyramine test

In a number of assays, the test was reassayed in the presence of Tyrode’s solution containing 3 ng/ml of mepyramine. A few doses of 3 ng/ml histamine standard were applied at first.

\[ \text{A more complete Mepyramine test under similar conditions was reported by Adam (1961). Using material from dog’s brain and purified for superfusion as described in the present work, it was found that a concentration of mepyramine which abolished the response of the ileum to equiactive doses of test and histamine standard, only slightly reduced the response to doses of 5-HT and substance P. On repeating the assay in the presence of low concentrations of mepyramine the estimates for the histamine equivalent agreed to within 10 per cent.} \]
The contractions to 3 ng/ml histamine soon became minimal. Doses of the test were then applied alternating with equiactive doses of histamine standards (those used beforehand for bracketing the test). The test was reassayed. In some cases it was difficult because the contractions to the test and the equiactive standards were almost abolished. In such cases the undiluted test was assayed, using stronger histamine solutions as standards.

RECOVERY EXPERIMENTS

The object of these experiments was to test the efficiency of the method. Histamine was added in quantities of 25, 50 and 100 ng to fresh samples of cerebellum and cerebral cortex weighing approximately 50 mg. The histamine was delivered in a small volume from a micrometer syringe ('Agla', Burroughs Wellcome). TCA was then added to each tube and the extraction and subsequent steps of the procedure were as described under methods (S. 2/3 and 2/4). Control samples were taken to estimate histamine originally present in the tissue. The final recovery was calculated from the difference between the total amount estimated and that
present in the control.

In similar experiments 25 to 100 ng of histamine were added to 10.0 ml buffer solution (0.05 molar, 100 mEq/l Na⁺). The recovery of histamine from buffer was thus compared with its recovery from tissue.

To investigate loss after the elution step (S. 2/3), 50 ng of histamine was added to each of the eluates obtained from five columns to which extracts of cerebellar tissue had been previously applied for adsorption.

The eluate in each of the above experiments was evaporated, heated with 6 N HCl and treated in the same way as described (S. 2/3). Histamine was estimated by superfusion as in S. 2/4.

PAPER CHROMATOGRAPHIC SEPARATION OF THE ACTIVITY IN THE ELUATE MATERIAL AND ITS ESTIMATION BY BIOASSAY

Experiments were carried out to isolate the activity in the dried residue of the eluate by paper chromatography. The possibility of the presence of β-N-acetylhistamine in the eluate was excluded, because even if it were present in the extract, it would not have been adsorbed (Adam et al., 1957). The possibility
of the presence of other biologically active imidazoles closely related to histamine could not be excluded (S. 1/6).

Particularly important were β-N-methylhistamine (MH) and β-N-dimethylhistamine (DMH), which are not destroyed by heating with strong HCl and which are not distinguishable from histamine in pharmacological tests (Vartiainen, 1935; Schild, 1947; Werle and Palm, 1952). Another possibility was the presence of 1,4-methylhistamine, which, however, is known to be much less active than histamine on the guinea-pig ileum (Lee and Jones, 1949).

The object of the experiment was to test whether or not the activity in the test solution was due solely to histamine or to a mixture of histamine and other pharmacologically active derivatives. Active substances in the eluate material were therefore separated on paper chromatograms and estimated biologically. Before this investigation could be undertaken it was desirable to study the following.

(a) The relative potencies of MH, DMH and 1,4-methylhistamine as compared with histamine on the superfused guinea-pig ileum. The results are given in Appendix I.f.
(b) The recoveries of these derivatives under the conditions used in the method for the purification of histamine. In other words, whether these substances are also adsorbed and eluted from the column at pH 8.0 along with histamine, and whether or not their activities withstand heating with 6 N HCl. The results are given in Appendix I.g.

(c) A paper chromatographic system suitable for the separation of the compounds from mixtures. This was investigated using authentic substances and under different conditions. The system which was finally adopted is described in Appendix I.h. along with the Rf values of the substances in the system.

2/6/1 Apparatus

Chromatography tank. All-glass chromatank (Aimer Products Ltd.). Volume: 52 x 29 x 19 cm (internal).

Chromatography oven. Shandon Scientific Co. (cat. no. 3121).

Sprayer. All-glass sprayer (Aimer)

2/6/2 Chemicals and solutions

Histamine dihydrochloride. m.w. 184.08, BDH.

Stock solutions: 5 µg/ml in water and 200 µg/ml in methanol.
6-N-methylhistamine dihydrobromide. m.w. 286.8, Eli Lilly. Stock solutions: 5 µg/ml in water and 200 µg/ml in methanol.

6-N-dimethylhistamine dihydrochloride. m.w. 212.1, Eli Lilly. Stock solutions: 5 µg/ml in water and 200 µg/ml in methanol.

(Values for the amines in the text refer to the base)

iso-Propyl alcohol. BDH
Ammonia. Sp. gr. 0.880, BDH.
Acidified ethanol. Twice redistilled ethanol, containing 0.1 per cent HCl (v/v).
Methanol. BDH
Fresh diazotized sulphanilic acid. For method of preparation and use, see Appendix I.h.

Chromatography paper. Whatman no. 1; 46 x 22 cm paper cut out to make 6 strips, each 3 cm wide. The strips were at 90° to the machine direction and were kept joined together at both ends.

2/6/3 Procedure

The following samples were collected from a cat's brain.

(1) Hypothalamus: this was from both sides of the brain and included the four samples
usually collected from the hypothalamus (S. 2/2). Total weight of the sample was 101.5 mg. This sample was for the chromatographic analysis of its activity.

(2) Thalamus: the sample was the medial thalamus as usually collected (S. 2/2), but from both sides of the brain. Total weight of the sample was 74.2 mg. This sample was for the use as marker on the chromatogram.

(3) Cerebellum: 104.0 mg of cerebellar tissue was from the region of the vermis. This sample was for the recovery of the substances added to it in a mixture. Histamine (200 ng), MH (200 ng) and DMH (200 ng) were added to the tissue grinder containing this sample before its extraction with TCA. Each substance was added in a volume of 0.04 ml from stock solutions (5 µg/ml in water) with the aid of a micrometer syringe (Agla).

The three samples (1 - 3) were extracted with TCA and purified as described earlier (S. 2/3). They were brought to the stage of final drying before biological assay. The eluate from a control column which had 10 ml buffer (0.05 molar) for adsorption was included as the 4th sample. Thus there were 4 tubes (Tubes 1 - 4), containing
the dried residues of the eluate from corresponding samples.

**Extraction of amines from the dried residue of the eluate.** It was possible to separate amines from most of the salt in the dried residue by extraction into acidified ethanol. The dried residue in each tube was taken up in 3 ml (3 x 1 ml) of acidified ethanol and transferred to a graduated centrifuge tube (conical, 10 ml). The final volume in the centrifuge tube was made up to 3 ml with the acid-ethanol extractant. The tubes were sealed with parafilm and centrifuged at 2000 r.p.m. at 4°C for 20 min.

2.7 ml of each supernatant was transferred to a corresponding miniature flask (Q.Q. stoppered round bottom, capacity 5 ml) and dried at 45°C under reduced pressure (~60 mm Hg). The residue was estimated to contain 1.1 mg of NaCl.

**Preparation of the marker.** 2 µg each of histamine, MH and DMH were added to flask (2) containing the extract from the thalamus. Each compound was delivered in 0.01 ml from stock solution (200 µg/ml in methanol), using separate micropipettes. The mixture (0.03 ml) was directly spotted on the marker strip (2) of the chromatogram. The flask was washed with three
Volumes of 0.1 ml methanol and this was also spotted on the paper.

**Spotting on the other strips.** Contents of each of the other three flasks (1, 3 and 4) were applied to the paper on three corresponding strips, using 3 x 0.1 ml methanol.

**Chromatographic separation.** This was by ascending chromatography, details of which are given in Appendix I.h. The solvent system was as follows.

- **iso-Propyl Alcohol** 150
- Ammonia (sp. gr. 0.88) 8
- Water 26

The chromatogram was run for 17 hours at room temperature (18° to 19°C). The paper was dried at 40°C for 10 min. The marker strip (Strip 2) was then cut out and the compounds on it were located with fresh diazotized sulphanilic acid solution (Appendix I.h.).

**Elution of substances from the chromatogram.** Strip 1 (hypothalamus) and Strip 3 (cerebellum + amines) were divided in the following way: the area from the 6th to the 16th cm from the starting line on each strip was divided transversely at intervals of either 1.0 or 0.5 cm. There were 12 pieces from each strip. These were
placed in separate glass stoppered tubes for elution.

From Strip 4 (control), only three pieces were cut out. Each of these was 2.0 cm wide and the centre of each piece corresponded with the centre of a spot on the marker strip.

Each piece of the strip was immersed in 4.0 ml of 0.02 N HCl for an hour. The piece of paper was then withdrawn and washed down the side of the tube with a further 1.0 ml of the eluent.

The solution was taken to dryness at 55°C under reduced pressure (20 - 30 mm Hg) and, to remove traces of acid, the tube was finally dried at 80°C under reduced pressure for 5 min; there was no visible residue left in the tube.

Biological Assay. 2 ml of Tyrode's solution was placed in the tube and after thorough shaking, the solution was assayed on the guinea-pig ileum by superfusion (S. 2/4).

2/6/4 Chromatographic separation of the activity in the hypophysis

In a similar experiment (as above) the hypophyses from two cats were pooled and the

*Elution of amines from the paper with water alone or with Tyrode's solution was not satisfactory.
extract was purified. The eluate material after extraction with acidified ethanol was subjected to chromatographic analysis. A marker strip containing histamine, MH and DMH was run in parallel. The strip containing the material from the hypophyses was divided (as in S. 2/6/3) and the activity was assayed. The position of the peak of activity was determined in relation to the spots on the marker strip.

**2/7**

**HISTOLOGICAL EXAMINATION OF THE HYPOPHYSIS AND THE HYPOTHALAMUS FOR MAST CELLS**

Mast cells have been described in the hypophysis of the cat (Gray, 1935). The object was to confirm the findings of Gray (1935) and to study the distribution of mast cells in the hypophysis by serial sections. An estimate was made of their number in different parts of a single gland. It was thus possible to compare the number of cells found with estimates of the concentration of histamine obtained from other glands.

**2/7/1 Method of examination**

The brain was fixed *in situ* by perfusion technique (Cammermeyer, 1960; Zeman and Innes, 1963). A cannula was introduced into the
aorta and the first perfusion (for flushing out the blood) was with 500 ml of Ringer-Locke solution. The second perfusion was with 600 ml of Duboscq-Brasil fixative solution (Gatenby and Beams, 1950). The brain was removed after 1 hour. A rectangular block of tissue containing the hypophysis and the tuber cinereum was removed. Most of the hypothalamus was included in this block. The tissue was left overnight in the fixative solution.

**Processing of the tissue.** The tissue block was dehydrated in graded ethanol and clarified in benzene. It was embedded in paraffin wax under vacuum. The block was cut into sections (5 μ thickness) with a rotary microtome. The sections were numbered.

**Staining.** Every tenth section was stained with aqueous toluidine blue (0.5 per cent). The commercial dye (Toluidine blue, Gurr's) was treated with saturated solution of potassium iodide and modified according to Robinson and Bacsich (1958). This improved the staining quality. The sections were clarified in xylol and mounted in D.P.X. (BDH).

Several other sections were stained with haematoxylin (Delafield's) and eosin (1 per cent).
These sections were also mounted.

*Scanning of the sections for mast cells.* The sections stained with toluidine blue were systematically examined for mast cells. The number of mast cells in the sections was counted and the position of each cell was noted. The number of mast cells occurring in different anatomical parts was recorded on a chart. In all, 140 sections stained with toluidine blue were examined for mast cells. Mast cells were seen and counted in 48 out of these 140 sections.
SECTION 3
RESULTS

3/1 SPECIFICITY OF THE METHOD

Several tests of the specificity of the method were carried out by Adam (1961), using material from dog's brain which had been extracted and purified for bioassay by superfusion as in the present method. The results obtained by Adam for such tests are in general applicable in the present method and some of these tests were not repeated. The original paper (Adam, 1961) should be consulted for the results of these tests. However, the following tests provide further evidence that the substance estimated was probably histamine.

3/1/1 Inhibition by mepyramine

Doses of the test and standard solution of histamine were always found to be equally inhibited or abolished under the effect of mepyramine in low concentration (3 ng/ml in Tyrode's solution). On repeating the assay in the presence of mepyramine (3 ng/ml), the estimates for the histamine equivalent in the test agreed to within 10 per cent.
Recovery experiments

The results of the recovery experiments are given in Table 3. The amount recovered in each case is shown and is also expressed as a percentage of the amount added. The average recovery of histamine when 25 to 100 ng of the amine was added to the tissue samples (Nos. 1 to 9 in the table), was 75 per cent (range 62 to 90 per cent). When similar quantities of histamine were added to buffer solution for adsorption (Nos. 10 to 15 in the table), the recovery was better. The mean recovery in this case was 88 per cent (range 84 to 90 per cent). When histamine was added to the eluates, after adsorption of tissue extracts (Nos. 16 to 20 in the table), the recovery was 89 or 90 per cent in all cases, and this was not much different from that when histamine was added to the buffer solution.

It seems that loss of histamine on the column was not very great. Some losses take place either before the adsorption step, or during the adsorption, when histamine is present in the tissue extract along with other constituents of the tissue and the trichloroacetate.
Table 3
Recovery of histamine

(Histamine added to brain tissue (approx. 50 mg.) before extraction)

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Histamine added (ng)</th>
<th>Estimated recovery* (ng)</th>
<th>Percentage recovery (approx.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cerebellum</td>
<td>100</td>
<td>89.8</td>
<td>90</td>
</tr>
<tr>
<td>2. Cerebral cortex</td>
<td>100</td>
<td>84.3</td>
<td>84</td>
</tr>
<tr>
<td>3. &quot; &quot;</td>
<td>100</td>
<td>79.3</td>
<td>79</td>
</tr>
<tr>
<td>4. &quot; &quot;</td>
<td>50</td>
<td>31.0</td>
<td>62</td>
</tr>
<tr>
<td>5. Cerebellum</td>
<td>50</td>
<td>37.9</td>
<td>76</td>
</tr>
<tr>
<td>6. &quot; &quot;</td>
<td>50</td>
<td>34.2</td>
<td>68</td>
</tr>
<tr>
<td>7. &quot; &quot;</td>
<td>50</td>
<td>38.0</td>
<td>76</td>
</tr>
<tr>
<td>8. &quot; &quot;</td>
<td>25</td>
<td>17.9</td>
<td>72</td>
</tr>
<tr>
<td>9. &quot; &quot;</td>
<td>25</td>
<td>17.2</td>
<td>69</td>
</tr>
</tbody>
</table>

(Histamine added to buffer solution for adsorption)

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Histamine added (ng)</th>
<th>Estimated recovery* (ng)</th>
<th>Percentage recovery (approx.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10. 10 ml buffer</td>
<td>100</td>
<td>87.6</td>
<td>88</td>
</tr>
<tr>
<td>11. &quot; &quot;</td>
<td>100</td>
<td>87.6</td>
<td>88</td>
</tr>
<tr>
<td>12. &quot; &quot;</td>
<td>50</td>
<td>42.0</td>
<td>84</td>
</tr>
<tr>
<td>13. &quot; &quot;</td>
<td>50</td>
<td>43.4</td>
<td>87</td>
</tr>
<tr>
<td>14. &quot; &quot;</td>
<td>25</td>
<td>22.5</td>
<td>90</td>
</tr>
<tr>
<td>15. &quot; &quot;</td>
<td>25</td>
<td>22.5</td>
<td>90</td>
</tr>
</tbody>
</table>

(Histamine added to eluate after adsorption of extracts from cerebellum (approx. 50 mg.))

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Histamine added (ng)</th>
<th>Estimated recovery* (ng)</th>
<th>Percentage recovery (approx.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16. 5 ml eluate</td>
<td>50</td>
<td>44.5</td>
<td>89</td>
</tr>
<tr>
<td>17. &quot; &quot;</td>
<td>50</td>
<td>45.0</td>
<td>90</td>
</tr>
<tr>
<td>18. &quot; &quot;</td>
<td>50</td>
<td>45.0</td>
<td>90</td>
</tr>
<tr>
<td>19. &quot; &quot;</td>
<td>50</td>
<td>44.5</td>
<td>89</td>
</tr>
<tr>
<td>20. &quot; &quot;</td>
<td>50</td>
<td>44.5</td>
<td>89</td>
</tr>
</tbody>
</table>

*In Experiments nos. 1-9 the values were corrected for the amount present in the control samples, as explained in the text (8.2/5).
Paper chromatographic analysis of the activity in the eluate material

When histamine, β-N-methylhistamine (MH) and β-N-dimethylhistamine (DMH) were added each in a quantity of 2 μg to an extract of brain tissue (thalamus), they could be separated from the mixture by paper chromatography, and clearly located by the Pauly reaction. The Rf values of the compounds on the marker strip (Strip 2) of the chromatogram were as follows:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rf x 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>42</td>
</tr>
<tr>
<td>β-N-methylhistamine</td>
<td>57</td>
</tr>
<tr>
<td>β-N-dimethylhistamine</td>
<td>67</td>
</tr>
</tbody>
</table>

In Strip 1 which had the extract of the hypothalamic tissue, the activity on bioassay was wholly confined to the region which corresponded with the histamine spot on the marker (Fig. 6). Total activity equivalent to 72 ng of histamine was estimated in Strip 1. Activity was not found in portions of the strip corresponding to the spots of MH and DMH.

For a more detailed study of the Rf values of the compounds in the same system, see Appendix I. h.
Fig. 6. Histogram showing chromatographic separation of the activity in the hypothalamus superimposed on the recovery of amines added to an extract of cerebellum. The vertical lines on the left and right show respectively the starting line and the solvent front of the chromatogram. Rf values are calculated from the location of spots on the marker strip. The location of histamine-like activity in the hypothalamus (72 ng histamine) corresponds closely with the spot of histamine on the marker and with the activity due to histamine added (200 ng) to the cerebellar extract.
In Strip 3 which had the extract of cerebellum plus the mixture of amines (histamine, MH and DMH, 200 ng of each) added to the tissue sample, three distinct peaks of histamine-like activity were detected on bioassay. These peaks corresponded with the spots of the compounds as located on the marker strip. The recoveries of the three compounds after the chromatographic separation could be roughly calculated from the peaks of activities after correcting for their relative potencies (Appendix I.f.). The recoveries were as follows:

<table>
<thead>
<tr>
<th>Mixture of compounds (200 ng of each)</th>
<th>Potency (histamine area = 100)</th>
<th>Activity (~ng histamine)</th>
<th>Recovery of substance (ng)</th>
<th>Percent recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>100</td>
<td>136</td>
<td>136</td>
<td>68</td>
</tr>
<tr>
<td>β-N-methylhistamine</td>
<td>76</td>
<td>86</td>
<td>112</td>
<td>56</td>
</tr>
<tr>
<td>β-N-dimethylhistamine</td>
<td>47</td>
<td>57</td>
<td>120</td>
<td>60</td>
</tr>
</tbody>
</table>

(The values for junctional areas, where there was some overlap of activity were excluded from this calculation).

The result is summarized in Fig. 6. Activity was not detected in Strip 4 (control) in the areas corresponding with the spots of the three active substances on the marker strip.
When the eluate material obtained from the pooled hypophyses of two cats was analysed in the same way, the histamine-like activity on the chromatogram was found to be confined to the area corresponding with the histame spot on the marker. Activity was not found in areas corresponding with the spots of MH or DMH. In this respect the activity from the hypophysial material was similar to that from the hypothalamus.

β-N-methylhistamine and β-N-dimethylhistamine were, like histamine, adsorbed on the column at pH 8.0. Most of their histamine-like activity survived heating in 6 N HCl (Appendix I.g.). On the basis of the recoveries of these substances from the chromatogram, it seems that if small quantities of these compounds were present in the hypothalamus or in the hypophysis, they would be detectable on bioassay after chromatographic separation on paper. The activity in the hypothalamus and hypophysis closely corresponded with the spot of authentic histamine on the chromatogram. The result of this experiment provides further evidence that the substance estimated in the brain tissue and hypophysis was probably histamine (see also Adam, 1961) and not a mixture of histamine and pharmacologically
active alkyl derivatives.

**3/2 DISTRIBUTION OF HISTAMINE IN THE HYPOPHYSIS AND BRAIN**

Estimates of the concentration of histamine in different parts of the hypophysis and the brain are presented in Table 4. The number of cats from which a particular tissue sample was collected is also shown. The concentrations are expressed as nanograms per gram of tissue weight and these have been rounded off to the nearest 10 ng after the calculations. The standard error (S.E.) of the mean value is given as the nearest whole number. Where the areas are represented by only a few cats, only the mean value and the range of the estimates are given.

Table 5 shows the average weights of the tissues as sampled and also their average histamine content. The four areas of the hypothalamus and the area postrema refer to the combined weights of corresponding samples removed from the right and left halves of the brain. Other samples from the brain were from the right half only.

**3/2/1 Hypophysis**

Values for the concentration in the three
### Table 4

Histamine in the cat's hypophysis and brain

Estimates of concentrations as nanogram per gram of tissue weight

<table>
<thead>
<tr>
<th>Region</th>
<th>No. of cats</th>
<th>Mean</th>
<th>Range</th>
<th>S.E. of mean</th>
<th>Limits ((P=0.05)) of the mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypophysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior lobe</td>
<td>25</td>
<td>2420</td>
<td>840-6530</td>
<td>277</td>
<td>1850 - 3000</td>
</tr>
<tr>
<td>Posterior lobe</td>
<td>25</td>
<td>1660</td>
<td>560-3450</td>
<td>142</td>
<td>1390 - 1980</td>
</tr>
<tr>
<td>Hypophysial stalk</td>
<td>14</td>
<td>5170</td>
<td>1230-9250</td>
<td>665</td>
<td>3740 - 6610</td>
</tr>
<tr>
<td><strong>Hypothalamus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpora mammillaria</td>
<td>14</td>
<td>1150</td>
<td>660-1840</td>
<td>91</td>
<td>950 - 1350</td>
</tr>
<tr>
<td>Ventral hypothalamus</td>
<td>15</td>
<td>800</td>
<td>390-1280</td>
<td>71</td>
<td>650 - 950</td>
</tr>
<tr>
<td>Dorsal hypothalamus</td>
<td>15</td>
<td>480</td>
<td>290-810</td>
<td>42</td>
<td>390 - 570</td>
</tr>
<tr>
<td>Preoptic region</td>
<td>15</td>
<td>430</td>
<td>230-730</td>
<td>35</td>
<td>350 - 510</td>
</tr>
<tr>
<td><strong>Thalamus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial</td>
<td>21</td>
<td>250</td>
<td>110-410</td>
<td>17</td>
<td>210 - 290</td>
</tr>
<tr>
<td>Dorsolateral</td>
<td>9</td>
<td>350</td>
<td>180-610</td>
<td>41</td>
<td>260 - 450</td>
</tr>
<tr>
<td>Ventrolateral</td>
<td>8</td>
<td>75</td>
<td>60-110</td>
<td>10</td>
<td>50 - 100</td>
</tr>
<tr>
<td>Med. geniculate body</td>
<td>12</td>
<td>370</td>
<td>180-740</td>
<td>49</td>
<td>260 - 480</td>
</tr>
<tr>
<td>Lat. geniculate body</td>
<td>12</td>
<td>180</td>
<td>90-400</td>
<td>27</td>
<td>120 - 240</td>
</tr>
<tr>
<td><strong>Midbrain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior colliculus</td>
<td>10</td>
<td>150</td>
<td>90-200</td>
<td>11</td>
<td>130 - 180</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>10</td>
<td>120</td>
<td>70-200</td>
<td>11</td>
<td>90 - 140</td>
</tr>
<tr>
<td>Central grey matter</td>
<td>13</td>
<td>160</td>
<td>80-240</td>
<td>12</td>
<td>130 - 180</td>
</tr>
<tr>
<td>Reg. of red nucleus</td>
<td>8</td>
<td>100</td>
<td>50-160</td>
<td>12</td>
<td>70 - 130</td>
</tr>
<tr>
<td>Region</td>
<td>No.</td>
<td>Mean</td>
<td>Range</td>
<td>S.E.</td>
<td>Limits of mean of the mean (P=0.05)</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----</td>
<td>------</td>
<td>-------------</td>
<td>------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td><strong>Pons and medulla</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floor of 4th ventricle</td>
<td>8</td>
<td>73</td>
<td>50 - 100</td>
<td>7</td>
<td>60 - 90</td>
</tr>
<tr>
<td>Reg. of reticular formation</td>
<td>4</td>
<td>53</td>
<td>40 - 60</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Brachium pontis</td>
<td>2</td>
<td>-</td>
<td>&lt;30, 30</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Nc. cuneatus</td>
<td>1</td>
<td>-</td>
<td>&lt;30</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Nc. gracialis</td>
<td>1</td>
<td>-</td>
<td>&lt;30</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Area postrema</td>
<td>5</td>
<td>-</td>
<td>1500, 1200, &lt;280, &lt;300, &lt;280</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Cerebrum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>8</td>
<td>90</td>
<td>40 - 130</td>
<td>15</td>
<td>60 - 130</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>9</td>
<td>50</td>
<td>30 - 70</td>
<td>5</td>
<td>40 - 60</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>3</td>
<td>60</td>
<td>40 - 90</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Corpus callosum</td>
<td>2</td>
<td>-</td>
<td>&lt;80, &lt;90</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Other parts</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum (vermis)</td>
<td>7</td>
<td>-</td>
<td>&lt;20 &lt;30</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Pineal body</td>
<td>1</td>
<td>-</td>
<td>380</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Optic nerve</td>
<td>3</td>
<td>-</td>
<td>&lt;60 &lt;70</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
### Table 5

Weight of tissue samples and estimates of the amount of histamine contained

(Number of cats same as in Table 4)

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Mean weight (mg)</th>
<th>Range (mg)</th>
<th>Mean histamine content (ng)</th>
<th>Range (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypophysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior lobe</td>
<td>18.8</td>
<td>13 - 28</td>
<td>45</td>
<td>16 - 120</td>
</tr>
<tr>
<td>Posterior lobe</td>
<td>12.7</td>
<td>8 - 19</td>
<td>22</td>
<td>8 - 41</td>
</tr>
<tr>
<td>Hypophysial stalk</td>
<td>3.3</td>
<td>1.5 - 4.0</td>
<td>17</td>
<td>6 - 38</td>
</tr>
<tr>
<td>Hypothalamus*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpora mammillaria</td>
<td>16.1</td>
<td>12 - 21</td>
<td>19</td>
<td>11 - 27</td>
</tr>
<tr>
<td>Ventral hypothalamus</td>
<td>34.2</td>
<td>29 - 46</td>
<td>28</td>
<td>14 - 49</td>
</tr>
<tr>
<td>Dorsal hypothalamus</td>
<td>30.6</td>
<td>22 - 43</td>
<td>15</td>
<td>7 - 22</td>
</tr>
<tr>
<td>Preoptic region</td>
<td>19.5</td>
<td>16 - 25</td>
<td>9</td>
<td>6 - 16</td>
</tr>
<tr>
<td>Thalamus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial thalamus</td>
<td>35.6</td>
<td>23 - 54</td>
<td>9</td>
<td>5 - 16</td>
</tr>
<tr>
<td>Dorsolateral thalamus</td>
<td>36.7</td>
<td>28 - 57</td>
<td>15</td>
<td>8 - 21</td>
</tr>
<tr>
<td>Med. geniculate body</td>
<td>46.2</td>
<td>34 - 55</td>
<td>17</td>
<td>11 - 41</td>
</tr>
<tr>
<td>Lat. geniculate body</td>
<td>49.1</td>
<td>37 - 61</td>
<td>10</td>
<td>14 - 24</td>
</tr>
<tr>
<td>Midbrain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior colliculus</td>
<td>40.0</td>
<td>30 - 54</td>
<td>6</td>
<td>4 - 10</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>58.3</td>
<td>39 - 66</td>
<td>7</td>
<td>3 - 10</td>
</tr>
<tr>
<td>Central grey matter</td>
<td>49.8</td>
<td>34 - 76</td>
<td>8</td>
<td>4 - 12</td>
</tr>
<tr>
<td>Reg. of red nucleus</td>
<td>58.4</td>
<td>41 - 74</td>
<td>5</td>
<td>3 - 9</td>
</tr>
<tr>
<td>Pons and Medulla</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floor of 4th vent.</td>
<td>56.8</td>
<td>46 - 73</td>
<td>4</td>
<td>3 - 6</td>
</tr>
<tr>
<td>Area postrema*</td>
<td>4.2</td>
<td>3.0 - 5.1</td>
<td>-</td>
<td>&lt; 1.0 - 4.6</td>
</tr>
<tr>
<td>Other parts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>47.5</td>
<td>36 - 60</td>
<td>4</td>
<td>2 - 5</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>60.0</td>
<td>52 - 73</td>
<td>3</td>
<td>2 - 5</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>64.0</td>
<td>41 - 90</td>
<td>-</td>
<td>&lt; 1.0 - 3</td>
</tr>
</tbody>
</table>

* Samples removed from the right and left halves of the brain and combined.
parts of the hypophysis varied by as much as 8 to 9 fold. The hypophysial stalk had the highest mean concentration of histamine among all the parts examined. The total amount of histamine (mean, 17 ng) present in this small tissue was considerable. The concentration of histamine in the anterior lobe was higher than in the posterior lobe.

3/2/2 Hypothalamus

The highest concentration in the brain was in the corpora mammillaria, where the mean value was above 1000 ng/g. In the adjoining ventral half of the hypothalamus the mean concentration was 800 ng/g, and in the dorsal half it had fallen to 480 ng/g. This last value does not differ significantly from the mean of 430 ng/g for the preoptic region. Beyond the hypothalamus, the concentration fell progressively and the mean concentration was nowhere above 400 ng/g. The estimates for the parts of the hypothalamus and also for most other parts of the brain were less variable than those for the hypophysis.

3/2/3 Rest of the brain

In the thalamus the concentration was
highest in the **medial geniculate body** and in the
dorsolateral part. By contrast, the **ventrolateral thalamus** contained very little histamine.
In the **medial thalamus** the mean concentration was 250 ng/g.

In the four parts sampled from the midbrain, the concentration was low and more or less uni-
form.

Some parts of the pons and medulla and various parts of the cerebral cortex always showed a low but measurable concentration. Histamine was not detectable in the **cerebellum**, **corpus callosum** and **optic nerve**.

In five samples of the **area postrema**, histamine was detected only in two. The results with this sample were not consistent.

**HISTOLOGICAL EXAMINATION OF THE HYPOPHYSIS AND THE HYPOTHALAMUS**

The hypophysis and adjoining parts of the brain are shown in a photomicrograph (Fig. 7). This was a sagittal section passing through the
Fig. 7. Medial sagittal section of cat's hypophysis and adjoining parts of the brain (x 20). Haematoxylin and eosin.
(c.m.) corpora mammillaria; (i.p.) infundibular process; (n.s.) neural stalk; (p.d.) pars distalis; (p.i.) pars intermedia; (p.t.) pars tuberalis; (vt.) third ventricle.

Nomenclature of parts (Rioch et al., 1940).

Adenohypophysis
1. pars distalis
2. pars tuberalis
3. pars intermedia

Neurohypophysis
1. infundibular process
2. infundibular stem

Neural stalk
1. infundibular stem
2. median eminence of the tuber cinereum

Hypophysial stalk
1. neural stalk
2. pars tuberalis of the adenohypophysis
middle part of the hypophysis and the median eminence of the tuber cinereum. The nomenclature for different anatomical parts (Rioch et al., 1940) are also shown in Fig. 7.

In sections stained with toluidine blue, mast cells were observed in the hypophysis and in the hypophysial stalk (Fig. 8). The greatest number of mast cells were seen in sections which passed through the middle part of the hypophysis and the stalk. The distribution of mast cells in different parts of the hypophysis is shown diagrammatically in Fig. 9. The localization of the mast cells and the total number counted in every tenth section were as follows:

**Anterior lobe (pars distalis).** Number of mast cells counted was 98. They were few and the maximum number in one section was 6. All the cells were found very close to the hypophysial cavity (cleft), sometimes actually lining the cavity.

**Posterior lobe (pars intermedia + infundibular process).** Number of cells counted was 543. Of these only 4 cells were found in the pars intermedia. The cells were found mainly in the central part of the infundibular process.
Fig. 8. Sagittal section of cat's hypophysis (x 1000): aqueous toluidine blue. Photomicrographs (1) and (2) show mast cells in the hypophysial stalk. (1) shows adjacent glandular tissue of the pars tuberalis. (3) shows mast cells in the middle part of the infundibular process.
Fig. 9. Distribution of mast cells in the cat's hypophysis. Number of crosses indicates approximately the number of cells in a section passing through the middle of the gland. The number of crosses multiplied by 200 also gives a rough indication of the total number of mast cells in a region (Table 6). **Key:** c.m., corpora mammillaria; h.c., hypophysial cavity (cleft); i.p. infundibular process; n.s., neural stalk; p.d., pars distalis; p.i., pars intermedia; p.t., pars tuberalis; vt., third ventricle.
and around the infundibular cavity. The maximum number of cells in one section was 36. The cells were often seen to lie close to blood vessels.

**Hypophysial stalk (pars tuberalis + neural stalk).** The total number of cells counted was 380. Of these 310 cells were found in the pars tuberalis and 70 cells in the neural stalk. Cells were present in all parts of the pars tuberalis but were particularly common where this part meets the infundibular stem. The cells were in close association with blood vessels. In the neural stalk they were found mainly in the infundibular stem near its border with the pars tuberalis and the infundibular process. Very few cells (only 4) were found in the median eminence of the tuber cinereum.

The total number of mast cells in the different parts of the gland was estimated by multiplying the total number counted in the sections by ten. The following values were obtained.
Mast cells were not found in the hypothalamus (excluding the median eminence of the tuber cinereum) or in the brain. None were seen in the ependymal lining of the third ventricle.
The presence of histamine in extracts of the brain and hypophysis has been reported for several species, including the cat (S. l/l and l/2). The object of the present work was to prepare a detailed map of its distribution in the cat's brain and hypophysis, thereby relating the anatomical area or region to the concentration of histamine extractable from it. Similar maps have been prepared for noradrenaline (Vogt, 1954), for 5-HT (Amin et al., 1954) and for histamine (Adam, 1961) in the dog's brain.

The method for estimating histamine in brain tissue was adopted from Adam (1961) but further experiments were done to test the specificity of this method. The results have led to the conclusion that the activity estimated in the assay was probably due only to histamine and not to a mixture of histamine and other similarly active alkyl derivatives. The method gave consistent recoveries in the range 25 to 100 ng histamine added to brain tissues. The relatively high values for the concentration of histamine in different parts of the hypophysis
and adjoining parts of the brain confirm those previously reported for the cat (Harris et al., 1952). The values obtained for the different parts of the brain do not agree with the higher values reported by McGeer (1964), who used a modification of the fluorometric method of Shore et al. (1959).

4/1 HYPOPHYSIS

Mast cells have been reported in the cat's hypophysis (Gray, 1935) and this finding has been confirmed in the present work (S. 3/3). In the dog, the high concentration of histamine in the gland is associated with the presence of mast cells (Adam, 1961). The wide variation in the values for the hypophysial histamine in the cat, as in the dog, might therefore depend on the number of mast cells present in the gland.

Estimates of histamine concentration in different parts of the hypophysis and the results of the histological examination are summarized in Table 6. The total number of mast cells in the single gland examined was estimated to be of the order of 10,000 and the content of histamine in an average gland, approximately 84 ng. Hence, the average content per mast cell might be
Table 6
Distribution of histamine and mast cells in the hypophysis

<table>
<thead>
<tr>
<th>Sample</th>
<th>Histamine</th>
<th>Mast cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean wt. mg</td>
<td>Mean conc. ng/g</td>
</tr>
<tr>
<td>Anterior lobe</td>
<td>18.8</td>
<td>2420</td>
</tr>
<tr>
<td>Posterior lobe</td>
<td>12.7</td>
<td>1680</td>
</tr>
<tr>
<td>Hypophysial stalk</td>
<td>3.3</td>
<td>5170</td>
</tr>
<tr>
<td>Total</td>
<td>34.8</td>
<td>-</td>
</tr>
</tbody>
</table>
between 8 and 9 ng, which is about one third of the amount in rat mast cells, as calculated by Riley (1959). It is doubtful, however, if all the histamine extractable from different parts of the hypophysis derives only from mast cells. Consider the anterior lobe. This part contains more than 50 per cent of the histamine extractable from the gland but less than 10 per cent of the mast cell population (Table 6). If histamine derives only from mast cells, those of the anterior lobe would be expected to contain 5 times more histamine than mast cells in other parts of the gland. A similar 'disproportion' is seen in the posterior lobe which is estimated to contain about 26 per cent of the total histamine but more than 50 per cent of the mast cell population. The highest concentration of mast cells is found in the hypophysial stalk which is estimated to contain 20 per cent of the total histamine and 37 per cent of the total population of mast cells in the gland. It is probable that in this part a large proportion, if not all, of the histamine derives from mast cells.

Evidence presented in Part II of this thesis, on the effect of compound 48/80, favours the view that in the posterior lobe and in the stalk
most of the histamine derives from mast cells and that in the anterior lobe most of the histamine derives from cells which are not mast cells. The subcellular distribution of histamine suggests that the hypophysial histamine in the dog derives from mast cells (Michaelson and Dowe, 1963). A similar study of the hypophysial histamine on a regional basis would be useful, since histamine in the anterior lobe might be contained in more than a single population of subcellular particles.

Mast cells are able to decarboxylate histidine (Schayer, 1956b; Hagen, Weiner, Ono and Lee, 1960; Weissbach et al., 1961; Day and Green, 1962a). It might therefore be expected that since the hypophysis contains mast cells, it should also be able to form histamine. Minced tissue from cat's hypophysis did not show decarboxylase activity when incubated with ^14C histamine (Adam et al., 1964). This finding could mean that the rate of formation of histamine in the hypophysis was too slow to be detectable under the conditions of the experiment.

Mast cells can take up extrinsic histamine (Day and Green, 1962b). The hypophysis in the cat can also take up ^14C histamine from the circulation (Adam et al., 1964). The uptake of
histamine by the hypophysis could be due to the mast cells in the gland. Thus part of the histamine extractable from the hypophysis could be extrinsic and stored in the mast cells.

The present experiments do not give any information as to the function of histamine in the hypophysis. But the occurrence of mast cells, particularly in association with the blood vessels in these regions, suggests that the function of histamine in these parts might be one of vascular control. In terms of 'nascent histamine' (Kahlson, 1960, 1962), histamine might be important for the development and growth of the glandular cells. The origin of histamine in the pars distalis (anterior lobe) of the adenohypophysis deserves further study.

4/2 BRAIN

As already mentioned (S. 1/3) mast cells have not been found in the brain. In the present work, examination of the cat's hypothalamus (excluding the eminentia media) in serial sections failed to reveal the presence of mast cells. Studies of the subcellular distribution of histamine in the brain of rat and dog indicate that histamine in the brain is present
in cells other than mast cells (Carlini and Green, 1963; Michaelson and Dowe, 1963).

The distribution of histamine in different parts of the cat's brain is found to be similar to that in the dog (Adam, 1961). Adam pointed out that the topographical distribution of histamine in the dog's brain was roughly similar to that of noradrenaline (Vogt, 1954) and 5-HT (Amin et al., 1954). This statement is also applicable to the cat. A detailed map of the distribution of noradrenaline in cat's brain is not available but Vogt (1954) has stated that it is similar to that in the dog. The mean concentration of noradrenaline in the cat's hypothalamus was estimated as 1380 ng/g (range 900 - 1920) (Vogt, 1954). The distribution of 5-HT in cat's brain was reported by Udenfriend, Weissbach and Bogdanski (1957), who found high concentrations in the hypothalamus (mean 1780 ng/g), intermediate concentrations in the midbrain (1230 ng/g) and thalamus (780 ng/g) and low concentrations in the cerebral cortex (240 ng/g) and in the cerebellum (270 ng/g). The values obtained for the concentration of histamine in these parts (S. 3/2) are lower but the mode of distribution is similar. The highest
concentration of the three amines is found in the hypothalamus, where the concentrations are comparable.

In the present study, it was possible to obtain values for histamine extractable from different parts of the hypothalamus and thalamus. The values appear to show a definite gradient of concentration. The highest concentration was found in the **corpora mammillaria** followed by the **ventral** half of the **hypothalamus**. In the **dorsal** half of the **hypothalamus** and in the **preoptic region** the concentration had fallen by about half. Similarly, in the thalamus the concentration was low in the **ventrolateral** part and nearly four times higher in the **medial thalamus** and higher still in the **dorsolateral** part. The gradient across these regions is difficult to explain but it might be related to morphological factors such as the density of neuronal or neuroglial cells or to differences in the number of cells which make and store histamine. It is possible that some cells of the **corpora mammillaria** and the **ventral hypothalamus** make and store more histamine than do cells elsewhere in the brain. This possibility could be tested by determining the histamine
forming capacity of these parts.

The high concentration of histamine in the hypothalamus agrees well with the high capacity of this part to form histamine from histidine (White, 1959, 1960). Parts of the brain in the cat, like the cerebral cortex and the cerebellum which show little capacity to form histamine in vitro (White, 1959), also contain very little histamine.

It was not possible to obtain consistent values for the concentration of histamine in the area postrema. The small weight of the tissue would not explain the irregularities, since in another tissue of similar weight, namely, the hypophysial stalk, the concentration was always observed to be high. In the dog, histamine was detectable in the area postrema and the concentration was usually high (Adam, 1961). In the cat, however, histamine formation was not detected in the area postrema and it did not show any evident uptake of \( ^{14} \text{C} \) histamine when this was infused into the vertebral artery (Adam et al., 1964).

"The uneven distribution of a pharmacologically active substance in the brain strongly suggests that the agent has a role to play in
the specialized function of those regions where its concentration is high" (Vogt, 1959). This statement quoted from a discussion of the catecholamines in the brain is also applicable to the histamine in the brain. Histamine is unevenly distributed in the brain. The histamine forming capacity of the brain appears to follow this uneven distribution (White, 1959, 1960). Brain tissue inactivates histamine by ring methylation (Brown et al., 1959) and the methylating enzyme is present in most parts of the brain (Axelrod et al., 1961). Besides its actions on the peripheral organs, histamine has pharmacological actions on the brain (S. 1/7). These facts would seem to support the hypothesis that histamine has a physiological function in the brain. "Histamine should now be included among the biologically active amines in the brain, such as the catecholamines and 5-HT" (White, 1961b). "It is tempting to assign functions to histamine extractable from particular regions of the brain, but until more is known about the exact site of its formation and storage, and the conditions necessary for its release, speculation is likely to be unprofitable" (Adam, 1961). A closer analysis of the
effects of histamine on brain functions is necessary. Study of the possible means of increasing and decreasing the histamine content and its formation and inactivation in the brain will shed more light on the functional significance of the amine in the brain.
PART II

EFFECT OF DRUGS ON THE CONCENTRATION OF HISTAMINE IN THE HYPOPHYSIS AND BRAIN IN THE CAT
INTRODUCTION

The object of this part of the work was to test whether certain drugs could alter the concentration of histamine in the cat's brain. Of particular interest were drugs which act to deplete the brain of other pharmacologically active amines. Drugs were also chosen for their known actions on the storage and metabolism of histamine. The effect of most of these drugs was also studied on the hypophysis where histamine is partly in the mast cells (S. 3/3, 4/1).

Ring methylation is known as the major pathway of histamine catabolism in the brain (S. 1/5). The various metabolic steps are shown schematically in Fig. 10. Theoretically, a drug acting on any part of this pathway could influence either directly or indirectly the concentration of histamine extractable from the brain or the hypophysis. Thus, drugs interfering with storage would be expected to lower the concentration of histamine and drugs which inhibit the methylating and oxidising enzymes, to raise the concentration. Since nothing is known about the function of histamine in the brain (S. 4/2), it seemed desirable as a first step to investigate the effect of such drugs.
Fig. 10. Schematic diagram of pathways for the storage and metabolism of histamine in the brain and hypophysis (based on Schayer, 1959). For details see S. 1/3, 1/4 and 1/5.
The drugs tested can be conveniently grouped as follows:

1) Compound 48/80
2) Reserpine
3) Phenothiazine derivatives
   Chlorpromazine
   Triflupromazine
   Thioproperazine
4) Monoamine oxidase inhibitors
   Iproniazid
   Pargyline
5) Miscellaneous drugs
   Bulbocapnine
   Morphine
   Pentobarbitone
   Adrenaline

Previous attempts (Waalkes, Coburn and Terry, 1959; Walaszek and Chapman, 1963) to observe the effect of drugs on the brain histamine have employed the whole brain of rabbit, guinea-pig or rat. This method can be criticized from the point of view that since histamine is so unevenly distributed in the brain, the inclusion of large parts of brain which contain very little histamine are likely to obscure the effect of the drug on those parts which contain most of the
amine. The technique used in the present work for the estimation of histamine in brain tissue (S. 2), allowed the investigation of the effect of drugs on the histamine concentration on a regional basis. Eight areas were chosen for the investigation: three in the hypophysis, four in the hypothalamus and one in the thalamus. In the cat, these areas contain relatively high concentrations of histamine as compared with the rest of the brain (S. 3/2). The eight areas selected were as follows and have already been defined (S. 2/2):

1) Anterior lobe
2) Posterior lobe
3) Hypophysial stalk
4) Corpora mammilaria
5) Ventral hypothalamus
6) Dorsal hypothalamus
7) Preoptic region
8) Medial thalamus

The samples were extracted immediately after collection. The extracts were purified and histamine concentration was estimated as described (S. 2/3 and 2/4). The results obtained in the untreated cats (S. 3/2, Table 4) for the concentration of histamine in these areas were
used in the present investigation as control values for comparing the effect of drugs. Where the effect of a drug is said to be significant, a test for the significance of the difference between two means (mean of control and that after treatment with the drug) was determined by 'Student's t test, and the value of P was noted only when it was less than 0.05.

The drugs, their administration and the results obtained after the treatments are described separately: the effects of the drugs are then summarized and discussed.
SECTION 5

COMPOUND 48/80

Compound 48/80 is a mixture of the products of condensation of p-methoxy-phenylethyl-methyl-amine with formaldehyde. It is known as a potent histamine liberator in animals like the rat, cat and dog, but is less active in the guinea-pig (for references, see Paton, 1957). Compound 48/80 releases histamine from mast cells (Fawcett, 1954; Riley and West, 1955). Various theories have been proposed for the mechanism of histamine release from mast cells by compound 48/80 (Uvnäs, 1958; Westerholm, 1960; Van Arsdel and Bray, 1961).

Compound 48/80 fails to liberate histamine that resides mostly outside mast cells. Thus in the rat, 48/80 did not release histamine of stomach and duodenum (Feldberg and Taelesnik, 1953) and released only a small fraction of the histamine from the peripheral nerves (Riley and West, 1955). In the cat and dog, on direct perfusion of the sciatic nerve, 48/80 released histamine from both the sheath of the nerve and the desheathed nerve (Feldberg and Greengard, 1956). But only between a quarter and a half of the histamine originally present in the nerve
was released.

The high toxicity of compound 48/80 in most species cannot be wholly explained in terms of histamine release (Mota, 1957). Compound 48/80 may also release 5-HT from mast cells (Bhattacharya and Lewis, 1956; Parratt and West, 1957), but the amounts of 5-HT released are too small to produce lethal effects. Compound 48/80 blocks transmission in the perfused ganglion (Gertner, 1955). Injections of 48/80 into the cerebral ventricles produce a peculiar motor excitement ('delirium ambulatorium') in mice (Rocha e Silva, 1959).

In the cat, mast cells are present in the hypophysis but not in the hypothalamus (Gray, 1935; also S. 3/3). Hence it was possible that 48/80 when given in a dose which would reduce histamine in the cat's skin (Smith, 1953) might also reduce the histamine concentration in the hypophysis, particularly in the posterior lobe and the stalk where mast cells are more abundant. It was also desirable to test the effect of 48/80 on the brain histamine, which is not contained in mast cells.
Method

Compound 48/80 (Burroughs and Wellcome) was dissolved in 0.9 per cent saline to make 0.5 per cent (5 mg/ml) solution. On the day before the first injection of the drug, two samples of skin (2 to 5 mg) from the tip of one ear were collected under ether anaesthesia. These were extracted and their histamine content was estimated.

Five cats received injections of 48/80 given intraperitoneally. On the first day a dose of 0.5 mg/kg was injected. Thereafter the daily dose was gradually increased by 0.25 or 0.5 mg/kg daily, or on alternate days, according to the condition of the cat. The aim was to give about 30 mg/kg over a period of two weeks.

In one cat treatment was not continued beyond 7 days. Although depletion probably fell short of the maximum, the value from this cat was included in the results. The animals were given 30 to 50 ml of normal saline (i.p.) daily from the 3rd day onwards to reduce the effects of fluid loss.

*At this dose the skin histamine in cat is known to be reduced by over 80 per cent (Smith, 1953).
At the end of the treatment, the cats were anaesthetized with ether and two more samples of the skin were collected from the ear. The animal was then bled to death and samples from the hypophysis and brain were taken as described (S. 2/2).

Results

Injections of 48/80 were usually followed by vomiting, salivation, lacrimation, purging and micturition. Soon after the injections the cats became restless, with signs of pruritis (scratching of ears and face), oedema of the face, dilatation of the pupil and sometimes severe prostration. They usually recovered from this acute distress in about 2 hours. The symptoms were more severe at the beginning of the treatment and usually after the first week the injections were better tolerated.

Effect on histamine concentration. Estimates of the histamine concentration in the hypophysis and in parts of the brain after 48/80 treatment are shown in Table 7/1. Control values for these regions are also presented in the table for comparison. Estimates of the histamine concentration in the ear skin, before
Effect of compound 48/80 on histamine in the cat's brain and hypophysis

<table>
<thead>
<tr>
<th></th>
<th>Total dose mg/kg</th>
<th>Days treated</th>
<th>Estimate of histamine concentration: ng/g of fresh tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat 1</td>
<td>10</td>
<td>7</td>
<td>Anterior lobe 3300, Posterior lobe 740, Hypophys. stalk 3100, Corpora mamill. 930, Ventral hypothal. 940, Dorsal hypothal. 500, Preoptic region 360, Medial thalamus 240</td>
</tr>
<tr>
<td>Cat 2</td>
<td>30</td>
<td>14</td>
<td>Anterior lobe 1100, Posterior lobe 810, Hypophys. stalk 1600, Corpora mamill. 1140, Ventral hypothal. 880, Dorsal hypothal. 400, Preoptic region 430, Medial thalamus 230</td>
</tr>
<tr>
<td>Cat 3</td>
<td>30</td>
<td>15</td>
<td>Anterior lobe 5300, Posterior lobe 280, Hypophys. stalk 1000, Corpora mamill. 1450, Ventral hypothal. 800, Dorsal hypothal. 590, Preoptic region 530, Medial thalamus 400</td>
</tr>
<tr>
<td>Cat 4</td>
<td>36</td>
<td>20</td>
<td>Anterior lobe 1700, Posterior lobe 1140, Hypophys. stalk 1900, Corpora mamill. 1360, Ventral hypothal. 650, Dorsal hypothal. 250, Preoptic region 320, Medial thalamus 300</td>
</tr>
<tr>
<td>Cat 5</td>
<td>41</td>
<td>15</td>
<td>Anterior lobe 1800, Posterior lobe 700, Hypophys. stalk 1080, Corpora mamill. 1060, Ventral hypothal. 590, Dorsal hypothal. 380, Preoptic region 300, Medial thalamus 240</td>
</tr>
</tbody>
</table>

Cat 1 - 5
Mean ± S.E. 2640 ± 798, 730 ± 138*, 1700 ± 379*, 1190 ± 92, 770 ± 68, 420 ± 58, 390 ± 42, 280 ± 32

Controls
Mean ± S.E. (no. of cats) 2420 ± 277 (23), 1680 ± 142 (25), 5170 ± 665 (14), 1150 ± 91 (14), 800 ± 71 (15), 480 ± 42 (15), 430 ± 35 (15), 250 ± 17 (21)

*Significantly different from the mean of control, P < 0.05
Table 7/2

Effect of compound 48/80 on skin histamine

<table>
<thead>
<tr>
<th></th>
<th>Estimate of histamine in ear skin: µg/g of fresh tissue</th>
<th>Per cent depletion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>After 48/80 treatment^</td>
</tr>
<tr>
<td>Cat 1</td>
<td>52.3, 51.2</td>
<td>26.4, 27.2</td>
</tr>
<tr>
<td>Cat 2</td>
<td>52.0, 56.4</td>
<td>35.0, 40.0</td>
</tr>
<tr>
<td>Cat 3</td>
<td>30.5, (lost)</td>
<td>3.7, 0.7</td>
</tr>
<tr>
<td>Cat 4</td>
<td>63.6, 55.3</td>
<td>6.0, 5.1</td>
</tr>
<tr>
<td>Cat 5</td>
<td>65.2, 68.0</td>
<td>21.2, 15.3</td>
</tr>
<tr>
<td>Mean Cat 1-5</td>
<td>55.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>

^For doses of compound 48/80 see Table 7/1.
and after treatment with 48/80, are shown in Table 7/2. The values for the concentration of histamine in the skin are expressed as μg/g of fresh tissue.

The concentration of histamine fell in the hypophysial stalk (to 33 per cent of the control) and in the posterior lobe (to 43 per cent of the control) after treatment with 48/80. These values were statistically significant (P<0.01). In the anterior lobe and in the brain areas, the mean concentration of histamine after treatment with 48/80 was unchanged as compared with the control values.

The mean percentage depletion of histamine in the skin after treatment with 48/80 was 66 per cent of the control (mean of 5 values; 31 to 91 per cent).

Discussion

The fall in the skin histamine to 34 per cent of the control after treatment with 48/80 indicates that the treatment was effective. Hence the parallel fall in the hypophysial stalk and the posterior lobe can be presumed to be due to the loss of histamine from mast cells in these tissues. The result agrees with the observation
that mast cells are found in these parts of the hypophysis (Gray, 1935; also S. 3/3). Gray (1935) did not observe any mast cells in the anterior lobe of the cat. In the present work, however, some mast cells were encountered in the anterior lobe near the hypophysial cavity (cleft). Nevertheless 48/80 failed to reduce the histamine concentration of the anterior lobe. The sinusoidal character of the blood capillaries in the anterior lobe would be expected to favour access to the cells of the gland, including mast cells. Hence the apparent lack of effect of 48/80 on the histamine concentration in the anterior lobe could be taken to indicate that most of the histamine in this part of the gland is not in mast cells. Since the depletion in the stalk and the posterior lobe (as in the skin) was only partial, it might be argued that all the mast cells in these parts were not destroyed by 48/80 or that the remaining histamine was contained in some other store. The latter explanation is more likely to be true, since 48/80 does not deplete histamine in tissues which do not contain mast cells.

The lack of effect of 48/80 on the concentration of histamine in the hypothalamus is
consistent with the observations that mast cells were not found in these areas (Gray, 1935; also S. 3/3). But the effect on the brain is difficult to interpret since there is no information on whether 48/80 passes from the blood into the brain. Effects have been observed when 48/80 was injected into the ventricles in mice (Rocha e Silva, 1959), but its mode of action is not known.
The discovery of the action of reserpine on the stores of 5-HT and catecholamines in the brain and other tissues has been frequently reviewed (for a recent review see Shore, 1962). A single dose of reserpine markedly reduces the level of 5-HT in the brain of rabbit (Brodie, Pletscher and Shore, 1955, 1956) and cat (Paasonen and Vogt, 1956). In the rabbit the low level persists for more than 24 hours and returns slowly to the original value in about 7 days. Reserpine depletes the cat's hypothalamus of noradrenaline (Holzbauer and Vogt, 1956; Vogt, 1959). A comparison of dose-response relations and the time of recovery of the brain noradrenaline and 5-HT in rabbit after reserpine, revealed that the effects of reserpine on the two amines were very similar and that the curves were superimposable (Shore and Brodie, 1957). But in the rat the depletion of noradrenaline in the brain was more prominent than that of 5-HT (Kärki and Paasonen, 1959). A considerable degree of species variation may be present in the action of reserpine and its analogues (Sanan and Vogt, 1962). The cat is particularly sensitive to
reserpine (Withrington and Zaimis, 1961).

Not much is known about the action of reserpine on brain histamine. Reserpine releases histamine from rabbit blood platelets both in vivo and in vitro (Waalkes and Weissbach, 1956; Waalkes et al., 1959; Burkhalter, Cohn and Shore, 1960). Waalkes et al. (1959) also found that histamine in some tissues was reduced after reserpine, but not as readily or to the same extent, as 5-HT. These workers estimated histamine in rabbit and guinea-pig brain by the spectrofluorimetric method (Shore et al., 1959) and found that reserpine had no apparent effect on the whole brain histamine. Parratt and West (1957) observed that after single or repeated doses of reserpine in rats, the maximum depletion of histamine was not more than 34 per cent in any tissue examined, except in the spleen where the reduction was 60 per cent. There was no visible degranulation in the mast cells after reserpine. Bertaccini (discussion to Adam, 1961) observed that reserpine released histamine from the optic ganglion of octopoda (Eledone moschata), and the depletion of histamine was similar in respect of rate and intensity to that observed for catecholamines and 5-HT.
There is some evidence that reserpine acts on the anterior lobe of the hypophysis to interfere with the formation or release of ACTH (Harwood and Mason, 1957; Kitay, Holub and Jaller, 1959; Saffran and Vogt, 1960; Maickel, Westermann and Brodie, 1961) and gonadotrophins (Hagen and Wallace, 1961).

It was desirable in the first instance to study the effect of various doses of reserpine on the histamine concentration in the hypothalamus and hypophysis of the cat. The investigation was later extended to the recovery of the concentration of histamine in the hypothalamus after a single dose of reserpine.

6/1 Method

Reserpine base ('Serpasil' pure substance, Ciba) was dissolved in 20 per cent (w/v) ascorbic acid solution to give a concentration of 10 mg/ml. For doses of less than 0.5 mg/kg, a more dilute solution (5 mg/ml or 1 mg/ml) of reserpine was prepared using the same solvent. The drug was prepared fresh and administered as a single intraperitoneal injection.

In the first series of experiments 14 cats were used. Each received a single dose of
reserpine in the range 0.1 to 10 mg/kg and was sacrificed 18 hours after injection. Samples from hypophysis, hypothalamus and the medial thalamus were collected from these cats.

In the second series of experiments, 20 cats were used. These were given reserpine in a dose of 0.5 mg/kg and were sacrificed at different time intervals ranging from 6 to 120 hours. Only the four samples from the hypothalamus were collected from these cats.

6/2 Results

When the dose of reserpine was above 0.5 mg/kg, the cats became very sedated within an hour after the injection. The respiration was rapid and laboured. They assumed a sleeping posture and avoided light. The eyes were tightly closed and pupils constricted. The cats at 18 hours were found in deep sleep and when aroused they were unsteady on their feet. By that time they always had diarrhoea.

With doses of 0.5 and 0.25 mg/kg, the cats were less sedated. Soon after the injection they became quiet and assumed a normal sleeping posture. The pupils were constricted. They were easily aroused. At 18 hours the cats were
sleeping and showed some diarrhoea.

With 0.1 mg/kg of reserpine, few symptoms were produced. At 18 hours the cats were quiet but otherwise normal. The two cats receiving this dose did not have diarrhoea.

**Effect on histamine concentration.** Histamine concentrations in the parts of hypophysis and brain at 18 hours after a single dose of reserpine (0.1 to 10 mg/kg) are shown in Table 8. Means of the estimates after reserpine are calculated from the results of 10 cats (Cats 5 to 14 in table) which received reserpine within the range of 0.5 to 10 mg/kg.

Within this dose range (0.5 to 10 mg/kg) reserpine reduced the histamine concentration in the hypothalamus and the medial thalamus. The effect in these parts seemed not to be influenced by the dose of reserpine within this range. After reserpine the values for the histamine concentration in these parts became less variable, as compared with the controls. In the hypothalamus the concentration fell significantly (P < 0.001) in all the four parts: in the ventral hypothalamus it fell to 34 per cent of the control; in the corpora mammillaria, to 35 per cent; in the dorsal hypothalamus, to 42 per cent
Table 8

Effect of reserpine (at 18 hrs after single injection) on histamine concentration in brain and hypophysis

| Dose mg/kg | Anterior lobe | Posterior lobe | Hypophyseal stalk | Corpora mamill.
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat 1 0.1</td>
<td>3300</td>
<td>2500</td>
<td>6500</td>
<td>720</td>
</tr>
<tr>
<td>Cat 2 0.1</td>
<td>260</td>
<td>1020</td>
<td>890</td>
<td>670</td>
</tr>
<tr>
<td>Cat 3 0.25</td>
<td>490</td>
<td>750</td>
<td>780</td>
<td>680</td>
</tr>
<tr>
<td>Cat 4 0.25</td>
<td>470</td>
<td>880</td>
<td>900</td>
<td>530</td>
</tr>
<tr>
<td>Cat 5 0.5</td>
<td>1000</td>
<td>6200</td>
<td>2600</td>
<td>300</td>
</tr>
<tr>
<td>Cat 6 0.5</td>
<td>870</td>
<td>1120</td>
<td>2200</td>
<td>540</td>
</tr>
<tr>
<td>Cat 7 1.0</td>
<td>1500</td>
<td>1600</td>
<td>6800</td>
<td>420</td>
</tr>
<tr>
<td>Cat 8 1.0</td>
<td>200</td>
<td>400</td>
<td>800</td>
<td>290</td>
</tr>
<tr>
<td>Cat 9 2.5</td>
<td>5500</td>
<td>1100</td>
<td>2600</td>
<td>460</td>
</tr>
<tr>
<td>Cat 10 5.0</td>
<td>760</td>
<td>2600</td>
<td>750</td>
<td>380</td>
</tr>
<tr>
<td>Cat 11 5.0</td>
<td>830</td>
<td>1500</td>
<td>3500</td>
<td>400</td>
</tr>
<tr>
<td>Cat 12 5.0</td>
<td>480</td>
<td>3200</td>
<td>8900</td>
<td>380</td>
</tr>
<tr>
<td>Cat 13 5.0</td>
<td>4400</td>
<td>3900</td>
<td>3900</td>
<td>440</td>
</tr>
<tr>
<td>Cat 14 10.0</td>
<td>700</td>
<td>1700</td>
<td>6000</td>
<td>340</td>
</tr>
</tbody>
</table>

Cat 5 - 14
Mean ± S.E. 1730±629 2330±544 4500±632 400±24* 270±19* 200±6* 190±5* 160±12*

Controls
Mean ± S.E. (no. of cats) 2420±277 1680±42 (25) 5170±665 (14) 1180±91 (14) 800±71 (15) 480±42 (15) 430±35 (15) 250±17 (21)

*Mean significantly different from control, P < 0.05.
and in the preoptic region to 44 per cent.

In the medial thalamus the concentration fell to 64 per cent of the control (P<0.01). After reserpine (0.5 to 10 mg/kg) the concentration of histamine in the hypophysis was not altered significantly. Nevertheless, the concentration appeared to fall in the anterior lobe and to rise somewhat in the posterior lobe. Further, the concentration in all three parts varied more widely than in the controls.

When the dose of reserpine was below 0.5 mg/kg (Cats 1 to 4 in Table 9), the effect was not definite. The concentration in the hypothalamus was on the low side and in the medial thalamus it was unaffected. In three out of four cats low values were obtained in the anterior lobe and the hypophysial stalk.

Recovery of histamine concentration after reserpine (0.5 mg/kg). The results are given in Table 9 and are also shown graphically in Fig. 11.

After a single dose of reserpine (0.5 mg/kg), the histamine concentration fell rapidly in all parts of the hypothalamus. The maximum reduction was at 18 hours after the injection. At 24 hours, the concentration had begun to rise. Recovery in all the four regions was slow and
Table 9

Depletion and recovery of histamine in the hypothalamus after a single dose of reserpine (0.5 mg/kg, i.p.)

<table>
<thead>
<tr>
<th></th>
<th>Controls Mean ± S.E.</th>
<th>6 hours (2)</th>
<th>12 hours (2)</th>
<th>18 hours (4)</th>
<th>24 hours (4)</th>
<th>48 hours (4)</th>
<th>72 hours (2)</th>
<th>96 hours (2)</th>
<th>120 hours (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corpora mamillaria</td>
<td>1150 ± 91 (14)</td>
<td>980</td>
<td>600</td>
<td>540</td>
<td>720</td>
<td>800</td>
<td>1000</td>
<td>1030</td>
<td>1010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>920</td>
<td>750</td>
<td>300</td>
<td>600</td>
<td>440</td>
<td>760</td>
<td>1350</td>
<td>800</td>
</tr>
<tr>
<td>Ventral hypothalamus</td>
<td>800 ± 71 (15)</td>
<td>640</td>
<td>490</td>
<td>410</td>
<td>350</td>
<td>430</td>
<td>550</td>
<td>600</td>
<td>560</td>
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<tr>
<td></td>
<td></td>
<td>510</td>
<td>440</td>
<td>220</td>
<td>350</td>
<td>270</td>
<td>360</td>
<td>730</td>
<td>550</td>
</tr>
<tr>
<td>Dorsal hypothalamus</td>
<td>480 ± 42 (15)</td>
<td>410</td>
<td>370</td>
<td>220</td>
<td>260</td>
<td>320</td>
<td>290</td>
<td>290</td>
<td>330</td>
</tr>
<tr>
<td></td>
<td></td>
<td>400</td>
<td>370</td>
<td>220</td>
<td>260</td>
<td>210</td>
<td>230</td>
<td>410</td>
<td>380</td>
</tr>
<tr>
<td>Preoptic region</td>
<td>430 ± 35 (15)</td>
<td>360</td>
<td>310</td>
<td>170</td>
<td>220</td>
<td>240</td>
<td>210</td>
<td>220</td>
<td>270</td>
</tr>
<tr>
<td></td>
<td></td>
<td>380</td>
<td>320</td>
<td>140</td>
<td>190</td>
<td>150</td>
<td>240</td>
<td>270</td>
<td>310</td>
</tr>
</tbody>
</table>

( ) No. of cats
Fig. 11. Depletion and recovery of histamine in cat's hypothalamus after a single dose of reserpine (0.5 mg/kg i.p.). The horizontal line at 100 per cent represents the control values for the concentration of histamine. The columns represent the mean concentration of histamine expressed as a percentage of the control in the four regions of the hypothalamus at different time intervals after the injection of reserpine.
not complete at 120 hours. The fall of histamine concentration was more marked in the corpora mammillaria and in the ventral hypothalamus. There was also a tendency for an earlier recovery in these two parts.

6/3 Discussion

The results show that reserpine lowers the histamine in the brain but not in the hypophysis. The concentration in parts of the hypothalamus was reduced to values in the range 35 to 44 percent of the controls. The effect is not related to dose in the range of 0.5 to 10 mg/kg. This suggests that only part of the histamine in the brain is accessible to the action of reserpine. It is possible that two different pools of histamine exist in the brain and only one of these is affected by reserpine. The dose for the maximum effect is about 0.5 mg/kg, but the effect is detectable in the corpora mammillaria and ventral hypothalamus even after 0.25 mg/kg of reserpine. This would seem to indicate that the action of reserpine on the histamine content of the hypothalamus is related to the central actions of the drug.

In the cat, noradrenaline and 5-HT in the
hypothalamus almost disappear after similar doses of reserpine (Holzbauer and Vogt, 1956; Paasonen and Vogt, 1956). Also in the rabbit after reserpine (5 mg/kg i.v.) the depletion of the two monoamines in the brain is nearly complete (Shore and Brodie, 1957). By comparison, the present results show that reserpine does not deplete histamine in the brain to the same extent as it depletes noradrenaline and 5-HT.

The rate of recovery of histamine in the hypothalamus after reserpine was slow. The concentration remained low for several days, and even when it could be expected that reserpine had disappeared from the brain (Hess, Shore and Brodie, 1956). After this smaller dose (0.5 mg/kg) the recovery of histamine in the cat's hypothalamus was found to be essentially similar to the recovery of 5-HT and noradrenaline in the rabbit's brain after a much larger dose (5 mg/kg) of reserpine (Pletscher, Shore and Brodie, 1956; Shore and Brodie, 1957). It has been suggested that reserpine lowers the content of 5-HT and noradrenaline in the brain by blocking a transport mechanism which normally pumps the amines across a membrane into particles (Pletscher et al., 1956; Brodie and Shore, 1957). According to
this hypothesis the depletion of the amines is pictured as a consequence of passive leakage of the amines from their intracellular pool (or pools), and the low level is a balance between their synthesis and rapid destruction. Reserpine might have acted on the storage mechanisms of histamine in the brain in a similar way. The difference in the intensity of the action on the brain histamine and the other monoamines could be related to the different rates of synthesis and destruction of the amines.

Reserpine releases histamine from the platelets (Waalkes et al., 1959; Burkhalter et al., 1960). It also releases 5-HT from rabbit platelets in vitro (Carlsson, Shore and Brodie, 1957). The binding of histamine in the brain could be similar to the binding of the amine in the platelets, since reserpine reduced the concentration of histamine in both the places.

Reserpine is known to release monoamines of the brain: the present results show that it also releases histamine of the brain. If the other monoamines are involved in the action of reserpine, so also is histamine. It would be interesting to study the effect on the brain histamine of agents, other than reserpine, which
are known to release the monoamines in brain. In contrast with the effect of reserpine on the brain histamine is the apparent absence of its effect on the hypophysial histamine. Mast cells are present in the cat's hypophysis (Gray, 1935; also S. 3/3), but according to Parratt and West (1957) reserpine does not act to release histamine from mast cells. Hence the lack of action of reserpine on the hypophysial histamine might be interpreted to mean that histamine in the hypophysis is mainly derived from mast cells. The anterior lobe, however, differs from other parts of the gland in that it contains fewer mast cells and treatment with compound 48/80 failed to lower the concentration of histamine extractable from it (S. 5/2). The failure of reserpine as well as of compound 48/80 to lower the histamine concentration in the anterior lobe suggests that in this part the binding of histamine differs from that in the brain and in the mast cells.
A large number of phenothiazine derivatives show varying degrees of tranquillizing, anti-emetic and antihistaminic properties (for reference see Parkes, 1961). Chlorpromazine (CPZ), however, has little antihistaminic activity and is better known for its central actions (Courvoisier, Fournel, Ducrot, Kolsky and Koetschot, 1953).

Chlorpromazine has no apparent effect on the concentration of monoamines in the brain (Brodie, Shore and Pletscher, 1956; Vogt, 1957; Gey and Pletscher, 1961) but some authors have reported an increase of 5-HT in the brain of mice and rats after chlorpromazine (Bartlet, 1960; Costa, Garattini and Valzelli, 1960). Chlorpromazine is reported to influence the changes of monoamine contents of rat brain, brought about by other drugs (Gey and Pletscher, 1961).

Chlorpromazine is known to inhibit ring methylation of histamine in vitro (Brown et al., 1959). Further, large doses of chlorpromazine given by intramuscular injection have been shown to reduce the turnover of $^{14}$C 1,4-methylhistamine in the cat's brain when $^{14}$C histamine is perfused
through the cerebral ventricles (White, 1961a, 1961b). It has been found in the rat that chlorpromazine is detectable in the brain long after injection of the dose (Wechsler and Forrest, 1959), and that it is concentrated in the hypothalamus (Wase, Christensen and Polley, 1956). It is therefore possible that, in the cat, large doses of chlorpromazine would raise the concentration of the drug in the hypothalamus sufficiently to inhibit the methylation of histamine. A prolonged inhibition of the methylation process might be expected to produce an increase in the concentration of histamine extractable from the hypothalamus. The present experiments were undertaken mainly to find out whether a large dose of chlorpromazine would increase the concentration of histamine extractable from the hypophysis and brain of the cat.

Two other phenothiazines were tested for their effect on the histamine concentration in the cat's hypothalamus. These were triflupromazine (TFP) and thioproperazine (TPZ). It is not known whether, like chlorpromazine, these drugs also inhibit ring methylation of histamine. Triflupromazine is more active than chlorpromazine as a 'tranquillizer' in monkeys and rats.
(Piala, High, Hassert, Burke and Craver, 1959; Bhargava and Chandra, 1964). It also antagonizes emesis induced by apomorphine in the dog. Thioproperazine (syn. Thioperazine) is a sulphonamide derivative of perphenazine. Its pharmacological properties were described by various authors (Courvoisier, Ducrot, Fournel and Julon, 1958; Delay, Deniker, Ropert, Beek, Barande and Emriault, 1959; Lambert, 1962). It is one of the most powerful anti-emetic drugs. In the rat, it produces 'catalepsy' in low doses (5 to 10 mg/kg) and is about 150 times more active than chlorpromazine in this respect.

Materials and method

Chlorpromazine hydrochloride (CPZ) (May and Baker Ltd.) was dissolved in sterile distilled water to make 100 mg/ml solution. The drug was freshly prepared before injection. Seven cats (Cats 1 to 7) received 3 x 50 mg/kg of CPZ (calculated as hydrochloride) in three divided doses in 24 hours. The last dose was given 2 hours before the animal was killed at 24 hours. Injections were given intramuscularly into the buttock on either side.

One cat (Cat 8) received 3 x 25 mg/kg of
Another cat (Cat 9) was given 5 x 50 mg/kg of the drug in 48 hours and killed at the end of that period. The cats were kept in a heated room at 25°C.

*Triflupromazine hydrochloride* (TFP) ('Vespral', Squibb and Sons) was dissolved in water to make 100 mg/ml of the drug. Six cats (Cats 10 to 15) received injections of 3 x 10 to 3 x 50 mg/kg (i.m.) of TFP calculated as hydrochloride) in 24 hours. The animals were killed at 24 hours, and 2 hours after the last injection. Only four samples from the hypothalamus were collected from these cats.

*Thioproperazine methane-Sulphonate* (TPZ) ('Majeptil', May and Baker Ltd.) was dissolved in 0.02 N NaOH to make 50 mg/ml solution of the drug, calculated as the base. The drug is soluble in water but forms an acidic solution. When dissolved in the alkali as above, the pH of the solution was about 6. Three cats (Cats 16 to 18) received 3 x 50 mg/kg of TPZ (base) intraperitoneally in 24 hours. The cats were killed at 24 hours, 2 hours after the last injection. Only the four hypothalamic samples were collected from these cats.
Results

Chlorpromazine hydrochloride

Within two hours after the first injection, the cats became quiet but remained alert and responded to stimuli. Later on there was shivering and a fall of body temperature. The nictitating membranes were fully relaxed. The animals took their food and drink.

At the time of killing (24 hours) the cats were more sedated but could be easily roused. When made to move they dragged their hind limbs. (This was a local effect of the drug which was injected i.m. in the buttocks). Sometimes they also showed tremor.

Effect on the histamine concentration.

Estimates of the histamine concentration in the parts of hypophysis and brain after CPZ treatment are presented in Table 10. The mean concentration of histamine increased in the brain but not in the hypophysis. After chlorpromazine (3 x 50 mg/kg), in the hypothalamus the concentration increased significantly (*P < 0.01) in all the four parts: in the ventral hypothalamus it rose to 163 per cent of the control; in the corpora mammillaria, to 148 per cent; in the dorsal hypothalamus to 150 per cent and in the preoptic
### Table 10

Effect of chlorpromazine hydrochloride on histamine in the hypophysis and brain

<table>
<thead>
<tr>
<th></th>
<th>Killed at 24 hours after total dose, mg/kg i.m.</th>
<th>Estimates of concentration as ng/g fresh tissue</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anterior lobe</td>
<td>Posterior lobe</td>
<td>Hypophysial stalk</td>
</tr>
<tr>
<td>Cat 1</td>
<td>3 x 50</td>
<td>1400</td>
<td>1300</td>
</tr>
<tr>
<td>Cat 2</td>
<td>3 x 50</td>
<td>1800</td>
<td>2200</td>
</tr>
<tr>
<td>Cat 3</td>
<td>3 x 50</td>
<td>1200</td>
<td>1100</td>
</tr>
<tr>
<td>Cat 4</td>
<td>3 x 50</td>
<td>1600</td>
<td>1200</td>
</tr>
<tr>
<td>Cat 5</td>
<td>3 x 50</td>
<td>1300</td>
<td>1600</td>
</tr>
<tr>
<td>Cat 6</td>
<td>3 x 50</td>
<td>3500</td>
<td>1400</td>
</tr>
<tr>
<td>Cat 7</td>
<td>3 x 50</td>
<td>4700</td>
<td>1300</td>
</tr>
<tr>
<td>Cat 8</td>
<td>3 x 25</td>
<td>1500</td>
<td>1300</td>
</tr>
<tr>
<td>Cat 9</td>
<td>5 x 50*</td>
<td>1200</td>
<td>2500</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>After CPZ Mean ± S.E. (Cats 1-7)</th>
<th>After CPZ Mean ± S.E. (Cats 1-7)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2210±509</td>
<td>1140±139</td>
<td>3690±319</td>
<td>1720±177*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1280±128*</td>
<td>720±64*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>650±88*</td>
<td>330±20*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Controls Mean ± S.E. (No. of cats)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2420±277</td>
<td>1680±142</td>
<td>5170±665</td>
</tr>
</tbody>
</table>

*Killed at 48 hours

*Significantly different from the control, P<0.05
region to 151 per cent. In the medial thalamus the concentration rose to only 132 per cent of the control \((P<0.05)\). One among the seven cats which received the dose \(3 \times 50 \text{ mg/kg of CPZ}\) (in 24 hours) did not show any rise of histamine concentration in the brain. This cat (Cat 7) was found to be more sedated than were the other cats after the same dose.

In a cat (Cat 8) which received \(3 \times 25 \text{ mg/kg of CPZ}\) (in 24 hours), the concentration of histamine in the brain did not show much change. But in another animal (Cat 9) which was given \(5 \times 50 \text{ mg/kg of CPZ}\) in 48 hours, the histamine concentration rose by more than 3-fold in the corpora mammillaria and in the ventral hypothalamus. In other parts of the hypothalamus and in the medial thalamus, the concentration was nearly doubled.

**Triflupromazine (TFP) and Thioproperazine (TPZ)**

After triflupromazine the cats were not sedated but showed some restlessness, and became rather unsteady. The nictitating membranes were fully relaxed and the pupils were somewhat dilated.

With thioproperazine, the cats showed
Table 11

Effect of triflupromazine (TFP) and thioproperazine (TPZ) on histamine in the hypothalamus

Estimates of concentration as ng/g fresh tissue

<table>
<thead>
<tr>
<th>Cat</th>
<th>Drug</th>
<th>Dose mg/kg at 24 hours</th>
<th>Corpora mamill.</th>
<th>Ventral hypothal.</th>
<th>Dorsal hypothal.</th>
<th>Preoptic region</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>TFP (HCl)</td>
<td>3 x 10</td>
<td>1800</td>
<td>1000</td>
<td>640</td>
<td>460</td>
</tr>
<tr>
<td>11</td>
<td>TFP (HCl)</td>
<td>3 x 20</td>
<td>2470</td>
<td>1560</td>
<td>680</td>
<td>880</td>
</tr>
<tr>
<td>12</td>
<td>TFP (HCl)</td>
<td>3 x 20</td>
<td>1230</td>
<td>1000</td>
<td>500</td>
<td>420</td>
</tr>
<tr>
<td>13</td>
<td>TFP (HCl)</td>
<td>3 x 20</td>
<td>1600</td>
<td>940</td>
<td>590</td>
<td>640</td>
</tr>
<tr>
<td>14</td>
<td>TFP (HCl)</td>
<td>3 x 50</td>
<td>1200</td>
<td>950</td>
<td>490</td>
<td>540</td>
</tr>
<tr>
<td>15</td>
<td>TFP (HCl)</td>
<td>3 x 50</td>
<td>2600</td>
<td>2120</td>
<td>1030</td>
<td>990</td>
</tr>
<tr>
<td>16</td>
<td>TPZ</td>
<td>3 x 50</td>
<td>1900</td>
<td>1200</td>
<td>740</td>
<td>540</td>
</tr>
<tr>
<td>17</td>
<td>TPZ</td>
<td>3 x 50</td>
<td>1760</td>
<td>1140</td>
<td>600</td>
<td>610</td>
</tr>
<tr>
<td>18</td>
<td>TPZ</td>
<td>3 x 50</td>
<td>1420</td>
<td>1120</td>
<td>540</td>
<td>590</td>
</tr>
</tbody>
</table>

After TFP: Mean ± S.E., Cats 10 - 15
1830 ± 246* 1260 ± 197* 655 ± 81* 655 ± 95*

After TPZ: Mean, Cats 16 - 18
1690 1150 630 580

Controls: Mean ± S.E. (no. of cats)
1150 ± 91 (14) 800 ± 71 (15) 480 ± 42 (15) 430 ± 35 (15)

*Significantly different from Control, P < 0.05
marked sedation and 'catatonia'. The nictitating membranes were relaxed.

The estimated concentrations of histamine in the four hypothalamic regions after the treatment are shown in Table 11. The control values for these regions are also shown.

After TFP the histamine concentration rose in most of the cats, but the results show some variation in the effect. However the mean values for the four areas calculated from all six cats which received different doses, were significantly different from the controls.

After TPZ there was a definite rise of the histamine concentration in the hypothalamus. But this was not as large as with chlorpromazine or TFP. The mean values from the three cats are shown in Table 11 but a test of significance was not performed since the number of animals used was too small.

7/3 Discussion

The complexity of the actions of chlorpromazine in the intact animal makes the interpretation of any observed effect of the drug difficult. There is even some doubt concerning the specificity of its central actions, since some effects
of the drug on the brain amines in the rat are lessened when the pronounced hypothermia produced by the drug is prevented (Hornykiewicz, Elringer and Lechner, 1961). Chlorpromazine inhibits several enzyme systems (Aboud, 1955; Magee, Berry and Rossiter, 1956; Century and Horwitt, 1956; Brown et al., 1959; Deesi, 1961). Moreover, it depresses respiratory exchange (Courvoisier et al., 1953) and the lactic acid formation (Larsson, 1961) in isolated brain tissues. It also inhibits the metabolic response to electrical stimulation in the isolated cerebral tissue (McIlwain and Greengard, 1957).

Doses of chlorpromazine used in the present work were probably sufficient to produce a concentration of drug in the brain of the same order as that which is known to inhibit ring methylation of histamine in vitro (Brown et al., 1959). It can thus be assumed, on the basis of White's (1961a) experiments, that chlorpromazine acted in this way to increase the concentration of histamine in the brain. However, histamine methylating activity is also present in the hypophysis (Axelrod et al., 1961), where the
concentration of the amine remained unchanged. In fact, in the monkey, the activity was found to be several times more in the posterior lobe than elsewhere in the brain. Why did the hypophysial histamine not rise after treatment with CPZ? The absence of a 'barrier' to histamine in the hypophysis (Adam et al., 1964) might be the explanation. These authors gave 'H histamine by intra arterial injections and showed that it was taken up by the hypophysis but not by the brain. If CPZ inhibited the methylation enzyme in the hypophysis it is possible that free histamine never accumulated because it was able to diffuse out into the circulation. In the brain however, since there is a 'barrier' to histamine, an accumulation of 'free' histamine would be easily detected.

The lack of action of CPZ on the hypophysial histamine could be due to the nature of storage of the amine in the gland. Since the methylation enzyme is present only in the soluble supernatant fraction (Brown et al., 1959; Lindahl, 1960), it could destroy histamine only when histamine has been released from its storage. Hypophysial histamine, which is partly derived from mast cells (Gray, 1935; S. 3/3), would
remain unaffected even if the methylation process was inhibited, so long as histamine was not liberated from the cells that contain it. The action of CPZ suggests that histamine in the brain is continually being released from its storage sites, so that CPZ by protecting the free histamine from methylation, produced an increase of its concentration.

Apart from inhibiting methylation of histamine in the brain, CPZ may have acted on the storage mechanism of histamine, as has been suggested for other amines in rat's brain (Gey and Pletscher, 1961). According to these authors, CPZ may act on the brain to decrease the permeability of the storage granules for some amines, and thereby to reduce both the storage and release of these amines. In support of this hypothesis is also the finding that CPZ reduces the permeability of certain cell membranes (Bartholini, Pletscher and Gey, 1961; Nathan and Friedman, 1962).

The doses of CPZ used in the present experiments were high, and so could have produced other changes in the brain. However, with a very large dose (5 x 50 mg/kg in 48 hours) the histamine concentration was greatly increased
and was more than doubled in the hypothalamus. Whereas with a smaller dose (3 x 25 mg/kg in 24 hours) very little change was detectable. This indicates that the actual concentration of the drug in the brain was probably the deciding factor for the effect on the histamine concentration. It can be assumed that the same mechanism of action of the drug would be present even with lower doses, but the present method of estimating histamine in the brain is capable of detecting only large changes in the brain histamine.

Like CPZ, triflupromazine and thioproperazine increased the histamine concentration in the hypothalamus. The effect was not so marked or consistent after these drugs, as it was with CPZ. It is not yet known whether TFP or TPZ also inhibit imidazole N-methyl transferase. It would be interesting to know this and, if they were found to be active, to compare their activity with that of CPZ. In the absence of direct evidence, it would seem fair to assume that these drugs raised the brain histamine by inhibiting the methylating enzyme.

Intramuscular injections of CPZ reduce the turnover of 1,4-methylhistamine from 14C histamine given intraventricularly (White, 1961a).
In the present experiments along with the rise of histamine after CPZ, there was probably a corresponding decrease of 1,4-methylhistamine in the brain. J.P. Green (personal communication) has estimated 1,4-methylhistamine in rat's brain and found the concentration to be approximately 25 ng/g. He used a method based on the coupling of 1,4-methylhistamine with dinitrofluorobenzene. It would be interesting to test the effect of CPZ on the concentration of 1,4-methylhistamine in the brain.
Monoamine oxidase (MAO) is probably involved in the metabolism of histamine (Davison, 1958; Schayer, 1959). Iproniazid (1-isonicotyl-2-isopropyl hydrazine) is a well known MAO inhibitor (Zeller, Barsky, Fouts, Kirchheimer and Van Orden, 1952). In the intact cat iproniazid influences the catabolism of $^{14}$C histamine, so that the excretion of a radioactive histamine metabolite, later identified as methylimidazole-acetic acid (MeImAA), is suppressed in the urine (Schayer, Kennedy and Smiley, 1953; Schayer, 1953). The oxidation of 1,4-methylhistamine to MeImAA in intact mice is blocked by a MAO inhibitor (Isobutyl-isonicotinyl hydrazide: IBINH) but not by aminoguanidine (Rothschild and Schayer, 1958). White (1960) observed in the cat, that subcutaneous injections of iproniazid completely inhibited the formation of MeImAA from $^{14}$C histamine which was perfused through the cerebral ventricles, and that under such conditions more 1,4-methylhistamine ($^{14}$C) was recovered from the brain. The question arose whether inhibition of the oxidative deamination of 1,4-methylhistamine could influence the histamine concentration in
the brain. The accumulation of 1,4-methylhistamine might be expected to block further methylation and so lead to an increase in free histamine.

Iproniazid is known to increase the content of 5-HT and noradrenaline in the brain of the rabbit, rat and mouse (Udenfriend, Weissbach and Bogdanski, 1957; Brodie, Spector and Shore, 1959; Leroy and Schaepdryver, 1961). In the cat and dog, however, treatment with iproniazid did not produce a detectable rise of the noradrenaline (Vogt, 1959; Maling, Highman and Spector, 1962). 5-HT is a potent inhibitor (in vitro) of imidazole N-methyl transferase (Brown et al., 1959). It is therefore possible that MAO inhibitors, by increasing 5-HT in the brain, could act indirectly to inhibit the ring methylation of histamine.

MAO inhibitors with a hydrazine structure have high reactivity and also inhibit other enzymes including diamine oxidase (Zeller et al., 1952; Gey, Pletscher and Burkard, 1963). It was desirable in the present work to test MAO inhibitors of both hydrazine and non-hydrazine type. Iproniazid was chosen as a hydrazine type MAO inhibitor. Pargyline (N-benzyl-N-methyl-2-propynylamine) was chosen as a non-hydrazine
type MAO inhibitor (Taylor, Wykes, Gladish and Martin, 1960; Spector, 1963). It has been reported that pargyline treatment causes a rise of 5-HT in the brain of different species including the cat (Spector, 1963; Wiegand and Rinaldi, 1963). The object of the present experiment was to test, in the first instance, whether treatment with MAO inhibitors would produce a detectable rise of the histamine concentration in brain.

8/1 Materials and method

**Iproniazid.** Iproniazid phosphate ('Marsilid', Roche) was dissolved in water to make 200 mg/ml solution of the drug calculated as the base. Three cats received iproniazid. One of these (Cat 1) was given a single dose of 125 mg/kg intraperitoneally and the cat was killed at 24 hours. Two other cats also received the same total dose of 125 mg/kg, but this was given as a daily injection of 25 mg/kg iproniazid (base) i.p. on 5 successive days and the cats were killed on the 5th day, two hours after the last injection. Samples were collected from hypophysis and brain of these three cats.

**Pargyline.** Pargyline hydrochloride (MO 911, 'Eutonyl', Abbott) was dissolved in 0.9 per cent
saline to make 100 mg/ml solution of the drug (calculated as base). 20 mg/kg pargyline was injected daily intraperitoneally for 5 days into 4 cats. The animals were killed on the 5th day, two hours after the last injection. The total dose was thus 100 mg/kg. Only the four samples from the hypothalamus were collected from the pargyline treated cats.

8/2 Results

After repeated injections of iproniazid, the cats remained alert but showed signs of weakness. Their mucous membranes and paws turned pale and later quite dark in colour. On bleeding, the blood was found to be very dark in colour. Pargyline produced few symptoms. The cats after pargyline treatment were alert and behaved normally. The pupils, however, were dilated.

**Effect on histamine concentration.** The results for the two drugs are shown in Table 12.

At 24 hours iproniazid did not produce any change in the histamine concentration (Cat 1). When the dose was divided over 5 days, the histamine concentration rose in the hypothalamus and [*medial thalamus* but not in the hypophysis.}
Table 12

Effect of monoamine oxidase inhibitors on histamine
in the hypophysis and brain

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</thead>
<tbody>
<tr>
<td>Cat 1</td>
<td>Iproniazid</td>
<td>24 hours</td>
<td>2170</td>
<td>1190</td>
<td>4000</td>
<td>970</td>
<td>1060</td>
<td>490</td>
<td>480</td>
<td>220</td>
</tr>
<tr>
<td>Cat 2</td>
<td>Iproniazid</td>
<td>5 days</td>
<td>570</td>
<td>1300</td>
<td>1240</td>
<td>2240</td>
<td>1510</td>
<td>600</td>
<td>620</td>
<td>350</td>
</tr>
<tr>
<td>Cat 3</td>
<td>Iproniazid</td>
<td>5 days</td>
<td>1290</td>
<td>300</td>
<td>1050</td>
<td>2130</td>
<td>1260</td>
<td>650</td>
<td>590</td>
<td>310</td>
</tr>
<tr>
<td>Cat 4</td>
<td>Pargyline</td>
<td>5 days</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2800</td>
<td>1420</td>
<td>640</td>
<td>420</td>
<td>-</td>
</tr>
<tr>
<td>Cat 5</td>
<td>Pargyline</td>
<td>5 days</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2020</td>
<td>930</td>
<td>420</td>
<td>450</td>
<td>-</td>
</tr>
<tr>
<td>Cat 6</td>
<td>Pargyline</td>
<td>5 days</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1430</td>
<td>700</td>
<td>400</td>
<td>370</td>
<td>-</td>
</tr>
<tr>
<td>Cat 7</td>
<td>Pargyline</td>
<td>5 days</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>970</td>
<td>490</td>
<td>400</td>
<td>390</td>
<td>-</td>
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<tr>
<td></td>
<td>After Iproniazid</td>
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<td></td>
<td>Mean Cats 1-3</td>
<td></td>
<td>1340</td>
<td>930</td>
<td>2100</td>
<td>1780</td>
<td>1280</td>
<td>570</td>
<td>560</td>
<td>290</td>
</tr>
<tr>
<td></td>
<td>After Pargyline</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1810</td>
<td>890</td>
<td>470</td>
<td>410</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mean Cats 4-7</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>Controls Mean ± S.E. (no. of cats)</td>
<td>2420±277 (25)</td>
<td>1680±162 (25)</td>
<td>5170±665 (14)</td>
<td>1150±91 (14)</td>
<td>800±71 (13)</td>
<td>460±42 (13)</td>
<td>430±35 (13)</td>
<td>290±17 (21)</td>
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</table>
The rise was most marked in the corpora mammillaria, where in both the cats the concentration was nearly doubled.

After pargyline the effect on the hypothalamic histamine was not very marked, except in the corpora mammillaria. In two of the cats (Cats 4 and 5) the concentration in the corpora mammillaria was nearly doubled after pargyline, whereas in one cat (Cat 7) there was no rise. In other parts of the hypothalamus the values were close to the controls (Table 12).

8/3 Discussion

Iproniazid increased the concentration of histamine in the brain but not in the hypophysis. Pargyline, a MAO inhibitor of non-hydrazine type, increased the concentration of histamine mainly in the corpora mammillaria. Whether the effect of the two drugs is related or not to their property of MAO inhibition remains to be tested. The difference in the distribution of their effect on the histamine concentration could be due to the dose and to other factors such as penetration into the brain. A single dose of iproniazid was not effective, whereas the same dose given over a longer period in divided doses
was effective.

Iproniazid does not inhibit ring methylation of histamine (Schayer and Karjala, 1956; Lindahl, 1960). As a MAO inhibitor, however, it may inhibit the oxidative deamination of 1,4-methylhistamine and so suppress the formation of MeImAA (Rothschild and Schayer, 1958; White, 1960). It would be interesting to know whether after iproniazid there was a rise in the concentration of 1,4-methylhistamine in the brain.

MAO inhibitors when given in doses comparable with those used in the present experiment can be expected to raise the content of 5-HT in the cat's brain (Maling et al., 1962; Spector, 1963; Schoepke and Wiegand, 1963). 5-HT is known to inhibit methylation of histamine (Brown et al., 1959). (At $5 \times 10^{-5}$ M, 5-HT inhibited methylation of histamine in vitro by about 75 per cent). It is therefore possible that the rise of histamine in the brain after the MAO inhibitors was due to the rise of 5-HT in the brain which protected histamine from methylation. A parallel estimation of 5-HT and histamine in the brain would show more clearly whether or not a rise of brain 5-HT is accompanied or followed by a rise of the brain histamine.
SECTION 9

MISCELLANEOUS DRUGS

The following drugs were also tested for their effect on the histamine concentration of the brain and hypophysis: bulbocapnine, morphine, adrenaline and pentobarbitone.

Bulbocapnine, the alkaloid from *Corydalis cava*, is known to produce 'catatonia' in various species including the cat (De Jong, 1945). On intraventricular injection 1 mg (less than 0.5 mg/kg) of bulbocapnine is sufficient to produce 'catatonic stupor' in the cat (Feldberg and Sherwood, 1955). It has been reported that anti-histaminics antagonize this effect of bulbocapnine in mice (Zetler and Moog, 1958). Walaszek and Chapman (1963) reported that after bulbocapnine the histamine content in the rat brain increased and they suggested that it might have been due to the inhibition of diamine oxidase by bulbocapnine (Chapman and Walaszek, 1962). It was therefore of interest to test in the cat whether bulbocapnine in a dose which produced 'catatonia' also increases the histamine concentration in the brain.

Morphine is known to release histamine from
cat skin and after intravenous injection of the drug the plasma histamine is raised (Feldberg and Paton, 1951). The fall of blood pressure in the cat after morphine can be prevented by mepyramine (Evans, Nasmyth and Stewart, 1952). Morphine also releases noradrenaline from the hypothalamus of the cat (Vogt, 1954). It was desirable to test whether morphine could influence the histamine content of the brain and the hypophysis.

Schayer reported on the 'adaptive increase' of mammalian histidine decarboxylase activity after injections of adrenaline (S. 1/4). Adrenaline was tested in the present experiment from this point of view. It was supposed that an increase in the formation of histamine might lead to more being stored and hence to a rise of its concentration in the brain.

Pentobarbitone is known to raise the content of 5-HT in the rat's brain (Bonnycastle, Giarman and Paasonen, 1957; Bonnycastle, Bonnycastle and Anderson, 1962). Three cats were therefore anaesthetized with pentobarbitone instead of ether.
**Materials and method**

**Bulbocapnine.** Bulbocapnine hydrochloride (Merck) was dissolved in warm saline (0.9 per cent) to make 20 mg/ml solution of the drug calculated as salt. Only a small quantity of the drug was available. One cat was given 3 x 30 mg/kg bulbocapnine-HCl (calculated as salt) and was killed 4 hours after the injection. Another cat received 90 mg/kg bulbocapnine-HCl, given in divided doses (3 x 30 mg/kg) in 24 hours, and was killed 2 hours after the last injection. The injections were given subcutaneously.

**Morphine.** Morphine hydrochloride was dissolved in water to make 50 mg/ml solution of the salt. Cats were injected intraperitoneally with 3 x 30 mg/kg morphine-HCl (calculated as salt), and killed at 24 hours, two hours after the last injection.

**Adrenaline.** Adrenaline in oil (2 mg/kg in oil, Roche) was injected intramuscularly. A total dose of 6.0 mg was given in 24 hours to a cat weighing 3.5 kg.

**Pentobarbitone.** Pentobarbitone sodium (Nembutal powder, Abbott) was dissolved in normal saline for injection. The solution was kept
cold to prevent precipitation. 60 mg/kg of the drug was injected intraperitoneally and the cats were bled to death 1 hour after the injection, without any ether.

Samples from hypophysis and brain were collected after treatment with these drugs.

9/2 Results

The results for the four drugs have been grouped in Table 13.

Bulbocapnine. Soon after the first injection of 30 mg/kg bulbocapnine-HCl, the cat became quiet and showed diminished motor activity. One hour after the injection the cat assumed a characteristic static posture and made no effort to move even when its position was changed. The animal closed its eyes and did not respond to loud sounds. On repeating the injection at 2 hours the cat became quite aggressive. This violent stage lasted for an hour. Soon after the third injection there was a convolution which lasted for about 10 minutes. When it recovered from the convolution, it was still aggressive and there was salivation and relaxation of the nictitating membrane.

When the injections were given at longer
### Table 13

Effect of miscellaneous drugs on histamine in the hypophysis and brain

<table>
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</thead>
<tbody>
<tr>
<td>Cat 1</td>
<td>Bulbocapnine (HC1)</td>
<td>3 x 30</td>
<td>4 hours</td>
<td>1160</td>
<td>5320</td>
<td>7070</td>
<td>1500</td>
<td>770</td>
<td>420</td>
<td>440</td>
</tr>
<tr>
<td>Cat 2</td>
<td>Bulbocapnine (HC1)</td>
<td>3 x 30</td>
<td>24 hours</td>
<td>570</td>
<td>540</td>
<td>700</td>
<td>1760</td>
<td>1220</td>
<td>600</td>
<td>540</td>
</tr>
<tr>
<td>Cat 3</td>
<td>Morphine (HC1)</td>
<td>3 x 30</td>
<td>24 hours</td>
<td>850</td>
<td>1050</td>
<td>2930</td>
<td>1490</td>
<td>900</td>
<td>560</td>
<td>480</td>
</tr>
<tr>
<td>Cat 4</td>
<td>Morphine (HC1)</td>
<td>3 x 30</td>
<td>24 hours</td>
<td>5330</td>
<td>1930</td>
<td>4600</td>
<td>1240</td>
<td>990</td>
<td>350</td>
<td>550</td>
</tr>
<tr>
<td>Cat 5</td>
<td>Pentobarbitone (Na)</td>
<td>60</td>
<td>1 hour</td>
<td>820</td>
<td>1360</td>
<td>3230</td>
<td>1420</td>
<td>1090</td>
<td>600</td>
<td>690</td>
</tr>
<tr>
<td>Cat 6</td>
<td>Pentobarbitone (Na)</td>
<td>60</td>
<td>1 hour</td>
<td>690</td>
<td>780</td>
<td>2630</td>
<td>1240</td>
<td>970</td>
<td>560</td>
<td>580</td>
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<tr>
<td>Cat 7</td>
<td>Pentobarbitone (Na)</td>
<td>60</td>
<td>1 hour</td>
<td>500</td>
<td>690</td>
<td>4300</td>
<td>1260</td>
<td>1030</td>
<td>610</td>
<td>710</td>
</tr>
<tr>
<td>Cat 8</td>
<td>Adrenaline (in oil)</td>
<td>1.7 approx. (5 divided doses)</td>
<td>24 hours</td>
<td>1130</td>
<td>2430</td>
<td>3950</td>
<td>660</td>
<td>530</td>
<td>330</td>
<td>240</td>
</tr>
</tbody>
</table>

Controls: Mean ± S.E. (no. of cats)  
2420±277 (25)  1680±142 (25)  5170±165 (14)  1150±91 (14)  800±71 (15)  480±42 (15)  430±35 (15)  250±17 (21)
intervals (Cat 2) the static posture (as observed in the first cat) was found to continue for about 4 hours. At the time of the second injection the cat had recovered and was apparently normal, but the same symptoms reappeared after the injection. At the time of killing, the cat showed these symptoms as a result of the third injection.

There was no major change in the concentration of histamine in the two cats, except in the corpora mammillaria where the concentration was considerably higher after bulbocapnine.

Morphine. After morphine, the cats were very restless and moved about in the cage. There was salivation and the pupils were dilated. The animals later on assumed a posture with a predominant flexor tone. They showed jerking movements and arched their bodies at intervals. No major change of the histamine concentration was observed after the morphine treatment in any of the parts examined.

Adrenaline. There was no marked change of the histamine concentration after adrenaline in one cat. The concentration in the parts of the brain examined are, however, somewhat lower than those of the controls.
Pentobarbitone. As compared with control values obtained in cats anaesthetized with ether, the values obtained from cats anaesthetized with pentobarbitone, appeared to be higher. A possible exception was the anterior lobe, where the concentration was low.

9/3 Discussion

The number of cats used to test these miscellaneous drugs was too small in each case to permit any definite conclusion as to their effects on the histamine concentration in the brain and hypophysis. However, the results suggest that none of these drugs has any major effect on the histamine concentration in the parts examined. Bulbocapnine increased the concentration of histamine in the corpora mammillaria but not in the rest of the brain. It is difficult to say if this action is due to inhibition of diamine oxidase as has been suggested by Walaszek and Chapman (1963). Bulbocapnine has been shown to inhibit diamine oxidase in vitro (Chapman and Walaszek, 1962), but there is so far no clear evidence that the enzyme is present in brain. Nor is it known whether bulbocapnine inhibits ring methylation of histamine.
Pentobarbitone had some effect on the histamine concentration of the brain, particularly in the **preoptic region** and in the **medial thalamus**. The dose of pentobarbitone known to increase 5-HT in the brain of rats was 125 mg/kg (Bonnycastle et al., 1962). Possibly with a higher dose and a longer period of treatment than used in the present experiment, pentobarbitone might have produced a larger increase in the histamine concentration of the brain.

The single result with adrenaline is difficult to interpret. There was no sign of increased formation of histamine in the hypothalamus where the values were lower than in the controls.
The study of the concentration of histamine in the hypophysis and brain on a regional basis has made it possible to detect changes in this concentration after treatment with drugs. Since histamine is unevenly distributed in the brain, it would have been difficult to observe any effect of drugs on the whole brain histamine. Moreover, with several drugs the effect seemed to be more prominent in the hypothalamus than elsewhere in the brain. In the present study it was possible to observe the effect of drugs on different parts of the hypothalamus.

10/1 HYPOPHYSIS

Histamine concentration in the cat's hypophysis is very variable, which may be explained by the presence of mast cells in the gland (S. 4/1). This variability makes it difficult to interpret the effect of drugs on the hypophysial histamine. Among the drugs tested in the present work, only compound 48/80 reduced the concentration of histamine in the posterior lobe and in the hypophysial stalk.
It had no significant effect on the histamine concentration in the **anterior lobe** or in the brain. The reduction of the concentration of histamine in the **posterior lobe** and in the **hypophyseal stalk** after 48/80 agrees with the finding that mast cells are present in both of these regions in the cat (Gray, 1935; also S. 3/3). It was concluded from mast cell counts in different parts of the hypophysis that the **anterior lobe**, which contains about 50 per cent of the total histamine extractable from the gland, contains only about 10 per cent of the total number of mast cells in the gland (S. 4/1). Hence it can be suggested that most of the histamine in the **anterior lobe** is held in cells other than mast cells, and that these cells are not susceptible to the action of 48/80.

The question arises why some drugs which produced effects on the concentration of histamine in the hypothalamus, failed to do so in the hypophysis. A rise of concentration of histamine in the hypophysis may be difficult to detect because of the absence of a barrier to histamine in the hypophysis (Adam et al., 1964). For example, it may be that a rise of histamine concentration was not detected in the hypophysis
after chlorpromazine because excess histamine passed into the circulation (S. 7/3). However, the hypophysis differs from the hypothalamus in several ways as regards histamine. The hypophysis contains mast cells but the hypothalamus does not (S. 3/3). The hypophysis takes up \(^{14}\)C histamine from the circulation but the hypothalamus does not (Adam et al., 1964). The hypophysis has no detectable capacity to form histamine from \(^{14}\)C histidine, yet the hypothalamus has this capacity (White, 1959; Adam et al., 1964). In the dog the subcellular distribution of hypophysial histamine is different as compared with that in the hypothalamus (Michaelson and Dowe, 1963). These differences suggest that the physiological role of histamine in the hypophysis may not be the same as that in the brain.

10/2 BRAIN

The effect of drugs was studied mainly on the histamine concentration of the hypothalamus and medial part of the thalamus. The drugs can be conveniently grouped according to their effects.

(1) Drugs which reduced the histamine concentration: reserpine is the only drug which belongs
to this group.

(2) Drugs which increased the histamine concentration: chlorpromazine and other phenothiazines. Iproniazid and bulbocapnine increased the concentration in the corpora mammillaria and ventral hypothalamus but apparently not in other parts of the brain. Pentobarbitone may also belong to this group.

(3) Drugs which did not alter the histamine concentration: compound 48/80 and morphine.

Fig. 12 shows the effect of drugs on the hypothalamic histamine. The effect is shown as percentage increase or decrease of the histamine concentration in the four hypothalamic areas with the control values at 100 per cent. Reserpine and the phenothiazines produced significant changes in the histamine concentration of the four parts of the hypothalamus. By contrast, the effect of iproniazid, pargyline and bulbocapnine was confined mainly to the corpora mammillaria and the ventral hypothalamus.

Quantitative differences in the effects of drugs were seen not only within the different parts of the hypothalamus but also between the hypothalamus and thalamus. With nearly all the drugs that had a significant effect, the change
Fig. 12. Effect of various drugs on the concentration of histamine in the cat's hypothalamus. The horizontal line at 100 per cent represents the control values for the concentration of histamine (Table 4). The columns represent the mean concentration of histamine expressed as a percentage of the control in the four regions of the hypothalamus after various drugs: R, reserpine; CPZ, chlorpromazine; TFP, triflupromazine; TPZ, thioproperazine; IPZ, iproniazid; PG, pargyline; B, bulbocapnine; M, morphine; PB, pentobarbitone. For details see Tables 7/1, 8, 10, 11, 12 and 13.
of concentration in the medial thalamus was less than in the hypothalamus. Pentobarbitone was the exception. This difference in the magnitude of the effect may have depended on regional differences in the penetration of the drugs or the metabolism of histamine. So far, little is known about regional differences in the penetration of the drugs used. There is, however, evidence that the metabolism of histamine may vary in different parts of the brain. Both the formation and the inactivation of histamine have been shown to proceed more actively in the hypothalamus than elsewhere in the brain (White, 1959, 1960, 1961a; Axelrod et al., 1961).

This is also the region where the concentration of histamine is highest (S. 3/2, Table 4). These facts may explain why the concentration of histamine in the hypothalamus rises and falls more readily than in the medial thalamus, under the influence of drugs. The histamine concentration fell after reserpine (S. 6/2) and in this respect histamine in the brain behaved in the same way as did the noradrenaline (Holzbauer and Vogt, 1956) and 5-HT (Brodie et al., 1955; Paasonen and Vogt, 1956; Shore and Brodie, 1957) in brain except that the depletion was incomplete.
It has been suggested that reserpine acts on the mechanism of storage of the monoamines (Pletscher et al., 1956; Brodie and Shore, 1957). Reserpine might have acted on histamine in brain (as on the monoamines), by inhibiting the process of concentration of histamine or its retention in some type of particle.

Binding of the amines has been reviewed recently (Green, 1962). In the particles, the amines are present in a relatively high concentration which is apparently maintained by active metabolic processes. In the brain the biologically active amines are present in the neurones (or their synaptic endings) and not in the glia cells (Gray and Whittaker, 1962; Falck, 1962). Histamine in rat brain and also in the dog's hypothalamus is present in particles which are similar to those containing 5-HT and acetylcholine (Carlini and Green, 1963; Michaelson and Dowe, 1963). A bimodal distribution of histamine in the guinea-pig brain has also been suggested (Michaelson and Dowe, 1963). The action of reserpine on brain histamine shows that part of the histamine (about 35 per cent) in the brain is not accessible to the drug whereas the rest of it is easily released. This suggests that
histamine in the cat's brain may be stored in two different ways. A study of the subcellular distribution of histamine in brain after reserpine would be useful in this respect.

The concentration of histamine in the brain was increased after large doses of chlorpromazine and other phenothiazines (S. 7/2). Chlorpromazine probably acted by inhibiting methylation of histamine (Brown et al., 1959; White, 1961a) in the brain. Whether other phenothiazines are also active inhibitors of histamine methylation is not yet known. It is difficult to relate any central action of the phenothiazines with the rise of histamine in the brain, since the doses required to produce this effect were very large (S. 7/1). It is known from the clinical use of chlorpromazine in the treatment of mental diseases that in most cases the administration of the drug must be continued for several days before the full therapeutic effects are obtained. It is therefore possible that the rise of brain histamine might be achieved by giving smaller doses of the drug over a longer period of time.

After repeated injections of iproniazid and pargyline the histamine concentration rose
mainly in the **corpora mammillaria** and in the **ventral hypothalamus**. Whether this action is wholly related to inhibition of monoamine oxidase is not clear. The inhibition of the formation of methylimidazoleacetic acid and the possible increase in the concentration of 1,4-methylhistamine in the brain after iproniazid (White, 1960), may be related to the rise of histamine in the brain. However, the rise seen after treatment with the MAO inhibitors, could also be due to the rise of 5-HT in the brain (Brodie *et al.*, 1959; Spector, 1963), since 5-HT is known to inhibit effectively the methylation of histamine *in vitro* (Brown *et al.*, 1959).

Compound 48/80, bulbocapnine, morphine and pentobarbitone did not affect the histamine concentration in the brain to any extent. The lack of action of 48/80 agrees with the finding that in the cat the hypothalamus does not contain mast cells (Gray, 1935; also S. 3/3). However, it is not known whether 48/80 penetrated into the brain. After bulbocapnine and pentobarbitone, the histamine concentration increased in certain parts of the hypothalamus (Fig. 12). But the number of cats treated with these drugs was too small to allow definite conclusions. The same
applies to the low value for the histamine concentration in the hypothalamus in the single cat after treatment with adrenaline (S.9/2).

The present work shows that the concentration of histamine in the brain, particularly in the hypothalamus, can be altered by several drugs but does not give precise information on their mechanism of action. The results do not indicate in any way the function of histamine in the brain, but suggest that histamine may have a functional relationship with other pharmacologically active amines in the brain. In spite of much research in recent years on the role of these amines in the brain, it is not yet possible to ascribe definite functions in the brain to any of them, or to their inter-relationship. The functional significance of histamine in the brain still remains to be defined.
APPENDIX I.a.

EFFECT OF STORAGE OF THE TISSUES

Parallel samples from the two sides of the brain were collected. The sample from one side was extracted fresh and that from the other side was frozen and stored overnight (see page 36). The frozen sample was extracted after 24 hours with TCA.

Assays of the parallel samples were carried out on the same preparation. The results are shown below:

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Histamine concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh</td>
</tr>
<tr>
<td>1. Hypothalamus (ventral and dorsal parts only)</td>
<td>850</td>
</tr>
<tr>
<td>2. &quot;</td>
<td>640</td>
</tr>
<tr>
<td>3. &quot;</td>
<td>600</td>
</tr>
<tr>
<td>4. Medial thalamus</td>
<td>210</td>
</tr>
<tr>
<td>5. &quot;</td>
<td>150</td>
</tr>
<tr>
<td>6. &quot;</td>
<td>150</td>
</tr>
<tr>
<td>7. &quot;</td>
<td>320</td>
</tr>
<tr>
<td>8. &quot;</td>
<td>180</td>
</tr>
<tr>
<td>9. Central grey matter</td>
<td>170</td>
</tr>
<tr>
<td>10. Med. geniculate body</td>
<td>200</td>
</tr>
<tr>
<td>11. Superior colliculus</td>
<td>140</td>
</tr>
</tbody>
</table>
The differences between the fresh and the stored samples were not large, and except in No. 7, could have been due to error of the assay. Some of the differences could also partly be due to the dissection.
APPENDIX I.b.

TEST OF EFFICIENCY OF THE EXTRACTION WITH TCA

The volume of 6 per cent (w/v) TCA used in the method for extraction of histamine from brain tissue was 5 µl/mg of tissue weight (page 43). The present experiment was performed to test the efficiency of the TCA extraction.

A sample from the hypothalamus (ventral and dorsal parts: weight 24.3 mg) was extracted three times with the same volume of 6 per cent TCA (5 µl/mg tissue in each extraction). During each extraction the tissue was ground and after making the volume in the grinder to 5 ml with water, the suspension was left at room temperature for 10 min, before it was centrifuged. 4.6 ml of the supernatant was collected after each extraction. The extracts were purified and the histamine content was estimated as described (S. 2/3 and 2/4).

The result of the bioassay was as follows:

<table>
<thead>
<tr>
<th>Extraction</th>
<th>Histamine Estimated in the Purified Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>First extraction</td>
<td>16.3 ng</td>
</tr>
<tr>
<td>Second extraction</td>
<td>&lt;1.0 ng</td>
</tr>
<tr>
<td>Third extraction</td>
<td>&lt;1.0 ng</td>
</tr>
</tbody>
</table>
It was concluded from this experiment that all the histamine extractable from the brain tissue was present in solution after the first extraction with the TCA (6 per cent TCA w/v, 5 μl/mg of tissue).
ESTIMATION OF SODIUM IN THE ELUATE MATERIAL

It was essential that the amount of NaCl present in the dried residue of the eluate material should remain nearly constant. Otherwise the reconstitution of the dried residue for bioassay by superfusion would be uncertain and so cause errors in the assay (see page 54). The amount of NaCl present in the dried residue of the eluate depended on several factors: (1) the pH and ionic strength of the solution for adsorption, (2) complete elution of sodium, and (3) possible losses of sodium chloride during evaporations (S. 2/3/3). The present experiment was carried out to confirm the total amount of NaCl present in the eluate material (Adam, 1961) and to observe whether replicates of the eluate material agreed or not as to their content of NaCl.

Eluate material was collected on different occasions and was from tissue extracts, the tissue being either from the cerebellum or the cerebral cortex. The weights of the tissue samples ranged from 32 to 58 mg. The sodium content of the eluate was determined with a flame photometer (Eel, Model A, Evans Electroselenium Ltd.). On each occasion fresh Na⁺
standards (six) were prepared from NaCl, which had concentrations ranging from 0.05 to 0.3 μEq/ml of Na⁺.

The eluate (5 ml; see page 47) was diluted with water (1 in 500) and its Na⁺ concentration determined, or it was evaporated and treated with 6 N HCl and the acid evaporated as described (S. 2/3/3). The dried residue was dissolved in 5 ml water and an aliquot diluted 1 in 500 to determine the Na⁺ concentration. The total Na⁺ found in the eluate was also calculated as NaCl, on the assumption that all the Na⁺ present was in this form.

The results are given in Table 14. The values for Na⁺ present in the eluate (5 ml) or in its dried residue after heating with 6 N HCl did not vary widely. This shows that there was no detectable loss of NaCl in the process of evaporation of the eluate (S. 2/3/3). The mean content of Na⁺ in the eluate (from 10 observations) was 405 μEq of Na⁺ (range: 390 to 425 μEq Na⁺), or from 22.8 to 24.75 mg (mean 23.64 mg) as NaCl.

The result confirms that of Adam (1961), who estimated that the dried residue of the eluate under similar conditions contained about 24 mg of NaCl. The modified Tyrode's solution
Table 14

Estimation of sodium in the eluate

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Total Na(^+) estimated µEq</th>
<th>Total NaCl calculated mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>400</td>
<td>23.38</td>
</tr>
<tr>
<td>2</td>
<td>390</td>
<td>22.80</td>
</tr>
<tr>
<td>3</td>
<td>400</td>
<td>23.38</td>
</tr>
<tr>
<td>4</td>
<td>400</td>
<td>23.38</td>
</tr>
<tr>
<td>5</td>
<td>420</td>
<td>24.55</td>
</tr>
<tr>
<td>6</td>
<td>420</td>
<td>24.55</td>
</tr>
<tr>
<td>7</td>
<td>400</td>
<td>23.38</td>
</tr>
<tr>
<td>8</td>
<td>425</td>
<td>24.75</td>
</tr>
<tr>
<td>10</td>
<td>390</td>
<td>22.80</td>
</tr>
<tr>
<td>Mean</td>
<td>405</td>
<td>23.64</td>
</tr>
<tr>
<td>Range</td>
<td>390 - 425</td>
<td>22.8 - 24.75</td>
</tr>
</tbody>
</table>

*Expts. 1 - 5: material tested was the eluate (5 ml)*

*Expts. 6 - 10: material tested was the dried residue of the eluate (after heating with 6 N HCl).*
(S. 2/4/1) was prepared on this basis and was routinely used for dissolving the dried residue in making the test solutions for bioassay (S. 2/4/4).
APPENDIX I.d.

ESTIMATION OF POTASSIUM IN THE ELUATE MATERIAL

Presence of excess quantities of K⁺ in the test solution, compared with the concentration of K⁺ in the Tyrode's solution, could interfere with the assay of histamine by superfusion (Adam et al., 1954). The K⁺ present in the tissue would probably be adsorbed on the column and eluted with HCl.

Total K⁺ present in the eluate was estimated with the Flame Photometer. K⁺ standards were prepared (0.05 – 0.3 μEq/ml K⁺) which also contained 2.3 mg/ml of NaCl. The dried residue of the eluate containing about 23 mg of NaCl was dissolved and made up to 10 ml with water. Without further dilution this was tested in the Flame Photometer.

The eluates were from three tissue extracts. The extracts were purified by adsorption on columns in the usual way. The eluates were dried, heated with 6 N HCl and dried. Total K⁺ estimated in the dried residues are shown on the following page.
<table>
<thead>
<tr>
<th>Sample</th>
<th>Weight (mg)</th>
<th>Total $K^+$ in dried residue ($\mu$Eq $K^+$)</th>
<th>Total as KCl ($\mu$g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Cerebral cortex</td>
<td>58.5</td>
<td>1.80</td>
<td>134</td>
</tr>
<tr>
<td>(area 2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2) Cerebellum</td>
<td>74.2</td>
<td>2.05</td>
<td>153</td>
</tr>
<tr>
<td>(vermis)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) Lateral geniculate</td>
<td>52.0</td>
<td>1.53</td>
<td>114</td>
</tr>
<tr>
<td>body</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The amount of $K^+$ eluted from the column probably depended on the weight of the tissue sample. With tissue samples weighing less than 50 mg, the amount of $K^+$ present as excess in the test solution would hardly interfere with the assay of histamine by superfusion. Moreover, as with most samples the test solution required further dilution with the Tyrode's solution, any slight excess of $K^+$ in the test solution would also be diluted. However, it was desirable not to collect too heavy a tissue sample, particularly when the concentration of histamine expected in the sample was very low.
APPENDIX I.e.

FRACTIONAL ELUTION OF SODIUM AND HISTAMINE: pH OF THE FRACTIONS

Adam et al. (1957) had studied the fractional elution of histamine from columns of Amberlite IRC-50 in bead form but not from composite columns as used in the present investigation. It was therefore desirable to make a detailed study of the elution process. Fractions of the eluate were studied for pH and their content of histamine and sodium.

Experiments with buffer solution. Four columns were prepared (Columns 1 - 4) for adsorption (S. 2/3/3). 100 ng of histamine was added to 10 ml buffer solution (pH 8, 100 mEq/1 Na+) and this was applied to Column 3. 10 ml of the same buffer solution alone was applied to each of the other three columns. After the adsorption and wash steps (S. 2/3/3), the eluate (2 ml 0.25 N HCl + 4 ml water) from Columns 1, 2 and 3 was collected as six fractions of 1 ml each. Eluate from Column 4 was collected as a whole (6 ml).

Column 1: Fractions (1 ml) were used to measure pH.

Column 2: Fractions (1 ml) were used to determine Na+ content.

*The volume of eluate in this experiment was increased by 1 ml of water.
**Column 3:** Fractions from this column were used to estimate histamine content. Assuming that the same amount of NaCl was present in similar fractions from two columns (Columns 2 and 3), the corresponding fractions from Column 3 were restored to contain 23.5 mg of NaCl, on the basis of Na⁺ found in the fractions of Column 2. This was done by adding the required volume of a NaCl solution (containing 5.7 mg/ml NaCl) to each of the fractions of Column 3. The solutions were then evaporated, treated with 6 N HCl and prepared for bioassay as described in the method. Histamine present in the fractions was estimated by bioassay (S. 2/4).

**Column 4:** This was a control. 6 ml eluate was dried and after heating with 6 N HCl, prepared for bioassay (S. 2/3). This was assayed with the fractions from Column 3.

After the assay, the Na⁺ concentration was measured in the test solutions from Columns 3 (fractions) and 4. The values were compared with that for Tyrode's solution.

The result is shown in Table 15/1. The Na⁺ content of the fractions of Column 2 are also shown, calculated as NaCl.
Table 15/1

Fractional elution of sodium and histamine: pH of the fractions
(Adsorption from buffer pH 8.0; 0.05 molar; elution
with 2 ml 0.25N HCl followed by 4.0 ml water)

<table>
<thead>
<tr>
<th></th>
<th>Column 1 pH</th>
<th>Column 2 Na⁺ (NaCl) (mg)</th>
<th>Column 3 Histamine (Na⁺ in test solution) (μEq/ml)</th>
<th>Column 4 (control) Histamine (Na⁺ in test solution) (μEq/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st ml</td>
<td>9.10</td>
<td>0.5 (0.03)</td>
<td>nil</td>
<td>(150)</td>
</tr>
<tr>
<td>2nd ml</td>
<td>7.54</td>
<td>78.5 (4.55)</td>
<td>nil</td>
<td>(150)</td>
</tr>
<tr>
<td>3rd ml</td>
<td>4.72</td>
<td>215.0 (12.55)</td>
<td>3.0</td>
<td>(152)</td>
</tr>
<tr>
<td>4th ml</td>
<td>1.16</td>
<td>105.0 (6.15)</td>
<td>87.0</td>
<td>(150)</td>
</tr>
<tr>
<td>5th ml</td>
<td>2.74</td>
<td>0.18 (0.01)</td>
<td>1.25</td>
<td>(148)</td>
</tr>
<tr>
<td>6th ml</td>
<td>4.05</td>
<td>0.10 (0.006)</td>
<td>nil</td>
<td>(150)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>399.3 (23.3)</td>
<td>91.3</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>

N.B. Na⁺ concentration in Tyrode's solution was 145 μEq/ml

Total histamine recovered = 91.3 ng out of 100 ng added

Dead space in the capillary and tap of the tube below the column (Fig. 3) = 0.3 ± 0.05 ml
**Experiments with tissue extracts.** In a parallel experiment (Columns 5 - 8), tissue extract was used for adsorption instead of buffer solution as above. Four tissue samples from the posterior ectosylvian area of cerebral cortex were extracted separately with TCA. An aliquot of 4.6 ml was taken from each supernatant and pooled. The pooled aliquot was neutralized and prepared for adsorption (8.2/3/3). The buffered solution was made up to a final volume of 32 ml. This was divided into four equal parts of 8.0 ml each. 100 ng of histamine was added to one part and, after mixing, the solution was applied to Column 7. The other parts were applied to Columns 5, 6 and 8.

The eluate from Columns 5 - 7 was collected as fractions (6 x 1 ml); that from Column 8 was collected as a whole (6 ml). As in the previous experiment (see above), the fractions from different columns were tested for pH (Column 5), Na⁺ content (Column 6) and for the recovery of the histamine (Column 7). The eluate from Column 8 was used to estimate histamine originally present in one part of the pooled sample. The Na⁺ concentration was measured in the test solutions from Columns 7 and 8, after their bioassay. The values were compared with that for Tyrode's solution.
Table 15/2

Fractional elution of sodium and histamine: pH of the fractions

(Adsorption from tissue extract; elution with 2 ml 0.25N HCl followed by 4.0 ml water)

<table>
<thead>
<tr>
<th>Column 5 pH</th>
<th>Na(^+) µEq</th>
<th>(NaCl) (mg)</th>
<th>Column 7 Histamine (Na(^+) in test solution) (µEq/ml)</th>
<th>Column 8 (control) Histamine (Na(^+) in test solution) (µEq/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st ml</td>
<td>9.06</td>
<td>0.66 (0.04)</td>
<td>nil (150)</td>
<td></td>
</tr>
<tr>
<td>2nd ml</td>
<td>7.10</td>
<td>64.0 (3.74)</td>
<td>nil (155)</td>
<td></td>
</tr>
<tr>
<td>3rd ml</td>
<td>5.22</td>
<td>220.0 (12.85)</td>
<td>1.5 (145)</td>
<td></td>
</tr>
<tr>
<td>4th ml</td>
<td>1.18</td>
<td>122.5 (7.15)</td>
<td>87.0 (150)</td>
<td></td>
</tr>
<tr>
<td>5th ml</td>
<td>2.88</td>
<td>0.64 (0.04)</td>
<td>1.25 (150)</td>
<td></td>
</tr>
<tr>
<td>6th ml</td>
<td>4.00</td>
<td>0.08 (0.005)</td>
<td>nil (145)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>407.9 (23.8)</td>
<td>89.75</td>
<td>3.75 (145)</td>
<td></td>
</tr>
</tbody>
</table>

N.B. Na\(^+\) concentration in the Tyrode's solution was 145 µEq/ml

Total histamine recovered was 89.75 - 3.75 = 86.0 ng out of 100 ng added.

Dead space in the capillary and tap of the tube below the column (Fig. 3) = 0.3 ml + 0.05 ml
The result is shown in Table 15/2. The result has been arranged in the same order as in Table 15/1.

Thus nearly the whole of the histamine recovered in both the experiments was present in the 4th ml fraction of the eluate. The Na+ present in the eluate was found in the 2nd, 3rd and 4th ml fractions but most of it was present in the 3rd and 4th ml fractions. The Na+ concentration in the fractions reconstituted for bioassay was close to that of Tyrode's solution.
APPENDIX I.f.

POTENCIES OF β-N-METHYLHISTAMINE, β-N-DIMETHYLHISTAMINE AND 1,4-METHYLHISTAMINE AS COMPARED WITH HISTAMINE

The biological activity of these compounds was compared with that of histamine on the superfused guinea-pig ileum. This was carried out by 4-point assays (Schild, 1942). In all the assays the high and low doses of histamine were 2.0 and 1.0 ng/ml. Suitable concentrations of the other compounds were prepared in each case, so that their activities were close to those of the histamine standards. The weights of the compounds are expressed as the base.

The activity of these compounds was also tested in the presence of a low concentration of mepyramine (3 ng/ml in the Tyrode's solution).

The results of the experiment were as follows:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Activity as percentage of that of histamine</th>
<th>P 0.99 limits of error of the percentage activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>β-N-methylhistamine</td>
<td>75.8</td>
<td>69.7 to 81.8</td>
</tr>
<tr>
<td>β-N-dimethylhistamine</td>
<td>46.7</td>
<td>41.5 to 52.0</td>
</tr>
<tr>
<td>1,4-methylhistamine*</td>
<td>2.8</td>
<td>2.5 to 3.1</td>
</tr>
</tbody>
</table>

*Source: 1,4-methylhistamine dihydrochloride, m.w. 198, from the laboratories of R.W. Schayer.
The responses to equiactive doses of the four compounds were equally inhibited by mepyramine (3 ng/ml).

The histamine-like activity of 1,4-methylhistamine was not due to any contamination of the substance with traces of histamine. This was confirmed by chromatographic analysis of the substance and subsequent assay. Moreover no apparent potentiation or antagonism between histamine and 1,4-methylhistamine was observed when these were applied together on the ileum. Since 1,4-methylhistamine has a low activity it would not affect the assay of histamine unless it were present in μg quantities.
APPENDIX I. g.

RECOVERY OF \( \beta \)-N-METHYLHISTAMINE, \( \beta \)-N-DIMETHYLHISTAMINE AND 1,4-METHYLHISTAMINE: THEIR ACTIVITIES AFTER HEATING WITH STRONG HCl

The object of this experiment was to determine the fate of these derivatives of histamine under the conditions described in the method for the purification of histamine for bioassay by superfusion. It was desirable to test the possibility of their presence in the purified extract of brain tissue (S. 2/6).

Six columns (1 - 6) were prepared and equilibrated as described in the method (S. 2/3/3). Solutions of histamine and the other compounds were prepared in buffer solution (0.05 molar, 100 mEq/1 Na\(^+\), pH 8.0) for adsorption. This is shown below:

Solution for adsorption:
10 ml buffer (pH 8.0)
containing

<table>
<thead>
<tr>
<th>Column</th>
<th>Compound</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column 1</td>
<td>Histamine</td>
<td>100 ng</td>
</tr>
<tr>
<td>Column 2</td>
<td>( \beta )-N-methylhistamine</td>
<td>100 ng</td>
</tr>
<tr>
<td>Column 3</td>
<td>( \beta )-N-dimethylhistamine</td>
<td>100 ng</td>
</tr>
<tr>
<td>Column 4</td>
<td>1,4-methylhistamine</td>
<td>2.0 ( \mu )g</td>
</tr>
<tr>
<td>Column 5</td>
<td>&quot;</td>
<td>4.0 ( \mu )g</td>
</tr>
<tr>
<td>Column 6 (control)</td>
<td>(10 ml buffer only)</td>
<td></td>
</tr>
</tbody>
</table>
The eluate was taken to dryness, heated with 6 N HCl and the acid was evaporated (S. 2/3/3). The dried residues were assayed for histamine-like activity by superfusion (S. 2/4). Activity found in each case was converted to the weight of the substance concerned, taking into account its specific potency as compared with histamine (Appendix I.f.). This value was taken as the recovery of the substance.

The recoveries of the compounds after bioassay was as follows:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Added</th>
<th>Found histamine equivalent (ng)</th>
<th>Recovery corrected for potency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>100 ng</td>
<td>92 ng</td>
<td>92 ng</td>
<td>92</td>
</tr>
<tr>
<td>β-N-methyl histamine</td>
<td>100 ng</td>
<td>67 ng</td>
<td>89 ng</td>
<td>89</td>
</tr>
<tr>
<td>β-N-dimethylhistamine</td>
<td>100 ng</td>
<td>34 ng</td>
<td>72 ng</td>
<td>72</td>
</tr>
<tr>
<td>1,4-methylhistamine</td>
<td>2 µg</td>
<td>4.3</td>
<td>1.53 µg</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>4 µg</td>
<td>8.2</td>
<td>2.93 µg</td>
<td>73</td>
</tr>
</tbody>
</table>

It was concluded that these derivatives of histamine were also adsorbed on the column at pH 8 and that most of their activity, like that of histamine, survived heating in 6 N HCl. The lower recoveries of β-N-dimethylhistamine and 1,4-methylhistamine suggest that these may be
weaker bases than histamine and hence not completely adsorbed at pH 8. However, this could as well be due to the effect of heating with 6 N HCl.
APPENDIX I.h.

CHROMATOGRAPHIC SEPARATION OF HISTAMINE AND RELATED COMPOUNDS

Histamine and some of its related compounds were separated from one another in mixtures by paper chromatography. The solvent system was adopted from Smith (1962) with some modification.

Authentic compounds and their sources

Histamine dihydrochloride: BDH, m.w. 184.08
β-N-methylhistamine dihydrobromide: Eli Lilly, m.w. 286.8
β-N-dimethylhistamine dihydrochloride: Eli Lilly, m.w. 212.1
1,4-methylhistamine dihydrochloride: from the laboratories of R.W. Schayer, m.w. 198.1
β-N-acetylhistamine: Spencer, m.w. 153.18
1-histidine monohydrochloride: BDH, m.w. 191.62

Weights of the compounds given in the text are those of the base.

Stock solutions of compounds for spotting.
1-histidine solution was 200 μg/ml in water. All others were prepared as 200 μg/ml in methanol. Quantity spotted was 2 μg of each compound separately or in mixtures.

Chromatographic conditions. The system developed for the separation of the compounds
(except 1,4-methylhistamine) had the following conditions:

Ascending chromatography

Paper: Whatman No. 1, run at 90° to machine direction; 46 x 22 cm paper cut out to make 4 strips, each 3 cm wide and joined at both ends.

Time of run: 16 - 17 hours

Temperature: 18° - 19°C (at room)

Tank vol.: 52 x 29 x 19 cm

Solvent: iso-Propyl Alcohol 150
Ammonia (sp.gr. 0.88) 8
Water 26

Vol.: 250 ml in trough and 300 ml in tank; total 550 ml.

Paper after the run was dried at 40°C for 10 min.

Location Reagent: Fresh diazotized sulphanilic acid (Pauly, 1904) prepared in the following way:

Solution 1 = 1.25 g of NaNO₂ dissolved in 25 ml of ice-cold water was added slowly to 5 ml sulphanilic acid solution (0.9 per cent in 1 N HCl, w/v), kept on ice. The solution was stirred while being mixed.

Solution 2 = 5 per cent Na₂CO₃ in water (w/v).
The dry chromatogram was sprayed with Solution 1, followed by Solution 2, while still moist.

Fig. 13 shows the separation of the compounds on the chromatogram. The Rf values of acetylhistamine and N-methylhistamine were very close and they were not clearly separated in mixtures. However, histamine was clearly separated from the two biologically active imidazoles, namely \( \beta \)-N-methyl and \( \beta \)-N-dimethyl histamine.

The mean Rf values obtained for the different compounds from several observations are as follows:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mean Rf</th>
<th>Range</th>
<th>No. of observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>0.41</td>
<td>0.40 - 0.43</td>
<td>10</td>
</tr>
<tr>
<td>( \beta )-N-methylhistamine</td>
<td>0.57</td>
<td>0.56 - 0.58</td>
<td>7</td>
</tr>
<tr>
<td>( \beta )-N-dimethylhistamine</td>
<td>0.69</td>
<td>0.68 - 0.70</td>
<td>7</td>
</tr>
<tr>
<td>1-histidine</td>
<td>0.08</td>
<td>0.07 - 0.09</td>
<td>8</td>
</tr>
<tr>
<td>( \beta )-N-acetylhistamine</td>
<td>0.60</td>
<td>all 0.60</td>
<td>4</td>
</tr>
</tbody>
</table>

1,4-methylhistamine is not separated from histamine in the above system. Day and Green (1962a) described a method of chromatographic separation of the two compounds. In the present work, these two substances were separated from
Fig. 13. Chromatographic separation of histamine and other imidazoles from mixtures. Ascending chromatography was on Whatman no. 1 paper in iso-Propyl alcohol/ammonia/water. The arrow shows the direction of run. Substances in mixture (2 µg of each) were spotted on the areas marked with crosses. The solvent front (S.F.) is shown by the lines at the top each strip. The substances separated were: Hd, histidine; H, histamine; M, β-N-methylhistamine; A, acetylhistamine; D, β-N-dimethylhistamine. The three pharmacologically active imidazoles are shown separated on the strip on the extreme right.
one another in mixtures, by a modification of the system already described. The following changes were necessary for the separation of histamine from 1,4-methylhistamine:

**Solvent:** iso-Propyl alcohol 160
Chloroform 30
Ammonia (0.88) 16
Water 16

**Location reagent:** 1,4-methylhistamine cannot be located with the diazo reagent of Pauly. Ninhydrin, 0.2 per cent in acetone was used for locating. The dry chromatogram was sprayed with the reagent and kept in the oven at 50°C for 10 min. The bluish violet spots with the ninhydrin solution fade away with time.

Rf values of histamine and 1,4-methylhistamine in this modified system were as follows:

Histamine 0.40
1,4-methylhistamine 0.51
SUMMARY

1. Information on the occurrence and metabolism of histamine in the brain and hypophysis is reviewed.

2. Histamine was estimated by a method which depended on purification of the amine by ion-exchange chromatography and on biological assay. Further work was done to establish the specificity of the method.

3. A detailed map is given of the distribution of histamine in the cat's brain and hypophysis. The highest values for the concentration were found in the hypophysis, where part of the histamine derives from mast cells. Mast cells occur mainly in the posterior lobe and in the stalk; mast cells were not found in the hypothalamus.

4. In the brain, the highest concentrations of histamine were found in the corpora mammillaria and ventral part of the hypothalamus. Thereafter the concentration fell progressively in the direction of the dorsal part of the hypothalamus, thalamus and midbrain. None was detected in the cerebellum or in the white matter of the brain.
5. Various drugs were tested for their effect on the concentration of histamine in the hypophysis and adjoining parts of the brain. The drugs were chosen for their known actions on the storage and metabolism of histamine or other pharmacologically active amines.

6. Compound 48/80, in doses which depleted the skin histamine, reduced the concentration of histamine in the posterior lobe and in the hypophysial stalk, but not in the anterior lobe or in the brain.

7. After single doses of reserpine the histamine concentration fell in the brain but not in the hypophysis. The effect of reserpine was independent of dose in the range 0.5 to 10 mg/kg. After a single dose of 0.5 mg/kg, the effect was maximal at 18 hours and the recovery of the histamine concentration in the hypothalamus required several days.

8. Large doses of chlorpromazine and other similar phenothiazines increased the concentration of histamine in the brain but not in the hypophysis.
9. After iproniazid the histamine concentration in the hypothalamus was increased. After pargyline, a non-hydrazine type MAO inhibitor, the rise in concentration was seen only in the corpora mammillaria.

10. Histamine concentration in the brain and hypophysis was not altered to any great extent after bulbocapnine, morphine, pentobarbitone or adrenaline. The results of the experiments with these drugs were inconclusive owing to the small number of cats used.

11. The results are discussed and compared with our knowledge of the distribution of histamine and its metabolism in the brain. Possible mechanisms of the action of the drugs are also discussed.
REFERENCES


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