J. Physiol. (1956) 131, 617–626

THE EFFECT OF DRUGS ON THE AMOUNTS OF SUBSTANCE P AND 5-HYDROXYTRYPTAMINE IN MAMMALIAN BRAIN

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(*Received* 3 *August* 1955)

Among other active principles substance P (v. Euler & Gaddum, 1931) and 5-hydroxytryptamine (HT) are normally present in nervous tissue. The distribution of substance P in the nervous system of mammals has recently been studied by several investigators (Pernow, 1953; Lembeck, 1953; Kopera & Lazarini, 1953; Zetler & Schlosser, 1953, 1954*a*, 1955; Amin, Crawford & Gaddum, 1954); and the concentrations of HT in different regions of dog brain have been estimated (Twarog & Page, 1953; Amin *et al.* 1954; Zetler & Schlosser, 1954*b*).

It is natural that the question of the possible physiological significance of these substances arises, and more so because clear differences in concentration can be found between neighbouring areas. The substance P activity of the dorsal spinal roots is greater than that of the ventral roots, and, because of this finding, Lembeck (1953) considered substance P as a possible chemical transmitter liberated by the first sensory neurone. On the other hand, Zetler & Schlosser (1953) think that it is more likely to be the 'non-cholinergic' transmitter for the autonomic system than for sensory pathways. v. Euler & Pernow (1954) injected substance P into the third ventricle of anaesthetized cats and rabbits and found stimulation of the respiration and a slight rise in blood pressure.

Gaddum (1953*b*, 1954) has drawn attention to the fact that small doses of lysergic acid diethylamide (LSD) affect the brain and antagonize some actions of HT on smooth muscle; he suspected a connexion between the two phenomena. Welsh (1953, 1954) regards HT as a neurohormone occurring in invertebrates. He showed that the pooled ganglia of the bivalve molluscs *Venus mercenaria* and *Busycon canaliculatum* were rich in HT and acetylcholine and that, in low concentrations, HT stimulated and acetylcholine inhibited the heart of *Venus*. Florey & Florey (1953), who worked on crustaceans, consider

HT to be a transmitter of nervous impulses in this class. Amin *et al.* (1954) established that, in dog brain, HT occurs only in grey matter, and that its distribution resembles closely that of noradrenaline as mapped out by Vogt (1954). In contrast, there was no correlation between the localization of noradrenaline and of substance P, the latter being found in grey nuclei as well as in medullated fibres. However, the amount of HT and of substance P was high in the central grey matter, in the mid-brain and in the area postrema, parts which are all rich in noradrenaline.

It has been shown (Vogt, 1954) that sympathin (adrenaline and noradrenaline) was reduced in amount in the hypothalamus and the mid-brain of cats and dogs after treatment with certain drugs. The same drugs did not deplete the sympathin from the area postrema. The work presented here was undertaken in order to study the behaviour of substance P and HT under the influence of drugs.

METHODS

Assays. HT was assayed on the isolated heart of the marine bivalve *Spisula* (*Mactra*) *solida* as described elsewhere (Gaddum & Paasonen, 1955). This method gives usually the same results as assays on the uterus of the oestrous rat, but is superior for the assay of extracts which contain certain other substances which affect the rat's uterus. The greatest difference was found in the assay of extracts from the caudate nucleus; when these were assayed on the uterus, the presence of HT appeared to be masked by inhibitory substances which did not interfere with the assay on *Spisula* (Gaddum & Paasonen, 1955). The bath fluid used for *Spisula* was aerated artificial sea water containing benzoquinonium chloride (6 mg/l. $= 10^{-5}$ M) as an antagonist of acetylcholine. An overflow-bath of 2 ml. capacity was used at room temperature. Assays on the rat's uterus were carried out as described by Amin *et al.* (1954), but, in order to destroy any adrenaline, the samples were pretreated with mushroom extracts following the procedure of Garven (1955).

Substance P was assayed on the guinea-pig's ileum suspended in a 2 ml. bath of Tyrode's solution containing atropine sulphate (10^{-7}) and mepyramine maleate (10^{-6}). The dosage employed lay usually between 0·1 and 0·25 units.

Extractions. The procedure of Amin *et al.* (1954) was followed. The dry HT-containing residues from the evaporated extracts were stored overnight in a desiccator at $-17°$ C, and were usually assayed on the following day. The residues which contained substance P were kept up to a week at $-17°$ C.

Dissection. Dogs of both sexes and weighing between 1 and 20 kg were used. Many experiments were carried out on litters of puppies, so that brains from controls and from injected animals came from the same litter. Some estimations were made on brains of cattle and pigs dissected in the slaughter-house as rapidly as possible, and not later than 2 hr, after the death of the animals. The samples were transported to the laboratory packed in dry ice. The dogs were anaesthetized with chloroform and bled to death. The dissected parts were rapidly weighed and immersed in acetone (20 ml./g of fresh tissue). The 'hypothalamus' included the supraoptic nuclei, but not the infundibular stalk, the preoptic region, the corpora mammillaria or the optic chiasma. The region called 'the floor of the fourth ventricle' consisted only of its most caudal part, cut 1–2 mm deep and comprising the trigonum hypoglossi and the ala cinerea. The superior cervical and the stellate ganglia were pooled in the dog, but in cattle the stellate ganglia were examined separately, or together with other thoracic ganglia.

Drugs. All drugs were administered subcutaneously, except for reserpine which was given intraperitoneally. Ephedrine, insulin, caffeine and, occasionally, amphetamine were administered in divided doses at 2 hr intervals. Unless otherwise stated, the dogs were anaesthetized and bled

to death between 4½ and 5½ hr after the first injection. The insulin was injected after an overnight fast. The drugs were dissolved in 0·9% NaCl solution except for β-tetrahydronaphthylamine carbonate which was dissolved in a small excess of HCl and neutralized with $NaHCO_3$, and reserpine, of which the solution as provided by Ciba Laboratories Ltd. ('Serpasil') was employed. The benzoquinonium chloride ('Mytolon') was obtained from the Sterling Winthrop Research Institute and the substance P reference sample was a laboratory standard containing 13·8 u./mg and prepared by Amin *et al.* (1954).

RESULTS

Substance P. Normal values. The substance P values for hypothalamus and caudate nucleus of normal dogs agree essentially with those reported in the literature (Pernow, 1953; Amin *et al.* 1954). Table 2 shows, however, that there are large individual variations in the amounts determined; in Expts. 11 and 15 (Table 2), carried out on puppies, the figures are low and of the same order as those reported by Amin *et al.* (1954), whereas the concentrations found in the other litters and in the adult dogs are higher and agree with those given by Pernow (1953). It may be that the lower figures of Expts. 11 and 15 should be regarded as 'abnormal', and as possibly due to some dietary deficiency in the recently weaned puppies; that low values also occur in adult dogs, is shown by the figures of Amin *et al.* (1954). Within the same litter, the scatter was always small, and therefore most of the work on drugs was carried out on litter-mates.

TABLE 1. The amount of substance P (units/g) in the area postrema and the floor of the fourth ventricle of dog, cattle and pig

		Area postrema	Floor of fourth ventricle
Adult dog		790	184
		330	38
		456	37
		250	50
	Mean	457	77
Cattle		134	45
		210	30
		85	26
	Mean	143	34
Pig		167	50

A series of experiments was carried out in order to re-examine the relative amounts of substance P in the area postrema and the caudal part of the floor of the 4th ventricle. In a single experiment, Amin *et al.* (1954) had reported the area postrema of the dog to contain much more substance P than did the floor of the ventricle, whereas Zetler & Schlosser (1955), working with tissue from cattle and man, found little substance P in the area postrema and much in the ala cinerea. In confirmation of the finding by Amin *et al.* (1954), Table 1 shows that, in the three species examined, the concentration of substance P was between four and five times higher in the area postrema than in the ala cinerea combined with the trigonum hypoglossi.

The last two regions were examined separately in a single cow brain in which the floor of the rhomboid fossa was cut out to a greater depth than before. This gave higher total figures; the bulk of substance P (334u./g) was found in the ala cinerea, and only about one-sixth of the total (70u./g) in the trigonum hypoglossi. These findings agreed perfectly with those of Zetler & Schlosser (1955), but they did not explain the difference in the amounts found in the area postrema.

The specificity of the activities measured in these regions was then checked by the following test. According to Gaddum (1953*a*), a large dose of substance P desensitizes the ileum specifically to subsequent doses of this compound. Thus 10 units of substance P were added to the bath and left in for 14 min. After washing, the responses to substance P standard, as well as those to the two brain extracts, had disappeared. During repeated washings the responses to all three preparations returned gradually at the same speed. This result supports the identity of the active principle in the standard preparation and in the extracts of area postrema and floor of the 4th ventricle.

Finally, some of the regions reported by Amin *et al.* (1954) to contain very little substance P were retested in a few animals. The figures in dog tissue were: for cerebellar cortex, 10 and 15 u./g, for pooled sympathetic ganglia, 21 and 26 u./g, and for the olfactory bulbs, 15 u./g. Stellate ganglia of cattle gave values of 12 and 20 u./g. The figures, though low in comparison with the values in the hypothalamus, are higher than those reported by Amin *et al.* (1954).

The effect of drugs. All drugs tested (for details see Table 2) were found to be without effect on the amounts of substance P in the dog's brain; the regions examined were hypothalamus and caudate nucleus. Since some of the uninjected puppies showed lower values than normal adult dogs, comparison in young animals should always be made between litter-mates. The question whether inadequate dosage might account for the lack of change observed is partially answered by the fact that the same doses of insulin, ether and β-tetrahydronaphthylamine caused a fall in hypothalamic sympathin (Vogt, 1954); further, as will be shown below, the brains of the animals which had unaltered concentration of substance P after the administration of amphetamine and of reserpine, had profound changes in their content of HT.

HT. Normal dogs. Table 3 contains the estimations, on *Spisula*, of HT in different parts of normal dog brain. The differences between regions found by Amin *et al.* (1954) are confirmed. It is of special interest that the low values previously reported for the sympathetic ganglia were confirmed on a preparation which is insensitive to adrenaline; one objection to the validity of the figures obtained on the rat's uterus had been that some of the adrenaline present in the ganglia was extracted by the acetone and might have antagonized the stimulating effect of HT on the uterus.

Another task which could not have been achieved on the rat's uterus was to

TABLE 2. Amount of substance P (u./g) found in the hypothalamus and the caudate nucleus of dogs

Expt. no.	Drug	Dose (mg or i.u.* per kg)	Hypothalamus		Caudate nucleus	
			Control	Injected	Control	Injected
Adult dogs						
1	Amphetamine sulphate	15	114	184	142	167
2	Amphetamine sulphate	20	197	220	203	170
3	Amphetamine sulphate	20	116	114	167	276
4	Amphetamine sulphate	20†	248	193	216	210
5	Amphetamine sulphate	30‡	110	169	223	200
			139	—	254	—
6	Ephedrine HCl	100	—	180	—	166
7	Ephedrine HCl	250	—	116	—	112
8	Insulin	3	—	221	—	183
	Mean		154	175	201	186
Puppies						
9	Amphetamine sulphate	20	153	122	180	136
	Insulin	2	—	144	—	227
10	Insulin	2	120	130	132	141
	β-Tetra§	32	—	83	—	118
11	Insulin	3	83	102	70	84
	β-Tetra§	50	—	72	—	47
12	Ephedrine HCl	200	143	140	170	157
13	Ephedrine HCl	250	147	116	96	112
14	Ephedrine HCl	250	104	158	75	75
	Ephedrine HCl	300	—	124	—	84
15	Ephedrine HCl	250	37	41	69	92
	Caffeine and Na benzoate	300	68	71	100	86
	Caffeine and Na benzoate	400	94	128	122	93
16	Ether	—	253	192	178	173
17	Reserpine	0·5 (11 hr)	134	120	95	122
	Reserpine	0·5 (22 hr)	—	132	—	124
	Mean		121	121	133	116

* Refers to insulin.

† Died after 5 hr.

‡ Died after 2 hr.

§ β-Tetrahydronaphthylamine carbonate.

In Expts. 1–8, the dogs listed under the same experiment were examined on the same day; in Expts. 9–17, the dogs of one experiment were litter-mates.

TABLE 3. Estimations of HT (ng/g fresh tissue) in different parts of the brain of normal dogs

Exp. no.	Hypothalamus	Caudate nucleus	Olfactory bulb	Cerebellar cortex	Floor of 4th ventricle	Area postrema	Sympathetic ganglia	Pituitary	
								Anterior	Posterior
1	354	280	—	—	375	189	—	<37	<154
2	526	310	—	—	—	—	—	—	—
3	521	298	—	—	350	214	—	14	<22
4	317	480	62	12	178	384	<59	—	<156
5	280	88	31	7	—	—	<19	<22	
6	224	200	25	8	—	—	<29	94	
7*	83	187	—	—	58	75	—	<8	<24
Mean (7)	329	262	39	9	230	213	0		0
Mean (6)	370	274	39	9	301	262	0		0

* Dog no. 7 suffered from severe arteriosclerosis, and the figures for the HT content of its brain are not included in the second row of means.

test the posterior lobe of the pituitary for possible content of HT. The high oxytocic activity of posterior lobe extracts, a little of which is extracted by the acetone, would render any estimation of HT invalid. One such extract was, in fact, assayed for oxytocin on the rat's uterus desensitized to HT by lysergic acid diethylamide. The contractions had the long latent period typical of posterior lobe hormone, and the activity was equivalent to 1·8 u. of standard pituitary extract per g of fresh tissue. Thus about 0·5% of the total activity of posterior lobe tissue had been extracted by the acetone. The heart of *Spisula* is quite insensitive to oxytocin, and thus its lack of response to acetone extracts of the posterior lobe can be taken as proof that HT is absent from this tissue. Small quantities of HT were detected in one whole pituitary and one anterior lobe.

The values for the hypothalamus are a little higher than those found by Amin *et al.* (1954), and this is probably due to the high sympathin content of this region and the possible contamination of the acetone extracts with some of the sympathin.

A substance stimulating the heart of *Spisula* was also found in the caudate nucleus, in which little HT had previously been estimated by Amin *et al.* (1954). The reason for this discrepancy appears to be the presence, in this region, of substances interfering with the assay of HT on the rat's uterus. Evidence for this view is presented elsewhere (Gaddum & Paasonen, 1955). In the present paper, the activity shown on the heart of *Spisula* is described as HT, though it may, in fact, only be the HT equivalent of another substance.

The effect of drugs. In Table 4 adult dogs and puppies are again treated separately, since the figures for the concentrations of HT were higher in adult dogs. In the adult dogs insulin and 15 mg/kg amphetamine caused no change in the HT content of the brain, whereas in one animal (Expt. 2), the content was somewhat low after 100 mg ephedrine. The larger doses of amphetamine, however, caused a significant reduction of the amounts of HT in both the hypothalamus and the caudate nucleus. These doses proved lethal in two of the animals.

The experiments were continued on litter-mate puppies, in order to reduce individual variations. Table 4, part 2, shows that ephedrine in larger doses than that used in Expt. 2 had no effect on the HT content of the brain. In this it resembled ether, insulin and β-tetrahydronaphthylamine. Amphetamine, on the other hand, reduced the HT content of the hypothalamus to about one-half, and also lowered the HT content of the area postrema (one experiment).

By far the most dramatic effect was, however, obtained after an intraperitoneal injection of reserpine. This drug was tried because Shore, Silver & Brodie (1955) had shown that it had a profound effect on the metabolism of HT. The dogs used belonged to the same litter of nine puppies (Expt. 17), part of which had been used to study the effect of amphetamine. 11 and 22 hr after

reserpine (0·5 mg/kg), the HT in the three parts of the brain under investigation had very nearly disappeared. If the experiment was terminated earlier, or the dose of reserpine halved, the figures were low but the loss was less striking.

TABLE 4. Estimation of HT (ng/g) in hypothalamus, caudate nucleus and area postrema of dogs; effect of drugs

Expt. no.	Drug	Dose (mg or i.u.*/kg)	Hypothalamus		Caudate nucleus		Area postrema	
			Control	Injected	Control	Injected	Control	Injected
Adult dogs								
8	Insulin	3	—	421	—	196	—	—
6	Ephedrine HCl	100	—	270	—	170	—	—
1	Amphetamine sulphate	15	354	347	280	303	—	—
2	Amphetamine sulphate	20	526	217	310	47	—	—
3	Amphetamine sulphate	20	521	294	298	102	—	—
4	Amphetamine sulphate	20†	317	95	480	100	—	—
5	Amphetamine sulphate	30‡		150		260	—	—
	Mean of 2–5		455	189	363	127	—	—
Puppies								
14	Ephedrine HCl	250	363	366	152	168	—	—
	Ephedrine HCl	300	—	315	—	166	—	—
13§	Ephedrine HCl	250	309	234	105	95	—	—
16	Ether	—	325	340	150	132	—	—
10	Insulin	2	259	364	147	208	—	—
	β-Tetra ‖	32	—	398	—	151	—	—
9	Insulin	2	452	487	171	230	—	—
	Mean		342	358	145	164		
9	Amphetamine sulphate	20	452	250	171	142	—	—
17	Amphetamine sulphate	30	400	197	137¶	—	858¶	515¶
	Amphetamine sulphate	30	474	216				
			453	—	153	—	—	—
	Mean		443	221	150	—	—	—
17	Reserpine	0·5, 22 hr	As above	<31	As above	16	As above	<107
	Reserpine	0·5, 11 hr		39		20		<63
	Reserpine	0·5, 4½ hr		247		97		141
	Reserpine	0·25, 13 hr		105		63		—

* Refers to insulin.

† Died after 5 hr.

‡ Died after 2 hr.

§ Assays on rat's uterus.

¶ Pooled.

‖ β-Tetrahydronaphthylamine carbonate.

In Expts. 1–8 (adult dogs), the dogs listed under the same experiment were examined on the same day; in Expts. 9–17 (puppies), the dogs of one experiment were litter-mates. For purposes of identification, the experiments are given the same current number as in Table 2.

In the evaluation of the results, the factor of dosage is important, since some of the failures to alter the HT and substance P content of the brain might have been due to inadequate dosage. Ether was given in a concentration which maintained surgical anaesthesia, insulin in doses which produced weakness and sometimes a comatose state from which, however, the dogs could be roused. Both caffeine and β-tetrahydronaphthylamine (β-Tetra) were given in near-lethal doses. Caffeine produced ataxia as its main effect, and β-Tetra caused excitement: the puppies became noisy and slightly ataxic, respiration and pulse rate were accelerated. It was obviously not possible to increase the dose of any of these substances much further. Ephedrine, on the other hand, was

surprisingly well tolerated and the signs shown were mild. The dogs were quiet, salivated, the respiration was fast and peculiar postures were often assumed, twitches occurred but no convulsions: the pulse was slow and often irregular. In contrast, excitement was very noticeable with amphetamine: respiration was fast, but assumed Cheyne-Stokes character and stopped before the heart beat if the dose used was lethal. If the animal died, there was a short period of muscular twitches which was probably due to anoxia. Salivation and a tendency to run backwards were regular clinical signs.

Reserpine (0·5 mg/kg) caused slowly developing changes. Sleepiness from which the dog could be roused, miosis and loss of appetite were always present, also some shivering. Diarrhoea occurred, and there was loss in weight if the experiment lasted 11 hr or more. Half that dose produced very few clinical signs—some diarrhoea, a loss in body weight and diminished liveliness.

DISCUSSION

The relative values for substance P in different regions of the brain of animals not treated with drugs confirmed the uneven distribution reported by previous workers; one discrepancy remained unexplained, the high figures found by us in the area postrema of cattle where Zetler & Schlosser (1955) found low values in their experiments. Whether details of dissection, or diffusion taking place in the time elapsing between dissection and the death of the animal are possible explanations of this difference is unknown. Another fact, for which an explanation is needed, is the occurrence of a low content of substance P in the brain of some litters of young puppies and not in others. There may be a connexion between these findings and the observation by Palladin & Bjeljaewa (1924) that the ratio amino-N/total-N in the grey matter of dog brain falls during fasting. It might be that some nutritional deficiency in the growing puppy has the same effect as fasting and reduces the amino-N to which the polypeptide P would contribute.

All drugs used in this work appeared to be without effect on the substance P content of the brain. This was so in spite of the fact that some of these drugs (ether, β-Tetra and insulin) cause a fall in brain sympathin, and others (amphetamine and reserpine) a loss in cerebral HT.

Whereas amphetamine caused only a moderate reduction of the HT content of brain tissue, a drastic effect was obtained by reserpine; in some experiments the depletion was such that no HT was found at all. Neither of these drugs have been tested in experiments in which sympathin was estimated. It is not very likely that they would have affected the sympathin content of the brain, because, so far, only drugs causing stimulation of the autonomic sympathetic centres have been found to produce a fall in brain sympathin. Reserpine does not appear to stimulate the sympathetic centres and amphetamine is not certain to have such an effect. A difference between the action

of reserpine or amphetamine on HT and that of drugs depleting brain sympathin is the fact that the area postrema is depleted of HT by reserpine (and probably by amphetamine), whereas its sympathin resists the action of drugs which reduce it in other parts of the brain. The more generalized effect of reserpine is not surprising in view of the fact that its action is not confined to the HT in brain, but that it also causes a disappearance of HT from the intestine, as shown by Pletscher, Shore & Brodie (1955). Reserpine thus appears to act on HT in many tissues, whereas the depletion of sympathin by drugs which stimulate certain autonomic centres is restricted to the stimulated regions. Amphetamine also caused a fall in the HT content of dog brain. This effect, however, was only obtained with doses which were often lethal and were greater than 15 mg/kg, and even then the depletion was only partial. The clinical picture obtained with the two drugs had no features in common, in fact it offered more contrasts than similarities. One might be tempted to speculate that the clinical signs after reserpine, among which inhibitions are prominent, might be caused by a release of HT into the circulation, whereas the excitation caused by amphetamine may be associated with an excessive utilization of HT or a decreased synthesis, the site of synthesis not necessarily being the brain tissue itself. Shore *et al.* (1955) have also expressed the view that reserpine may act by releasing HT and based it on the finding that excretion of 5-hydroxyindoleacetic acid is increased after administration of reserpine to dogs. The question whether HT acts as a transmitter, as it might do in those invertebrates in which the heart exhibits a very high sensitivity to HT (Twarog & Page, 1953; Welsh, 1953, 1954; Fänge, 1955; Gaddum & Paasonen, 1955), cannot be answered by these observations: but they support the theory that HT plays some part in the normal function of certain regions of the brain.

SUMMARY

1. The concentration of substance P and of HT in different regions of the mammalian brain was determined. HT was assayed on the heart of *Spisula solida*. Except for the concentration of HT in the caudate nucleus, the figures were in agreement with those reported by other workers. This difference in the caudate nucleus was probably due to the presence of substances which interfere with the assay of HT on the uterus of the oestrous rat.

2. The drugs used had no effect on the concentration of substance P in dog brain.

3. Reserpine (0·5 mg/kg) reduced the concentration of HT in dog brain to vanishing amounts, and amphetamine (20–30 mg/kg) lowered it to approximately one-half.

Grateful acknowledgement is made of the fact that M. H. Paasonen's visit to Edinburgh was rendered possible by a grant from the Finnish Government, and by a grant from the Medical

Research Council to Prof. J. H. Gaddum. We also wish to thank Messrs Ciba Laboratories Ltd., Horsham, for a gift of reserpine and the Sterling Winthrop Research Institute for Mytolon. We are very grateful to Dr J. M. Dodd for supplying us with *Spisula*.

REFERENCES

AMIN, A. H., CRAWFORD, T. B. B. & GADDUM, J. H. (1954). The distribution of substance P and 5-hydroxytryptamine in the central nervous system of the dog. *J. Physiol.* **126**, 596–618.

VON EULER, U. S. & GADDUM, J. H. (1931). An unidentified depressor substance in certain tissue extracts. *J. Physiol.* **72**, 74–87.

VON EULER, U. S. & PERNOW, B. (1954). Effects of intraventricular administration of substance P. *Nature, Lond.*, **174**, 184.

FÄNGE, R. (1955). Use of isolated heart of freshwater mussel (*Anodonta cygnea* L.) for biological estimation of 5-hydroxytryptamine. *Experientia*, **11**, 156.

FLOREY, E. & FLOREY E. (1953). Über die Bedeutung von 5-Hydroxytryptamin als nervöser Aktionssubstanz bei Cephalopoden und Crustaceen. *Naturwissenschaften*, **40**, 413–414.

GADDUM, J. H. (1953*a*). Tryptamine receptors. *J. Physiol.* **119**, 363–368.

GADDUM, J. H. (1953*b*). Antagonism between lysergic acid diethylamide and 5-hydroxytryptamine. *J. Physiol.* **121**, 15*P*.

GADDUM, J. H. (1954). Drugs antagonistic to 5-hydroxytryptamine. *Ciba Coll. on Hypertension*, pp. 75–77.

GADDUM, J. H. & PAASONEN, M. K. (1955). The use of some molluscan hearts for the estimation of 5-hydroxytryptamine. *Brit. J. Pharmacol.* **10**, 474–483.

GARVEN, J. D. (1955). The pharmacological activity of extracts of animal tissues, with particular reference to their content of 5-hydroxytryptamine. Ph.D. Thesis, Edinburgh University.

KOPERA, H. & LAZARINI, W. (1953). Zur Frage der zentralen Übertragung afferenter Impulse. IV. Mitteilung. Die Verteilung der Substanz P im Zentralnervensystem. *Arch. exp. Path. Pharmak.* **219**, 214–222.

LEMBECK, F. (1953). Zur Frage der zentralen Übertragung afferenter Impulse. III. Mitteilung, Das Vorkommen und die Bedeutung der Substanz P in den dorsalen Wurzeln des Rückenmarks. *Arch. exp. Path. Pharmak.* **219**, 197–213.

PALLADIN, A. & BJELJAEWA, W. (1924). Zur Frage der Aminogenese in der grauen und weissen Gehirnsubstanz im Hungerzustande. *Hoppe-Seyl. Z.* **139**, 57–63.

PERNOW, B. (1953). Studies on substance P. Purification, occurrence and biological actions. *Acta physiol. scand.* **29**, Suppl. 105.

PLETSCHER, A., SHORE, P. A. & BRODIE, B. B. (1955). Serotonin release as a possible mechanism of reserpine action. *Science*, **122**, 374–375.

SHORE, P. A., SILVER, S. L. & BRODIE, B. B. (1955). Interaction of reserpine, serotonin, and lysergic acid diethylamide in brain. *Science*, **122**, 284–285.

TWAROG, B. M. & PAGE, I. H. (1953). Serotonin content of some mammalian tissues and urine and a method for its identification. *Amer. J. Physiol.* **175**, 157–161.

VOGT, M. (1954). The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. *J. Physiol.* **123**, 451–481.

WELSH, J. H. (1953). Excitation of the heart of *Venus mercenaria*. *Arch. exp. Path. Pharmak.* **219**, 23–29.

WELSH, J. H. (1954). Hydroxytryptamine: a neurohormone in the invertebrates. *Fed. Proc.* **13**, 162–163.

ZELTER, G. & SCHLOSSER, L. (1953). Substanz P im Zentralnervensystem des Rindes. *Naturwissenschaften*, **40**, 559–560.

ZETLER, G. & SCHLOSSER, L. (1954*a*). Substanz P im Gehirn des Menschen. *Naturwissenschaften*, **41**, 46.

ZETLER, G. & SCHLOSSER, L. (1954*b*). Über das Vorkommen von 5-Hydroxy-tryptamin (Enteramin oder Serotonin) im Gehirn von Säugetieren. *Arch. exp. Path. Pharmak.* **222**, 345–351.

ZETLER, G. & SCHLOSSER, L. (1955). Über die Verteilung von Substanz P und Cholinacetylase im Gehirn. *Arch. exp. Path. Pharmak.* **224**, 159–175.