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RELEASE OF HISTAMINE BY TRYPTAMINE AND 5-HYDROXYTRYPTAMINE

BY

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MacIntosh and Paton (1949) have shown that many amines have in common the property of causing histamine release. In the present experiments it will be shown that this applies to tryptamine and 5-hydroxytryptamine as well.

The following procedures were adopted for examining the histamine releasing properties of the tryptamine compounds. (1) Perfusion of isolated skin flaps and gastrocnemius muscles and assaying the histamine in the venous effluent after arterial injection of the tryptamine compounds. (2) Determining changes in histamine content of tissues after subcutaneous and intraperitoneal injections of tryptamine.

METHODS

The perfusion experiments were performed on skin flaps of the cat's and dog's hind leg and on the gastrocnemius muscle of the cat, according to the methods described by Feldberg and Paton (1951) and Feldberg and Schachter (1952). The presence in the venous samples of tryptamine and 5-hydroxytryptamine collected after their injection had to be taken into account when assaying the samples for histamine on the atropinized guinea-pig's ileum preparation, because these substances have themselves a contractile effect on this preparation. For this reason, the samples were first assayed for tryptamine or 5-hydroxytryptamine respectively on the atropinized rat's colon, according to the method described by Feldberg and Toh (1953). They were then assayed for histamine on the guinea-pig's ileum preparation by adding to the control solutions of histamine, tryptamine, or 5-hydroxytryptamine respectively. For instance, if a sample of perfusate was found to contain 100 μg. tryptamine per ml., and 0.05 ml. was required for producing a reasonable contraction of the guinea-pig's gut, the effect was compared with contractions produced by different amounts of histamine to each of which was added 5 μg. tryptamine.

Changes in histamine content of tissues after subcutaneous and intraperitoneal injections of tryptamine were studied in albino rats of about 100 g. body weight. The rats were killed by a blow on the head and samples of the tissue to be examined were removed, weighed, ground in $\frac{1}{4}$N HCl with saline solution, boiled and neutralized before testing. The method has been described elsewhere (Feldberg and Talesnik, 1953; Smith, 1953). Tissue samples from 4 to 6 rats were pooled and extracted together in order to minimize individual variations in the histamine content.

All values for histamine are expressed as base. We are indebted to Dr. R. K. Richards, Abbott Laboratories, for kindly providing us with the sample of 5-hydroxytryptamine creatine sulphate. Its molecular weight is 355, of which about half, i.e., 176, represents the base; all values given refer to the base. The tryptamine used was the hydrochloride. Its molecular weight is 196.5, of which about 80%, i.e. 160, represents the base; all values given refer to the salt. In some experiments the histamine releasing activity of tryptamine was compared with that of compound 48/80, kindly provided by the late Dr. C. H. Kellaway (Wellcome Research Laboratories).

RESULTS

Perfused Skin Flap of the Cat

Both tryptamine and 5-hydroxytryptamine caused a release of histamine from perfused skin flaps. There was, in addition, vasoconstriction and the development of oedema; the vasoconstriction, which was particularly strong after 5-hydroxytryptamine, was probably only to some degree due to the released histamine and was mainly a direct effect of the tryptamine compounds on the vessels. With the injection of either 2.5 mg. tryptamine or 5-hydroxytryptamine in 0.5 ml., the flow was either stopped or nearly stopped by a vasoconstriction which supervened about 2 min. after the injection. When the flow was not totally arrested, the vasoconstriction was found to reach a maximum in 5–7 min. and then very gradually subsided. The intensity of the oedema paralleled the output of histamine; it developed during the first 30 or 60 min. after the injection, was particularly pronounced in the subcutaneous tissue, and

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could amount to about six times the weight of the skin flap.

As was to be expected, the first samples after the injections contained most of the tryptamine or 5-hydroxytryptamine. They were recovered in the venous effluent to between 50–70%. Since the first drops of venous effluent after the injection, which probably contained the highest concentration of the tryptamine compounds, were discarded, the actual amounts escaping into the venous effluent were probably greater. Further, some destruction and inactivation of these compounds may have occurred during the passage through the skin and some may have been retained, as evidenced by their presence in the oedema fluid collected after cessation of the perfusion.

Table I shows the amounts of tryptamine and 5-hydroxytryptamine from skin flaps of the same cat in successive samples of venous effluent, and the histamine content of these samples when assayed against known histamine solutions but incorporating the amounts of the tryptamine compounds present in them.

TABLE I

ASSAY OF VENOUS EFFLUENT FOR TRYPTAMINE (T), 5-HYDROXYTRYPTAMINE (HT) AND HISTAMINE (Hi) FROM PERFUSED CAT'S SKIN FLAPS OF THE SAME CAT

2 mg. T injected into the right, and 2 mg. HT into the left skin flap (same experiment as No. 9, Table II)

Sample after Injection	Minutes of Collection	Right Skin Flap			Left Skin Flap		
		ml. Collected	μg. T	μg. Hi	ml. Collected	μg. HT	μg. Hi
1	1½–2	3·0	525	36·5	2·2	660	79·2
2	8–8½	4·4	396	145·2	1·3	78	35·8
3	10	5·0	100	25·0	11·4	160	43·3
4	20	22·0	143	66·0	15·0	63	60·0
5	10	14·7	28	7·4	12·7	13	0·6
6	10	13·5	11	1·4	20·5	17	0·8
7	20	24·2	—	1·6	16·5	12	0·5
8	20	15·0	—	0·5	—	—	—
Oedema fluid		75·0	75	7·5	68·0	54	7·2
Total ..			1,273	291·1		1,057	227·4

Table II shows the amounts of histamine released in eleven experiments on injection of 0.5–2 mg. of the two tryptamine compounds. Doses under 0.5 mg. were ineffective, but with 2 mg. usually between 93 and 283 μg. histamine was released. The low value of 30 μg. in experiment 6 represents only the histamine of the sample collected during the first 5 min. after the injection; in this experiment the vasoconstriction was so intense that the flow stopped after this time and attempts to restart it by increasing the perfusion pressure led to leakage from the arterial side, so that the experiment had to be discontinued.

In experiments 4, 5, 8, and 9 of Table II, the skin flaps of both hind legs were perfused and

TABLE II

OUTPUT OF HISTAMINE AFTER TRYPTAMINE (T) AND 5-HYDROXYTRYPTAMINE (HT) FROM PERFUSED SKIN PREPARATIONS OF THE CAT

Figures in brackets refer to the highest histamine concentration (μg./ml.) found in the initial samples after injection of the tryptamine compounds

Expt. No.	mg. Injected	Output of Histamine in μg.			
		After (T)		After (HT)	
1	0·5	1	(0·09)	—	—
2	0·5	—	—	28·5	(2)
3	1·0	11·2	(1·05)	—	—
4	1·0	46·8	(14)	68	(20)
5	2·0	93	(4)	115	(8)
6	2·0	106	(5)	30*	(6)
7	2·0	172	(20)	—	—
8	2·0	187	(32)	127	(28)
9	2·0	283	(33)	220	(36)
10	2·0	—	—	172	(20)
11	2·0	229	(22)	—	—

* Only one 5 min. sample collected.

tryptamine was examined on one side and the equivalent amount of 5-hydroxytryptamine on the other. It is evident that the two compounds have approximately the same histamine releasing ability; if there is any difference, tryptamine is slightly more active. Since we express 5-hydroxytryptamine as base and tryptamine as salt, 2 mg. of tryptamine corresponds to 1.6 mg. of base only, and the difference between the activity of the two compounds is thereby accentuated.

A similar conclusion is reached from experiments in which the effects of repeated injections are compared on the same skin preparation. In the experiments of Fig. 1, 2 mg. of 5-hydroxytryptamine and 2 mg. of tryptamine were injected into the skin flaps of both legs of the same cat, but in reversed order. In both perfusions the first injection, whatever the tryptamine compound injected, produced a greater histamine output than the subsequent injection. It was possible, however, to increase the histamine output considerably by doubling the dose injected. This is seen for tryptamine at the end of the perfusion of the left skin flap (Fig. 1).

The vasoconstriction interfered with the time course of the appearance of histamine in the venous outflow, and the trough seen in the record of the histamine output after 4 mg. tryptamine is explained by the fact that during the corresponding period the flow was greatly reduced and therefore only a small volume of venous effluent collected. The vasoconstriction interfered also with the output of histamine during some of the previous injections in the experiment of Fig. 1, by delaying the maximal output per minute until the vasoconstriction had subsided. However, the actual "release" of histamine probably occurs as

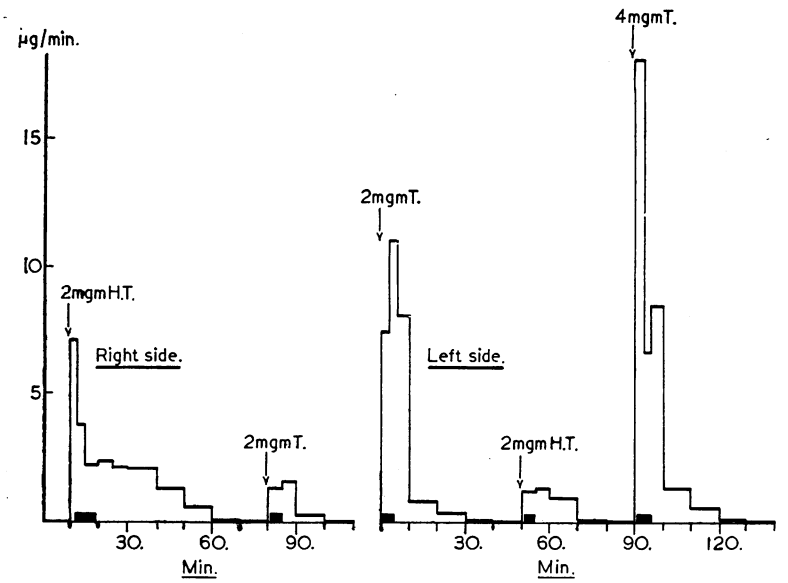

FIG. 1.—Histamine output from perfused skin preparations of the hind legs of the same cat after tryptamine (T) and 5-hydroxytryptamine (HT). The black areas above the base-line indicate periods of pronounced vasoconstriction. (For details see text.)

instantaneously as after the other known histamine liberators.

The histamine release was associated with a reduction in the histamine content of the central parts of the perfused skin. In one experiment a release of 173 µg. histamine after 2 mg. tryptamine reduced the histamine content in the central part of the skin flap by 75%, to 2.9 µg./g.; in a corresponding experiment with 2 mg. 5-hydroxytryptamine a release of 171 µg. histamine caused a reduction by 88%, to 2.6 µg./g.

When the histamine releasing property of the two tryptamine compounds is compared on the perfused cat's skin preparation with that of 48/80, it is found that, weight for weight, 48/80 is about 100 times more active. This is illustrated in the experiment of Fig. 2, in which the effect of 20 µg. 48/80 is compared with that of 2 mg. tryptamine, on two skin preparations from the same cat in which the order of injection was reversed. The tryptamine compounds are therefore about as active as, or perhaps even slightly more active than, propamidine. They are certainly more active than the other amines and amidines examined by MacIntosh and Paton (1949), and also more active than the opium alkaloids and morphine derivatives examined by Feldberg and Paton (1951).

Perfused Gastrocnemius Muscle of the Cat

The histamine releasing activity of tryptamine and 5-hydroxytryptamine from the perfused gastrocnemius muscle is illustrated in the two experiments of Fig. 3, which are from different cats. Since the gastrocnemius has a low histamine content, the amounts released from this prepara-

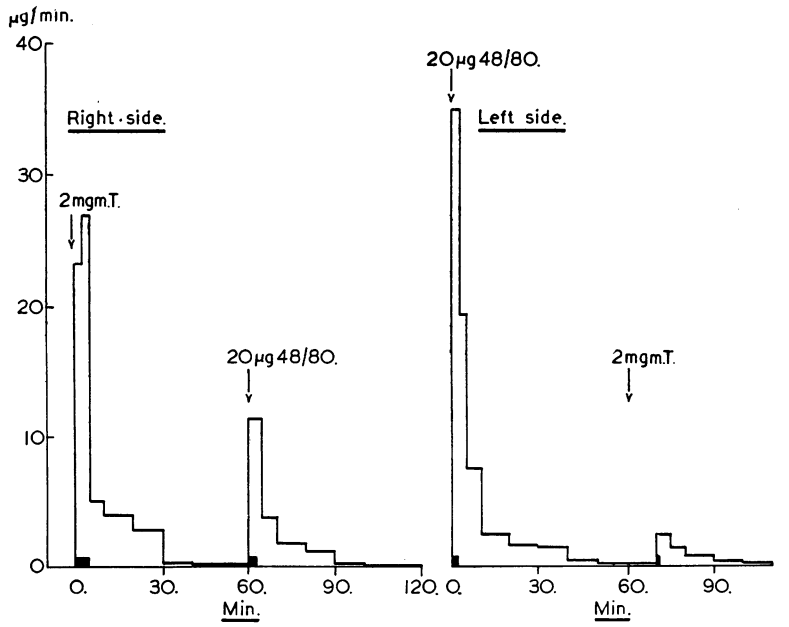

FIG. 2.—Comparison of the histamine output by 48/80 and tryptamine (T) from perfused skin preparations of the hind legs of the same cat. The black areas above the base-line indicate periods of pronounced vasoconstriction. (For details see text.)

tion are much smaller than those released from the cat's skin. In the first experiment, two successive injections of 2 mg. 5-hydroxytryptamine released 3.35 and 0.71 μg. histamine ; in the second experiment 2 mg. tryptamine released 2.59 μg. histamine, and a succeeding injection of 4 mg., 6.35 μg. Although the two experiments were from different cats, they suggest that for the perfused gastrocnemius muscle, as for the perfused skin of the cat, the histamine releasing property of the two compounds is of the same order.

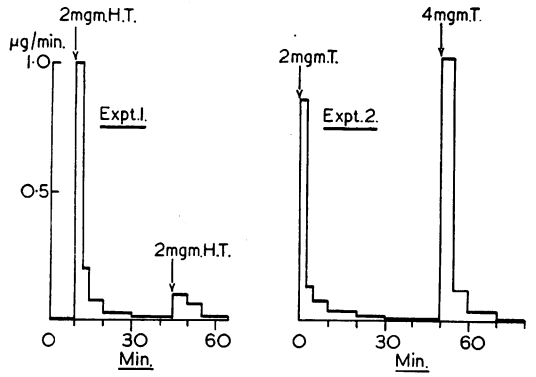

FIG. 3.—Histamine output from perfused gastrocnemius after 2 mg. 5-hydroxytryptamine (HT) in expt. 1 and after 2 and 4 mg. tryptamine (T) in expt. 2 which was from a different cat.

The amounts of histamine released in the two experiments of Fig. 3 represent only a fraction of the muscle histamine. In the experiment with 5-hydroxytryptamine, the gastrocnemius of the non-perfused leg weighed 30 g. and contained 1.38 μg./g. histamine ; its total histamine content was therefore about 41 μg. ; the amounts released by the two injections of 5-hydroxytryptamine correspond, therefore, to about 10% of this figure. In the other experiment, the weight of the non-perfused gastrocnemius was 28.5 g. and contained 1.64 μg./g. histamine ; its total histamine content was therefore about 47 μg., of which a little over 20% was released by the two tryptamine injections.

Perfused Skin Flap of the Dog

Tryptamine alone was examined, which produced some vasoconstriction, but to a less degree than on the cat's skin preparation. When assaying the effluent for tryptamine on the rat's colon, the values obtained for the combined first three samples, collected over a 10 min. period after the injection, were higher than the actual amounts of tryptamine injected. This may have resulted from the fact that these samples collected during the vasoconstriction were blood-stained, even if the effluent had become clear before the injection ; and it had been found that samples collected at the beginning of a perfusion, when they were yet blood-stained and cloudy but no tryptamine had been injected, had some activity on the rat's colon. It was not examined whether this activity was due to tryptamine or 5-hydroxytryptamine. In order to avoid the error of exaggerating, by this activity, the histamine assayed in the effluent, two methods were adopted : one (method 1) by taking the excessively high value actually assayed for tryptamine on the rat's colon as representing the tryptamine in the sample, and the other (method 2) by assuming that the first three samples contained the total amount of tryptamine injected. With both methods a histamine release could be demonstrated, but, as seen from Table III, the values

TABLE III

HISTAMINE OUTPUT BY TRYPTAMINE FROM PERFUSED SKIN PREPARATION OF THE DOG

Tryptamine Injected	μg. Histamine Assayed	
	By Method 1	By Method 2
2 mg. in 0·5 ml.	7·0	—
4 „ „ 2 „	5·3	5·8
8 „ „ 2 „	6·8	9·6

obtained with the second method were higher ; even these may be an underestimation of the actual amounts of histamine present in the samples, because it is unlikely that there was a 100% recovery of tryptamine.

Histamine Release from Rat's Tissue by Tryptamine

Acid saline extracts from rat tissue produced contraction of the rat's colon ; the activity corresponded to that of 10–20 μg. tryptamine per gramme tissue. Somewhat higher values were obtained from the animals treated with tryptamine. Although such amounts of tryptamine would not greatly interfere in the histamine assay, they were taken into account by first assaying the extracts against tryptamine on the rat colon, and, in the subsequent histamine assay on the guinea-pig's ileum, adding tryptamine to the control solution of histamine, as in the corresponding assays of perfusate (see Methods).

Subcutaneous Injections.—As with 48/80, the subcutaneous injection of tryptamine leads to a reduction of skin histamine at the site of injection. For this purpose 2 mg. tryptamine was injected in 0.5 ml. in previously demarcated areas of the abdominal skin in eight rats. The injections

caused locally a bluish cyanosed area surrounded by erythema which two hours later became cyanosed also. By this time a haemorrhagic weal had developed at the injection site. Half of the injected rats were killed two hours after the injection; the skin at the site of the injection contained blood cells and plasma. The other half of the rats were killed 24 hours after the injection; the site of injection was no longer oedematous but contained a crust of cells and dried serum. The histamine content of the skin areas at the site of injection from these rats is given in Table IV

TABLE IV

EFFECT OF SUBCUTANEOUS INJECTION OF 2 MG. TRYPTAMINE IN 0·5 ML. ON HISTAMINE CONTENT OF ABDOMINAL SKIN (POOLED SKIN SAMPLES) AT SITE OF INJECTION

No. of Rats	Treatment	μg./g Histamine	% Reduction
2	None	50	0
2	2 hr. after injection of saline	42	16
4	2 ,, ,, ,, ,, tryptamine	14	72
4	24 ,, ,, ,, ,, ,,	12·8	74

and compared with the histamine content from normal rats and from skin areas of rats having received a control injection of 0.5 ml. saline solution. Tryptamine produces a great reduction in histamine content which persists after all traces of oedema have disappeared. The small reduction two hours after the saline injection must be accounted for by an increased fluid content of the skin due to the fact that the injected fluid was not completely absorbed.

There is this difference between the effects of tryptamine and those of corresponding experiments with 48/80 (see Feldberg and Talesnik, 1953) that the injection of tryptamine, probably through its intense vasoconstrictor properties, produces local damage to the tissue which may contribute to the observed reduction in histamine content.

Intraperitoneal Injections.—Eight rats were each given a total of 13 mg. of tryptamine intraperitoneally. The injections were made twice daily, starting with 1 mg. and going up to 4 mg., as shown in Table V.

After the injection of 1 mg., respiration became accelerated, and after 2 and 3 mg. was, in addition, laboured. The rats became cyanotic, their coat was ruffled, but later the skin became pink, and in some animals oedema developed round the mouth and on the paws. This occurred after 2 mg. in three, and after 3 mg. in five out of eight rats about 45 min. after the injections. With 4 mg. the animals were first prostrated, cyanosed, and cold, and oedema developed in half the animals when they began to recover.

Table V gives the histamine content of various tissues from these animals killed 24 hours after the last injection. The values from the pooled samples are compared with those obtained from six control rats. There is a definite reduction in skin histamine and also, to a lesser degree, in the histamine content of the diaphragm, but the histamine content of the viscera showed no consistent

TABLE V

CHANGES IN HISTAMINE CONTENT OF RAT'S TISSUES AFTER INTRAPERITONEAL INJECTIONS OF TRYPTAMINE (FIRST DAY 1 MG. TWICE; SECOND DAY, 2 MG. TWICE; THIRD DAY, 3 MG. ONCE AND 4 MG. ONCE)

Area	μg./g. Histamine		% Change after Tryptamine
	Controls	After Tryptamine	
Skin of ear	50	35	−30
Skin of paw	55	30	−46
Skin of abdomen	32	18	−39
Diaphragm	11·5	8·5	−26
Liver	0·7	0·7	0
Lung	1·0	0·9	−10
Aorta	2·3	2·5	+9
Stomach	12·5	11·5	−8

change. The difference of 10% or less cannot be regarded as sufficient proof for an effect of the tryptamine.

DISCUSSION

The finding that tryptamine and 5-hydroxytryptamine have the ability to release histamine from living tissue brings them into line with the large group of amines having this property. Although the activity of tryptamine and 5-hydroxytryptamine in releasing histamine is somewhat greater than that of the other amines and amidines examined by MacIntosh and Paton (1949), and is, in fact, slightly more active than that of propamidine, the most active compound examined by these authors, this action must, nevertheless, be regarded as a side-effect of their pharmacological properties. This does not eliminate the possibility that histamine release is at the root of certain phenomena, seen after injection of tryptamine compounds and resembling histamine effects; for instance, it may account for some of the depressor effects on the arterial blood pressure obtained with 5-hydroxytryptamine in cats and dogs (Erspamer, 1952; Page, 1952; Freyburger, Graham, Rapport, Seay, Govier, Swoap, and Vander Brook, 1952) and is most likely the cause of the intense vasodilatation associated with itching seen in the rat after subcutaneous injection of a large dose of 5-hydroxytryptamine (Erspamer, 1952).

On the other hand, histamine release may occur without its usual signs, because the strong vasoconstrictor action of tryptamine or 5-hydroxytryptamine may override a histamine vasodilatation.

Recent work has shown that 5-hydroxytryptamine is a natural constituent of some animal tissues (Rapport, 1949; Erspamer and Asero, 1952; Bacq, Fischer, and Ghiretti, 1952; Dalgliesh, Toh, and Work, 1953; Feldberg and Toh, 1953); if released in the body it might well, in its turn, cause release of histamine. Although there is as yet no evidence that this sequence of events occurs in the body, the possibility cannot be excluded that such a mechanism participates in the histamine release by peptone and by the antigen-antibody reaction of anaphylaxis, because 5-hydroxytryptamine is released from platelets by the antigen-antibody reaction *in vitro* (Humphrey and Jaques, 1953) and because release of histamine from the dog's liver and the rabbit's lung by either antigen or peptone is, to a great extent, dependent on disintegration of platelets (Dragstedt, 1941; Rocha e Silva, 1950). Even if, under these special conditions, the release of 5-hydroxytryptamine from platelets by antigen or peptone should be an intermediate step for the release of histamine, not only from the platelets themselves, but perhaps also from the liver and lung cells with which the agglutinated platelets come in close contact when they are "trapped" in the capillary bed of these organs, 5-hydroxytryptamine is probably not the only intermediate histamine liberator set free. It is also necessary to keep in mind, in this connection, that the symptomatology following injections of 5-hydroxytryptamine does not resemble closely that of anaphylaxis.

Histamine is also released from the platelets during clotting (Code, 1952), which is, moreover, associated with release into the serum of 5-hydroxytryptamine (Rapport, Green, and Page, 1948; Rapport, 1949; Reid and Rand, 1951). The release of histamine in this process may again be subsequent to that of 5-hydroxytryptamine and, during intravascular thrombosis, release of 5-hydroxytryptamine from the thrombus may, in turn, lead to release of histamine from the endothelial lining of the vessel.

Summary

Tryptamine and 5-hydroxytryptamine release histamine from living tissue; this finding brings them into line with the large group of amines having this property. The histamine-releasing activity of tryptamine and 5-hydroxytryptamine is about 100 times less than that of compound 48/80. The release of histamine was demonstrated under the following experimental conditions:

(*a*) After arterial injection of either tryptamine or 5-hydroxytryptamine into the perfused skin flap or gastrocnemius muscle of the cat.

(*b*) After arterial injection of tryptamine into the perfused skin flap of the dog.

(*c*) After subcutaneous or repeated intraperitoneal injections of tryptamine to rats. The subcutaneous injection caused a local reduction in the histamine content of skin at the site of injection, the intraperitoneal injections a general reduction of the histamine content in skin and skeletal muscle.

References

Bacq, Z. M., Fischer, P., and Ghiretti, Fr. (1952). *Arch. int. Physiol.*, **60**, 165.

Code, C. F. (1952) *Physiol. Rev.*, **32**, 47.

Dalgliesh, C. E., Toh, C. C., and Work, T. S. (1953). *J. Physiol.*, **120**, 298.

Dragstedt, C. A. (1941). *Physiol. Rev.*, **21**, 563.

Erspamer, V. (1952). *Arch. intern. Med.*, **90**, 505.

—— and Asero, B. (1952). *Nature, Lond.*, **169**, 800.

Feldberg, W., and Paton, W. D. M. (1951). *J. Physiol.*, **114**, 490.

—— and Schachter, M. (1952). Ibid., **118**, 124.

—— and Talesnik, J. (1953). Ibid., **120**, 550.

—— and Toh, C. C. (1953). Ibid., **119**, 352.

Freyburger, W. A., Graham, B. E., Rapport, M. M., Seay, P. H., Govier, W. M., Swoap, O. F., and Vander Brook, M. J. (1952). *J. Pharmacol.*, **105**, 80.

Humphrey, J. H., and Jaques, R. (1953). *J. Physiol.*, **119**, 43*P*.

MacIntosh, F. C., and Paton, W. D. M. (1949). Ibid., **109**, 190.

Page, I. (1952). *J. Pharmacol.*, **105**, 58.

Rapport, M. M. (1949). *J. biol. Chem.*, **180**, 961.

—— Green, A. A., and Page, I. H. (1948). Ibid., **176**, 1243.

Reid, G., and Rand, M. (1951). *Aust. J. exp. Biol. med. Sci.*, **29**, 401.

Rocha e Silva, M. (1950). *Ann. N.Y. Acad. Sci.*, **50**, 1045.

Smith, A. N. (1953). *J. Physiol.* (in the press).