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THE SUBCELLULAR DISTRIBUTION OF HISTAMINE, SLOW-REACTING SUBSTANCE AND 5-HYDROXYTRYPTAMINE IN THE BRAIN OF THE RAT

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Various extracts of brain were prepared with dilute and concentrated acid, acetone and *n*-butanol. When the extracts were applied to the guinea-pig isolated ileum, they produced a slow contraction which was not prevented by an antihistamine ; in addition, the acid and acetone extracts inhibited the action of histamine. Histamine in extracts of brain was separated from other pharmacologically active substances by chromatography on a carboxylic-acid resin and estimated biologically. The bulk of this histamine was found in small particulate material, whereas slow-reacting substance was found in particulate material of greater density. 5-Hydroxytryptamine was in both the small and large particles. There was no relationship between the distribution of these substances and that of succinic dehydrogenase activity. The measurement of histamine in brain both by biological assay on the guinea-pig ileum and by chemical assay using a fluorimetric procedure gave mean values of 53 and 246 ng/g of wet tissue, respectively. The high values obtained by the chemical assay are attributable in part to substances other than histamine which become fluorescent after reaction with *o*-phthalaldehyde in this procedure.

The regional distribution of histamine in brain is similar to that of 5-hydroxytryptamine and noradrenaline (Adam, 1961). The purpose of the present experiments was to see whether or not histamine occurs in particulate material of brain ; this material contains most of the 5-hydroxytryptamine, acetylcholine (Whittaker, 1959) and noradrenaline (Chruściel, 1960) in brain. Accordingly, the subcellular distribution of histamine has been compared with that of 5-hydroxytryptamine. In addition, the subcellular distribution in brain of a slow-reacting substance is described, together with some of the characteristics of the substance ; this part of the work arose from difficulties encountered in the separation of histamine from the slow-reacting substances. Finally, the fluorimetric and biological methods for measuring histamine in brain are compared. A preliminary report of this work has appeared (Green, Furano & Carlini, 1962).

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METHODS

Fractionation of brain

All procedures were carried out at 0 to 4° C. In each experiment, brains of three or four rats, killed by decapitation, were pooled, homogenized with a Teflon pestle in 0.32 M-sucrose, and centrifuged by a method nearly identical with that of Whittaker (1959). The whole homogenate was centrifuged at 1,200 *g* for 20 min to yield a particulate fraction, P-1, which contained nuclei, debris, and unbroken cells, and a cloudy supernatant material, S-1. This supernatant fraction, containing large and small particulate material as well as soluble substance, was centrifuged at 10,300 *g* for 40 min to yield a deposit of large particulate material, P-2, and a cloudy supernatant material, S-2. Centrifugation of S-2 at 100,000 *g* for 60 min produced another deposit, P-3, containing small particulate material, and a clear supernatant material, S-3. In some experiments, S-1 was centrifuged for 60 min at 100,000 *g* to obtain both large and small particulate materials in one fraction, P-2 and P-3. In other experiments, P-2 was suspended in 0.32 M-sucrose and layered over a density gradient of sucrose, 0.8 to 1.2 M. The interfaces between these sucrose layers were marked on the tube, in order that the original boundaries could be identified after centrifugation. Centrifugation was carried out in the SW-25 rotor in a Spinco preparative ultra-centrifuge for 1 hr at 90,000 *g*; at the end of this time the suspension of particulate material had resolved into separate bands. A diagram was made of each tube, which was then cut at various levels with a Spinco tube-slicer to allow separate removal of the different fractions (referred to as F-1 to F-6, see Fig. 7).

Extraction of substances from homogenates of brain and its fractions for bioassay

Several methods were used:

(a) Dilute acid. The tissue was kept for 1 hr at room temperature in 0.1 N-HCl, after which the mixture was centrifuged and the supernatant material neutralized.

(b) Boiling dilute acid. The tissue was boiled for 3 min in 0.1 N-HCl, centrifuged and the supernatant material neutralized, as with *(a)*.

(c) Acetone. The tissue was extracted with twenty volumes of acetone by the method described for the extraction of 5-hydroxytryptamine (Amin, Crawford & Gaddum, 1954).

(d) Boiled dilute acid-acetone. The dilute acid extract *(a)* was boiled for 1 hr, centrifuged, and the soluble material was extracted three times with two volumes of diethyl ether. The aqueous and ether extracts were separately evaporated *in vacuo*. The residue of the aqueous extract was suspended in 20 ml. of 70% acetone; after removal of the residue by centrifugation, the acetone was evaporated *in vacuo* from the clear solution.

(e) n-*Butanol.* The method used to extract histamine for fluorimetric assay (Shore, Burkhalter & Cohn, 1959) was also used to extract histamine for bioassay. The final acid extract was concentrated *in vacuo* and neutralized.

(f) Ion exchange chromatography. Protein was removed from the homogenates with 5% trichloroacetic acid or 0.5 N-perchloric acid, and the supernatant material obtained after centrifugation was extracted with *n*-butanol, as recommended by Shore *et al.* (1959). Without extraction into acid, the butanol extract, *p*H 6.0, was allowed to percolate through a cation-exchange column, Amberlite CG-50, Type 2, which had been previously washed (Hirs, Moore & Stein, 1953). The column, 0.8×10 cm, was washed with a volume of water five times the volume of resin, and eluted with five volumes of 0.1 N-HCl and five volumes of 0.5 N-HCl. The eluates were evaporated *in vacuo*, suspended in Tyrode solution, and assayed.

(g) Ion exchange chromatography. The procedure of Adam (1961) was followed with only minor modifications. Tissue was extracted in 5% trichloroacetic acid, brought to *p*H 7.9 with 0.4 M-Na_2HPO_4 without using Neutral Red, and chromatographed on 4 ml. of Amberlite CG-50, Type 2, without cellulose. The column was washed with 20 ml. of water and eluted with 20 ml. of 0.25 N-HCl followed by 10 ml. of water. The last two fractions were pooled, dried *in vacuo*, and 4 ml. of 6 N-HCl was added to the residue. The solution was placed in a boiling water bath for 30 min and dried at 50° C *in vacuo*, a procedure that also removed

excess acid. In this procedure, a known solution of histamine was run concomitantly to permit compensation for loss of histamine and for any effects of residual inorganic salts on the guinea-pig ileum.

Extraction of 5-hydroxytryptamine

The method of Amin *et al.* (1954) was used.

Determination of substances

Histamine, slow-reacting substances and 5-hydroxytryptamine. Histamine was measured by a fluorimetric method (Shore *et al.*, 1959); the tissue blank was compensated for by adding *o*-phthalaldehyde to an acid extract, as described by these authors. Histamine was also measured by bioassay with guinea-pig ileum that was bathed at 35° C in a 5 ml. chamber with aerated Tyrode solution to which atropine (6 ng/ml.) and lysergic acid diethylamide (50 ng/ml.) were added. The slow-reacting substance was measured with the guinea-pig ileum under the same conditions; units were expressed in equivalents of histamine. 5-Hydroxytryptamine was measured with the isolated heart of *Venus mercenaria* suspended in a 5 ml. chamber and bathed with aerated natural sea-water at room temperature. All values of histamine and 5-hydroxytryptamine are expressed as the free base.

Succinic dehydrogenase. A spectrophotometric method (Slater & Bonner, 1952) was used.

Paper chromatography. Whatman 3 MM paper and a solvent system consisting of isopropanol:water:ammonium hydroxide (100:10:15) were used. The dried paper was dipped first into a 0.2% solution of *o*-phthalaldehyde in acetone and, after drying, into a 1% solution of potassium hydroxide in 95% ethanol; this method yields coloured and fluorescent products with several amino acids (Smith, 1960).

RESULTS

Measurement of histamine and slow-reacting substance by bioassay

A dilute acid extract of whole brain contracted the guinea-pig ileum. This activity (Fig. 1), although present in all fractions, was greatest in the F-4 fraction, obtained by density-gradient centrifugation of the large particulate material of brain. The

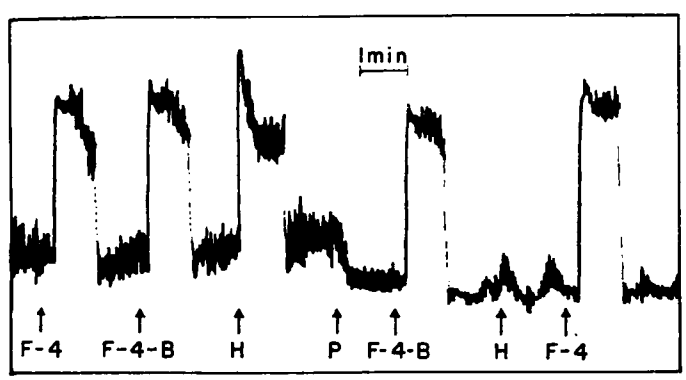

Fig. 1. Responses of guinea-pig ileum to histamine (15 ng at H), to 0.25 ml. of fraction F-4 of brain homogenate extracted with 0.1 N-HCl (at F-4), and to 0.25 ml. of this fraction boiled for 3 min (at F-4-B), before and after addition of mepyramine (0.1 μg at P).

activity of this extract was not decreased by boiling and was not blocked by the antihistamine, mepyramine; it differed from that of histamine in another respect, since the contractions began after a latent period of 10 to 20 sec, whereas histamine had an almost instantaneous effect (Fig. 1). Other experiments disclosed even more

clearly the slow-reacting activity of these extracts. In two other experiments, the unboiled extract slightly inhibited the action of histamine.

Fig. 2 shows the slow-reacting activity of an acetone-extract of F-4 which was not blocked by mepyramine. Other fractions, obtained in the density-gradient centrifugation of P-2, had only weak activity.

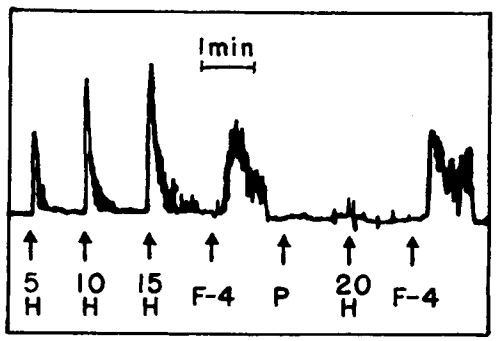

Fig. 2. Responses of the guinea-pig ileum to histamine (at H) and to 0.25 ml. of fraction F-4 of brain homogenate extracted with acetone (at F-4), before and after addition of mepyramine (0.1 μg at P). The doses of histamine are expressed in ng.

The same procedure of extraction with acetone (Amin *et al.*, 1954), when applied to lung, extracted over 90% of the histamine, since further extraction of the acetone residue with 0.1 N-HCl yielded only small additional amounts of histamine. The slow-reacting substance was not detected in lung.

Fig. 3 shows the effect of an acid-acetone extract of brain: it not only contracted the ileum but also inhibited the contraction due to histamine, whether the acid-acetone extract was boiled or unboiled. The ether-extract of the acid-extract was completely inactive. The duration of the inhibitory effect was proportional to the concentration of the extract: after the addition of brain extract diluted 1:2 (E/2)

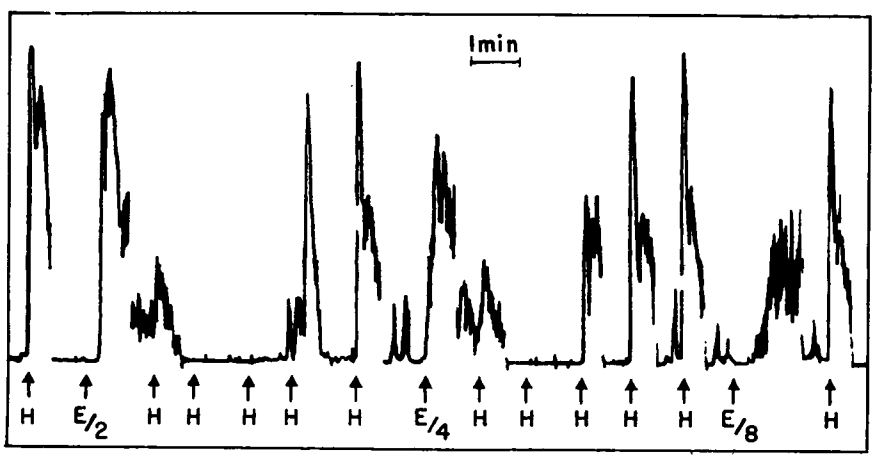

Fig. 3. Responses of the guinea-pig ileum to histamine (30 ng at H) and to 0.25 ml. of a boiled extract of whole brain homogenate in dilute acid and acetone, diluted two-, four- and eight-fold respectively at E/2, E/4 and E 8.

the response to histamine could not be elicited for 12 min and was not fully recovered for 14 min ; brain extract diluted 1:4 (E/4) prevented the action of histamine for 9 min and at a dilution of 1/8 (E/8) for, at most, 2 min.

If all the contractile effects of this whole brain extract were calculated as if due to histamine, the concentration of the active substance in brain would be equivalent to 253 ng/g. However, unlike histamine, the extract was active after the ileum had been treated with mepyramine, and showed a latent period between the application of the dose and the onset of response. The duration of the latent period and the speed of attaining maximal response depended on the dose. These observations suggested that in high concentrations slow-reacting substances appear to act rapidly, and to mimic histamine.

That slow-reacting substances are indeed capable of inducing an apparently rapid reaction, if present in sufficiently high concentrations, is shown in Fig. 4. Synthetic bradykinin had slow-reacting activity when 20 ng was added to the bath ; 40 ng acted less slowly, and 80 ng caused a relatively rapid response.

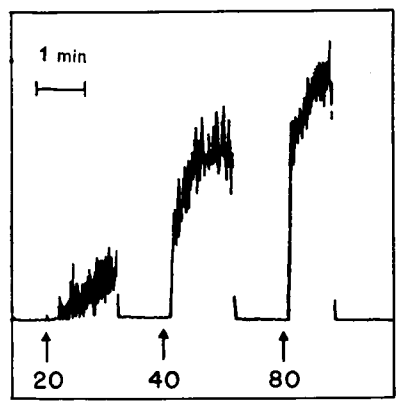

Fig. 4. Responses of the guinea-pig ileum to synthetic bradykinin (20, 40 and 80 ng).

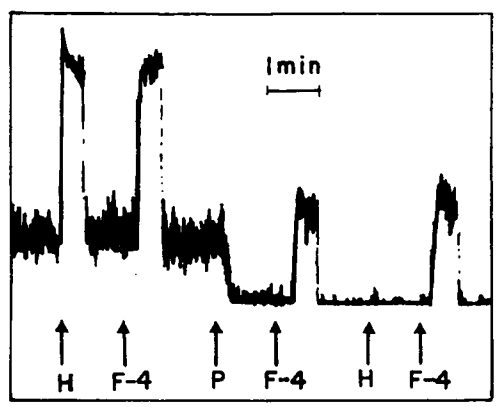

Fig. 5. Responses of the guinea-pig ileum to histamine (10 ng at H) and to 0.25 ml. of an extract in *n*-butanol of fraction F-4 of brain homogenate (at F-4), prepared by the method used for the fluorimetric assay of histamine (Shore *et al.*, 1959), before and after addition of mepyramine (0.2 μg at P).

Fig. 5 shows the effect of a butanol-extract of brain, prepared as for the fluorimetric assay (Shore *et al.*, 1959). The extract contracted the ileum, but this effect differed from that due to histamine in having a latent period of action and in being only partially diminished by mepyramine in a dose (0.2 μg) higher than usual and which completely prevented the action of histamine.

After chromatography of the butanol extract on a cation exchange column, biological activity was found in both acidic eluates. However, the results of the experiments were inconsistent: in some experiments, the slow-reacting substance was present; in others, only histamine, as inferred from the abolition of activity by mepyramine; in yet others, the extract relaxed the ileum. Material that inhibited the action of histamine on the gut was not detected in the eluate.

Fig. 6 shows the action of a brain fraction prepared by the method of Adam (1961). This extract had an action of rapid onset which was completely blocked by mepyramine, thus resembling histamine in both characteristics. As may be seen,

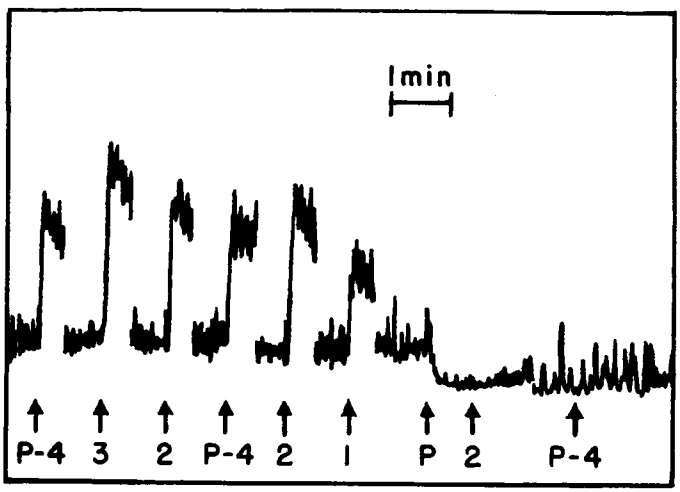

Fig. 6. Responses of guinea-pig ileum to histamine at the numbers (which correspond to the doses in ng) and to 0.25 ml. of an extract of fraction P-4 of brain, prepared by the chromatographic method of Adam (1961), before and after addition of mepyramine (0.1 μg at P).

slow-reacting material was not detectable in this extract, which also failed to inhibit the action of histamine. With this method, the concentration of histamine in whole brain was shown to be 53.5 ± 5.2 ng/g.

Measurement of histamine in brain by the fluorimetric method

Histamine in brain was also measured by the fluorimetric procedure, in which the high tissue-blank of brain was compensated for in the manner recommended (Shore *et al.*, 1959); a mean value of 246 ± 13.5 ng/g of brain was obtained. To learn whether these high levels could be attributed to the presence in brain of substances other than histamine which react with *o*-phthalaldehyde to yield fluorescent material, the final butanol extract from twenty rat brains (33.5 g), prepared by the method of Shore *et al.* (1959), was examined. The extract was adjusted

to 6.0 and passed through a column (2 × 7 cm) of Amberlite CG-50, Type 2, in an effort to remove some of the inorganic salts that impede the resolution of substances by paper chromatography. The column was then washed with 150 ml. of water; the effluent and the washing water were combined. Elution was carried out with 150 ml. of 0.25 N-HCl, followed by 150 ml. of 0.5 N-HCl. The combined effluent and washing water were evaporated *in vacuo*, as were the separate acid-eluates. The residues were dissolved in 3 ml. of 70% ethanol, and the alcohol-extracts concentrated to 0.2 ml. Paper chromatography was carried out with this material and, after the paper had dried, it was allowed to react with *o*-phthalaldehyde. Fluorescent materials were disclosed with R_F values of 0.02, 0.06, 0.25 and 0.47 (histamine) in the effluent-water fraction; with 0.03, 0.23, 0.25 and 0.46 in the 0.25 N-HCl-fraction; and with 0.05 and 0.30 in the 0.5 N-HCl-fraction. Thus, in one solvent system at least five substances that give fluorescent compounds with *o*-phthalaldehyde were demonstrable in the extract used to measure histamine in brain. No attempt was made to identify these substances.

Subcellular distribution of histamine, slow-reacting substance and 5-hydroxytryptamine in brain

Histamine in whole brain and its fractions was measured by bioassay after extraction by the chromatographic method (Adam, 1961). Table 1 shows that the major portions of both histamine (62.5%) and 5-hydroxytryptamine (54.9%) were in a

TABLE 1

THE CONCENTRATION AND PERCENTAGE DISTRIBUTION OF HISTAMINE AND 5-HYDROXYTRYPTAMINE IN THE BRAIN OF THE RAT

The values with the standard deviations are given and, in parentheses, the number of experiments, each of which included three or four brains. Both 5-hydroxytryptamine and histamine were measured by biological assay, the latter after extraction by the method of Adam (1961)

Fractions	Histamine	5-Hydroxytryptamine
Whole brain (ng/g/wet weight)		
Biological assay	53±5·2 (5)	279±170·2 (6)
Chemical assay	246±13·5 (3)	—
Percentage in P-1 (nuclei and debris)	17·6±7·0 (8)	6·1±3·2 (5)
Percentage in P-2+P-3 (large+small particles)	62·5±7·6 (8)	54·9±8·3 (5)
Percentage in S-3 (soluble material)	19·9±6·5 (8)	39·0±6·2 (5)

fraction containing both large and small particulate material. In one experiment the same amines from guinea-pig brain showed the same distribution. It should also be noted that the levels of 5-hydroxytryptamine in brain varied widely, as indicated by the standard deviation, but the distribution showed only slight variation.

When the small were separated from the large particles, the greatest portion (44.3%) of the histamine was found in P-3, the fraction containing the small particles (Table 2). When fraction P-3 was subjected to centrifugation in a density-gradient, the particles containing histamine were found in fraction F-2, which is at the interface between 0.3 and 0.8 M-sucrose (Fig. 7).

The distribution of 5-hydroxytryptamine was not so clearly defined. In all experiments, fraction P-2 contained the greatest portion of 5-hydroxytryptamine, and when

TABLE 2

DISTRIBUTION OF HISTAMINE IN FRACTIONS OF BRAIN HOMOGENATES

Values are percentages of the total histamine extracted for each group of four rats. The weights are the mean for each group

Mean weight of rats (g)	Sex	P-1 (nuclei and debris)	P-2 (large particles)	P-3 (small particles)	S-3 (soluble material)
335	M	13·0	23·3	51·9	11·7
340	F	15·4	23·0	38·6	23·0
115	M	22·2	17·7	43·4	16·6
155	F	14·4	15·4	37·5	32·7
200	M	11·8	19·9	48·7	19·6
340	M	23·5	18·1	44·5	13·9
365	M	30·4	9·2	41·1	19·3
315	F	10·0	18·8	48·4	22·8
Mean ± s.d.		17·6 ± 7·0	18·2 ± 4·4	44·3 ± 5·1	19·9 ± 6·5

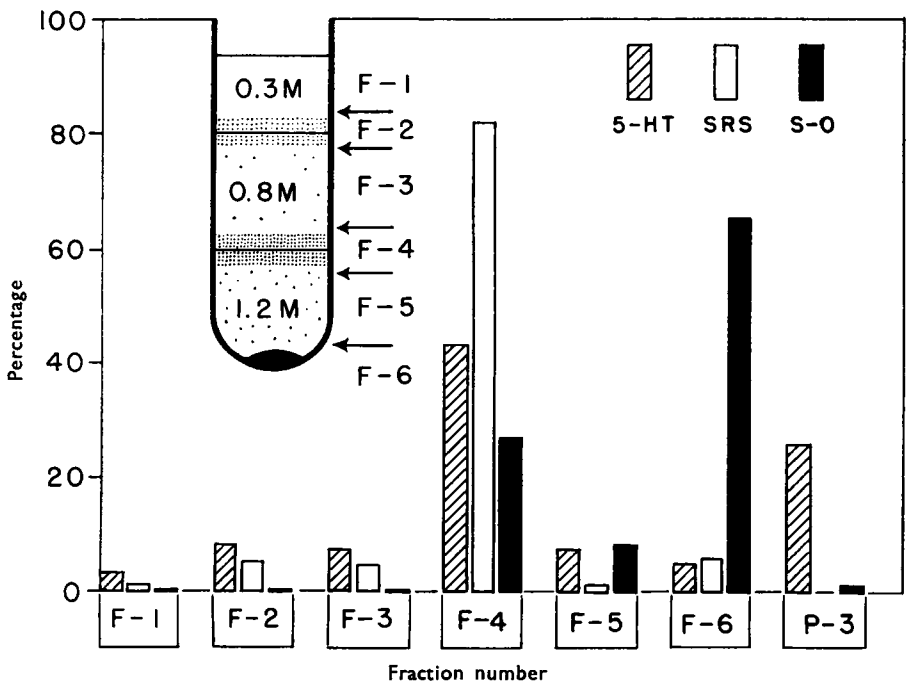

Fig. 7. Distribution of 5-hydroxytryptamine (5-HT), slow-reacting substance (SRS) and succinic dehydrogenase (S-O) in the particulate material of the brain. Fractions F-1 to F-6 are sub-fractions of P-2, the large particulate material. P-3 is the fraction containing small particulate material. Results are calculated as percentages present in particulate material.

this fraction was further fractionated by density-gradient centrifugation, the greatest portion of the 5-hydroxytryptamine was found in fraction F-4 (Table 3), which sedimented at the interface between 0.8 and 1.2 M-sucrose. There was a reciprocal relationship between the percentage of 5-hydroxytryptamine in fractions F-4 and P-3. The distribution of 5-hydroxytryptamine was not related to the total amount of 5-hydroxytryptamine or to the amount present in fraction P-1, which contained nuclei, debris, and unbroken cells (Table 3).

TABLE 3

DISTRIBUTION OF 5-HYDROXYTRYPTAMINE IN PARTICULATE MATERIAL OF BRAIN HOMOGENATES

Values are percentages of the total 5-hydroxytryptamine extracted for each group of four male rats. The weights are the means of each group

Mean weight of rats (g)	Total 5-hydroxy-tryptamine (ng/g wet weight of brain)	Percentage of 5-hydroxytryptamine in fractions						
		P-1	F-1	F-2	F-3	F-4	F-5	F-6	P-3
285	417	8·5	5·6	10·8	7·8	24·2	1·0	3·5	38·6
317	260	3·9	3·7	7·4	6·1	58·0	0·0	3·9	17·0
345	122	11·1	1·6	7·6	4·6	22·0	17·4	5·7	30·0
308	202	18·4	1·2	3·2	7·8	51·1	6·2	3·1	9·0
371	222	14·2	2·6	4·6	7·7	33·2	8·1	7·4	22·2

Fig. 7 shows the average distribution of 5-hydroxytryptamine in fractions P-2 and P-3, as well as the distribution of succinic dehydrogenase and of the slow-reacting substance. Fraction F-4 contained a greater average percentage of 5-hydroxytryptamine than did fraction P-3. There was no relationship between the distribution of 5-hydroxytryptamine and that of succinic dehydrogenase, of which 65.5% was found in fraction F-6.

Over 60% of the slow-reacting substance was present in fraction F-4. In two experiments, slow-reacting substance had been extracted with butanol by the method of Shore *et al.* (1959), in three with acetone (Amin *et al.*, 1954) and in four with dilute acid. The distributions were the same by the three methods, and the results shown in Fig. 7 are the averages of the nine experiments.

DISCUSSION

The measurement of histamine in the brain of the rat by bioassay after extraction by the method of Adam (1961) gave a mean value of 53 ng/g, compared with a mean of 246 ng/g obtained by the fluorimetric method of Shore *et al.* (1959). These authors obtained similar values in brains of rat, guinea-pig, rabbit and dog with this fluorimetric method. The lower levels with Adam's method can be partially explained by recoveries of only 68% of the total histamine (Adam, 1961), but the greater part of the discrepancy seems to be due to the high values obtained by the fluorimetric procedure. The high values are attributable to the presence in extracts of brain of substances other than histamine which yield fluorescent compounds after reaction with *o*-phthalaldehyde. These substances were demonstrated by paper chromatography of an extract of brain prepared by the method of Shore *et al.* (1959). No attempts have been made to identify them, but it is well known that many amines react with *o*-phthalaldehyde (see Shore *et al.*, 1959 ; Smith, 1960). In its present form the fluorimetric procedure for measuring histamine appears to be inadequate for brain and, as pointed out by Graham (1961), for plasma and liver, although it is suitable for the measurement of histamine in some other tissues (Green & Furano, 1962).

The presence of interfering substances probably explains the conflicting reports concerning the distribution of histamine in brain. Using the fluorimetric procedure, Shore *et al.* (1959) concluded that histamine was uniformly distributed in the brain, whereas biological methods of estimation have indicated that histamine is localized in specific portions of the brain (Kwiatkowski, 1943 ; Harris, Jacobsohn & Kahlson, 1952 ; Adam, 1961). Values in the cerebral cortex of several species (Kwiatkowski, 1943 ; Adam, 1961) are similar to that of 53 ng/g obtained by us on the whole brain, using bioassay. Similarly, Harris *et al.* (1952) obtained values of less than 100 ng/g for guinea-pig whole brain and less than 30 ng/g for cat whole brain. Others, however, using bioassay with whole brain, have reported higher values. Thus, Strengers & Maas (1956) obtained with one guinea-pig brain a value of 200 ng/g, similar to the values obtained by West (1957), Shore *et al.* (1959) and Crossland (1961) by bioassay of brains of cat, rabbit, dog, guinea-pig and rat ; Clouet, Gaitonde & Richter (1957) obtained values as high as 6,800 ng/g of rat brain. Cicardo & Stoppani (1949) measured depressor activity of extracts on arterial blood pressure and obtained values of 17,300 ng/g.

The tracings presented in Figs. 1 to 5 show that extracts of brain prepared by a variety of procedures contained a slow-reacting substance which interfered with the bioassay of histamine. The action of the slow-reacting substance may have been responsible for the apparently high values of brain histamine recorded in some of the other laboratories, for in high concentrations the slow-reacting substance mimics the action of histamine by acting relatively fast (Fig. 3), as can bradykinin, a known slow-reacting material (Fig. 4). In fact, if one attributes all the biological action of the extracts (for example, Fig. 3) to histamine, then one obtains values for histamine (250 ng/g) like those obtained by Shore *et al.* (1959) by bioassay, and equal to those obtained by the fluorimetric method (Shore *et al.*, 1959 ; Table 1) ; but in our experiments the extract of Shore *et al.* (1959) clearly showed slow-reacting activity (Fig. 5). Only after chromatography of the extract on a carboxylic acid resin and heating the eluate in acid as described by Adam (1961) was the slow-reacting material removed, thus permitting the unequivocal demonstration of histamine by its rapid action which was prevented by an antihistamine (Fig. 6).

The agreement between the bioassay and the fluorimetric assay observed by Shore *et al.* (1959) may be fortuitous. There is no reason to assume that the substances responsible for the high values obtained by bioassay (Fig. 5) and by fluorimetric assay (Table 1) are identical.

No attempt was made to identify the slow-reacting substance in brain extracts. It was not present in lung. It was soluble in dilute or concentrated hydrochloric acid, acetone or butanol, but not in diethyl ether ; it was stable on boiling for 3 min in 0.1 N-HCl. Its solubility in acetone suggests that it is not substance P (see Amin *et al.*, 1954), and its insolubility in ether would appear to rule out its identity with some pharmacologically active lipids (see Green, Robinson & Day, 1961). Similar material has been described before (Andrews, Price & West, 1960).

Some brain extracts possessed antihistamine activity (Fig. 3). It is noteworthy that neither the slow-reacting substance nor antihistamine activity was found in similar extracts of neoplastic murine mast cells (Green & Day, 1960) or of lung.

The nature of the substance or substances with antihistamine activity is unknown. It is almost certainly distinct from the slow-reacting substance, since antihistamine activity was found in acetone and acid extracts but, in contrast with the slow-reacting substance, not in the butanol extract.

The content of 5-hydroxytryptamine in brain was 279 ng/g. Also using the clam heart for assay, Paasonen & Giarman (1958) and Anderson, Markowitz & Bonnycastle (1962) have obtained similar values, as did Kivalo, Rinne & Karinkanta (1961) and Whittaker (1962) using the rat stomach ; the fluorimetric method has given values of 520 to 790 ng/g (Anderson *et al.*, 1962 ; Louttit, 1962 ; Hess & Doepfner, 1961 ; Werdinius, 1962). The 5-hydroxytryptamine concentration varied over a wide range from one brain to another (Table 1). The range of values noted here, 122 to 450 ng/g, has been obtained by others (Paasonen & Giarman, 1958 ; Whittaker, 1959, 1962 ; Anderson *et al.*, 1962 ; Werdinius, 1962) and is much wider than the range observed for histamine (Table 1). Werdinius (1962) used internal standards in measuring 5-hydroxytryptamine in brain and concluded that the large differences between animals were real. Brain is not unique in this respect, for a wide range was also noted in the concentration of 5-hydroxytryptamine in human blood (Green, Paasonen & Giarman, 1957).

Studies on subcellular distribution revealed that the greatest portion of the histamine in brain is in small (" microsomal ") particles, fraction P-3, with relatively little in the large particles, fraction P-2 (Table 2). Recently a different subcellular distribution of histamine in brain was described (Michaelson & Whittaker, 1962b), but in these experiments the fluorimetric method was used to measure histamine. Our finding that histamine in brain is in the " microsomal " fraction contrasts with analogous studies on the distribution of histamine in neoplastic mast cells (Hagen, Barrnett & Lee, 1959 ; Green & Furano, 1962) in which histamine was found in dense particles that sedimented even more rapidly than mitochondria. These observations support the conclusion (Adam, 1961) that the bulk of the histamine in brain is not in mast cells. In mast cells heparin and histamine are in the dense particles containing histamine, whereas in brain sulphomucopolysaccharides and histamine are in the light particles (Robinson & Green, 1962). This association may be fortuitous for, though sulphomucopolysaccharides may play a role in the binding of amines (see Green, 1962), the fact that these sulphur-containing compounds differ from histamine in their regional distribution in brain (Robinson & Green, 1962) would appear to rule out histamine-binding as their primary function.

The subcellular distribution of 5-hydroxytryptamine in brain also differed from that in neoplastic mast cells (Hagen *et al.*, 1959 ; Green & Furano, 1962) and from that in the intestine (Prusoff, 1960). In each of these studies, 5-hydroxytryptamine was found primarily in particles as dense or denser than mitochondria ; in brain 5-hydroxytryptamine was found in fractions less dense than mitochondria (Table 3 ; Fig. 7). The percentage of 5-hydroxytryptamine in total particulate material of brain (Table 1) was similar to that noted by others (Michaelson & Whittaker, 1962a ; Walaszek & Abood, 1959 ; Inouye, Kataoka & Shingawa, 1962 ; Schanberg & Giarman, 1962) and the fraction richest in 5-hydroxytryptamine was, on the average, F-4, a subfraction of the large particulate material (Fig. 7), as Whittaker (1959)

found. The distribution of 5-hydroxytryptamine varied in individual experiments: in some the highest percentage was in fraction F-4, in others in fraction P-3 (Table 3). The relationship between the amounts in these two fractions appears to be reciprocal (Table 3). The amount appearing in fraction P-3 may have been derived from the disruption of particles in fraction F-4 (see DeRobertis, Rodrigues de Lores & Pellegrino de Iraldi, 1962), but this seems improbable since concurrent experiments with histamine showed a consistent distribution in fraction P-3; it is equally unlikely that the paradoxical distribution reflects the extent of homogenization for, if this were so, fraction P-1, which contains unbroken cells, would show a proportionally higher percentage of 5-hydroxytryptamine when fraction F-4 showed little, a relationship that did not obtain (Table 3). The reason for the variable and seemingly reciprocal distribution of 5-hydroxytryptamine beween the small and large particles is not known, but such inter-particulate shifts may be of physiological importance. There is some evidence that the subcellular distribution of 5-hydroxytryptamine in brain homogenates (between particulate and free form) is correlated with drug-induced behavioural changes (Schanberg & Giarman, 1962).

The slow-reacting substance was found in fraction F-4 (Fig. 7). A similar localization has been described for substance P (Inouye & Kataoka, 1962).

Succinic dehydrogenase, which has been taken as an index of mitochondrial activity, was not associated with the slow-reacting substance, 5-hydroxytryptamine or histamine (Fig. 7).

Although the particulate fractions used in the present experiments were not examined by electron microscopy, the method of preparing them followed closely that used in such studies (Gray & Whittaker, 1962; DeRobertis *et al.*, 1962). It is, therefore, possible to suggest by comparison with the results of these studies that fraction F-4 of the present experiments contained pinched-off nerve endings; this fraction yielded the slow-reacting substance and sometimes 5-hydroxytryptamine. Similarly, it may be suggested that fraction P-3 contained synaptic vesicles; this fraction sometimes yielded 5-hydroxytryptamine and always histamine.

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