Histamine in Non-vertebrate Animals

SIR,—Histamine is widely distributed in nature. In the plant kingdom, histamine is formed in large amounts by several bacterial organisms, is present in various fungi, and is of common occurrence in the higher plants (Emmelin and Feldberg, 1947; Werle and Raub, 1948; Gale, 1953; Fowler, 1962). In the animal kingdom, investigation of the distribution of histamine has been confined mainly to the vertebrates: the amine is known to occur commonly in fish, and to be present in greater or lesser amount in almost every mammalian organ, tissue and fluid (Guggenheim, 1951; Feldberg, 1956). Among invertebrates, high concentrations of histamine have been detected in various stings and venoms (Marcou, Derevici and Derevici, 1937; Jaques and Schachter, 1954b; Rothschild and Parsons, 1962), in the gnat (Eckert, Paasonen and Vartiainen, 1951), in 2 coelenterates (Mathias, Ross and Schachter, 1960), and in a sponge (Ackermann and List, 1957). It is not known, however, whether histamine is of general as opposed to isolated occurrence among the lower animal phyla, particularly in the non-coelomates. Studies on the distribution and significance of histamine in comparatively simple organisms may provide valuable clues to the function of the amine in higher species, and a survey has therefore been made of its occurrence in a wide range of diplo- and triploblastic animals. A study of the ability to form histamine from histidine has also been made in some species.

Freshly collected organisms were extracted for 24 hr. in 10 per cent trichloroacetic acid, and after removal of excess acid with ether, the extracts were assayed for histamine or histamine-like substance on the isolated ileum of the guinea-pig and on the blood pressure of the anaesthetised dog. The specificity of the responses was checked on each preparation using mepyramine maleate. Histidine decarboxylase activity was measured as the amount of histamine formed from added histidine during an incubation period of 3 hr. in buffered physiological saline solution. The methods have been described in detail elsewhere (Telford and West, 1961a).

The results, presented in Table I, indicate that histamine is widely distribut in non-vertebrate animals. The species investigated are drawn from all the major phyla in the animal kingdom with the exception of the protozoa, and though the number of species studied is small, at least one species from each of the 10 phyla represented has been found to contain histamine. Furthermore, the amine has been detected in 19 of the 26 species examined. Particularly high concentrations have been found in the sea-anemone *Aiptasia tagetes* (Coelenterata: Anthozoa), in the parasite *Mesocoelium monodi* (Platyhelminthes: Trematoda) obtained from the intestine of *Bufo marinus*, and in the sea-urchin *Echinaster echinophorus* (Echinodermata: Echinoidea). The amount of histamine per unit weight in each of these species is comparable with the large amount of histamine present in certain mammalian tissues such as rat skin and guinea-pig lung. Some other species from the same phyla, however, contain little or no histamine. Hence, as may be seen from Table I where the species are classified according to Rothschild (1961), there is no relationship in the non-vertebrate animals between the distribution of histamine and biological complexity.

Calculation of histamine content as μg. histamine per unit weight gives little information about the total amount of amine that a single specimen contains. However, calculation as μg. histamine per specimen is impractical as the weights of individual specimens from any one species sometimes vary greatly. Such approximate estimates as can be made nevertheless indicate that among those species containing histamine there is no relation between total histamine content and size.

Histamine was found in all the parasitic species studied. Too few animals have been investigated to postulate that histamine is more widely distributed among parasitic species than among invertebrate free-living species, but among the species examined, the parasitic forms contain a significantly greater amount of histamine per unit weight than the free-living forms ($P > 0{\cdot}05$).

Histidine decarboxylase activity was detected in two histamine-containing species, *Aiptasia tagetes* and *Mesocoelium monodi*. In both, enzyme activity was found in the range pH 4·0–9·0, in contrast to bacterial histidine decarboxylases which are most active at an acid pH (Gale, 1953), and in contrast to mammalian histidine decarboxylases which are usually most active at an alkaline pH (Schayer, 1957; Telford and West, 1961a, 1961b; Weissbach, Lovenberg and Udenfriend, 1961). The addition of organic solvent did not increase enzyme activity in either species, neither did the addition of pyridoxal-5-phosphoric acid. The addition of organic solvent usually increases the histidine decarboxylase activity of mammalian tissues (Waton, 1956; Telford and West, 1961a, 1961b), whilst the addition of pyridoxal-5-phosphoric acid has been found to increase enzyme activity in some mammalian tissues (Rothschild and Schayer, 1958; Telford, unpublished observations) and in bacteria (Gale, 1953). It is possible, therefore, that the histamine-forming enzymes in *Aiptasia tagetes* and *Mesocoelium monodi* represent a different type of histidine decarboxylase from those which have been previously described.

TABLE I

HISTAMINE CONTENT (μg./g.) AND HISTIDINE DECARBOXYLASE ACTIVITY (μg. HISTAMINE FORMED/g./3 hr.) OF VARIOUS INVERTEBRATE SPECIES

Species	Mode of life	Histamine content	Histidine decarboxylase activity
Phylum Porifera			
Haliclona sp.	FL	3·0	—
Mycale cecilia	FL	1·0	—
Phylum Coelenterata			
Rhodactis sanctithomae	FL	0·4	—
Aiptasia tagetes	FL	32·5	15·0
Cassiopeia xaymacana	FL	1·8	—
Condactylis gigantea	FL	0	—
Phylum Platyhelminthes			
Stylochus megalops	FL	0	—
Styloplanocera fasciata	FL	0	—
Syndesmis franciscana	SP	6·9	0
Fasciola hepatica	P	5·2	0
Mesocoelium monodi	P	58·3	154·4
Oochoristica ameivae	P	13·0	0
Phylum Aschelminthes			
Stephanurus dentatus	P	1·9	0
Phylum Acanthocephala			
Macracanthorynchus hirudinaceus	P	3·2	0
Phylum Mollusca			
Australorbis glabratus	FL	0	—
Acra zebra	FL	1·0	—
Pleurodonta sp.	FL	0·1	0
Domax denticulatus	FL	0·6	—
Phylum Annelida			
Pheretima sp.	FL	0·2	—
Hermodice carunculata	FL	?	—
Phylum Arthropoda			
Mysidium columbiae	FL	0	—
Coenobita clypeatus	FL	4·3	—
Phylum Echinodermata			
Echinaster echinophorus	FL	28·3	—
Lytechinus variegatus	FL	0	0
Diadema antillarum	FL	2·5	—
Holothuria sp.	FL	18·0	—

FL, free living; P, parasitic; SP, semi-parasitic. ?, interfering substance —, not tested. All values of histamine refer to the base, and are the mean of at least 3 determinations.

One of the species studied, *Aiptasia tagetes*, is a sea-anemone whose tentacles release an unidentified substance which on contact with human skin produces symptoms resembling those produced by histamine. The symptoms consist of a burning sensation accompanied by itching, redness, and sometimes local oedema. The present experiments strongly suggest that these symptoms are in fact due, at least in part, to histamine being introduced into the skin. However, other substances such as histamine releasers, acetylcholine or 5-hydroxytryptamine may be involved; it should be noted that potent histamine releasers and 5-hydroxytryptamine have been extracted from a number of coelenterate species (Jaques and Schachter, 1954a; Mathias, Ross and Schachter, 1957; Mathias, Ross and Schachter, 1960; Uvnäs, 1960; Welsh, 1960).

Histamine may be widely distributed in sea-anemones. Large amounts have been found in the tentacles of *Actinia equina* and *Anemonica sulcata* (Mathias, Ross and Schachter, 1960), but *Aiptasia tagetes* differs from these species in that its histamine is localised mainly in the body of the animal. Only 20–30 per cent of the total histamine was found to be present in the tentacles, the remaining 70–80 per cent being present in the column and coelenteric structures. We have not so far established whether the histamine is confined to one type of cell structure, but it does not appear to be localised in the nematocysts. Thus, while the total number of nematocysts in the column and coelenteric structures is probably greater than in the tentacles, the number of nematocysts per unit area is higher in the tentacles than elsewhere.

The experiments reported here provide a further demonstration of the apparently haphazard fashion in which histamine is distributed in nature. At the present time it is not possible to say whether the amine serves a common function in all tissues which contain it, or whether during the course of evolution its presence has been adapted to serve new functions. However, the occurrence of particularly high concentrations of histamine in a variety of invertebrate poisonous secretions indicates that in the lower animal groups the amine may play a part in defence and attack. In mammals, it has been suggested by various authors (Feldberg, 1954; Kahlson, Nilsson, Rosengren and Zederfeldt, 1960; Riley, 1962) that the amine exerts an important role in tissue defence and repair, and it is possible that histamine serves a related function throughout the animal kingdom.

Department of Zoology, D. F. METTRICK

Department of Physiology, J. M. TELFORD
University of the West Indies,
Kingston 7, Jamaica.

August 20, 1963

REFERENCES

Ackermann, D. and List, P. H. (1957). *Hoppe-Seyl. Z.*, **308**, 274–276.
Eckert, D., Paasonen, M. and Vartiainen, A. (1951). *Acta pharm. tox., Kbh.*, **7**, 16–22.
Emmelin, H. and Feldberg, W. (1947). *J. Physiol.*, **106**, 440–448.
Feldberg, W. (1954). *J. Pharm. Pharmacol.*, **6**, 281–301.
Feldberg, W. (1956). *Ciba Symposium on Histamine*, London: Churchill.
Fowler, H. D. (1962). *Nature, Lond.*, **193**, 582–583.
Gale, E. F. (1953). *Brit. med. Bull.*, **9**, 135–136.
Guggenheim, M. (1951). *Die biogenen Amine*, Basle: Karger, and New York: Interscience.
Jaques, R. and Schachter, M. (1954a). *Brit. J. Pharmacol.*, **9**, 49–52.
Jaques, R. and Schachter, M. (1954b). *Brit. J. Pharmacol.*, **9**, 53–58.

Kahlson, G., Nilsson, K., Rosengren, E. and Zederfeldt, R. (1960). *Lancet*, **2**, 230–234.
Marcou, I., Derevici, A. and Derevici, M. (1937). *C.R. Soc. Biol., Paris*, **126**, 726–728.
Mathias, A. P., Ross, D. M. and Schachter, M. (1957). *Nature, Lond.*, **180**, 658–659.
Mathias, A. P., Ross, D. M. and Schachter, M. (1960). *J. Physiol.* **151**, 296–311.
Riley, J. F. (1962). *Lancet*, **2**, 40–41.
Rothschild, Lord. (1961). *A Classification of Living Animals*, London: Longmans.
Rothschild, The Hon. M. and Parsons, J. (1962). *Proc. R. Ent. Soc.* (*c*), **27**, 21–22.
Rothschild, A. M. and Schayer, R. W. (1958). *Fed. Proc.*, **17**, 136.
Schayer, R. W. (1957). *Amer. J. Physiol.*, **189**, 533–536.
Telford, J. M. and West, G. B. (1961a). *J. Pharm. Pharmacol.*, **13**, 75–82.
Telford, J. M. and West, G. B. (1961b). *J. Physiol.*, **157**, 306–314.
Uvnäs, B. (1960). *Ann. N.Y. Acad. Sci.*, **90**, 751–759.
Waton, N. G. (1956). *Brit. J. Pharmacol.*, **11**, 119–127.
Weissbach, H., Lovenberg, W. and Udenfriend, S. (1961). *Biochim. biophys. acta*, **50**, 177–179.
Welsh, J. H. (1960). *Nature, Lond.*, **186**, 811–812.
Werle, E. and Raub, A. (1948). *Biochem. Z.*, **318**, 538–544.

Papaverine-like Pharmacological Properties of Rotenone

SIR,—Recent investigations showed papaverine and some structurally related compounds to be powerful inhibitors of oxidative phosphorylation (Santi, Ferrari and Contessa, 1963). A relation also seemed possible between the spasmolytic activity of papaverine-like drugs and their inhibition of the respiratory chain, thus explaining the similarity of effects elicited by anoxia, by some enzyme inhibitors and by papaverine, on the drug-induced contraction of intestinal smooth muscle. Under these experimental conditions it was observed (West, Hadden and Farah, 1951; Santi Contessa and Ferrari, 1963) that the isolated gut failed to give normal tonic responses to acetylcholine, histamine and $BaCl_2$ showing only an immediate, short-lasting contraction. This inability of smooth muscle to maintain tone was considered as "the first evidence of interference with energy production" (West, Hadden and Farah, 1951). For papaverine and allied drugs we pointed out that a similar effect may be elicited through a strong inhibition of electron-transfer reactions, between DPN and cytochrome b. The site of action is therefore the same as the one recognised for amytal (Ernster, Jalling, Low and Lindberg, 1955), rotenone (Ernster, Dallner and Azzone, 1963) and allyloxibenzamide (Bruni and Contessa, 1961). With regard to the selectivity of the action and to the degree of activity, among the inhibitors of oxidative phosphorylation rotenone appears as the one most closely related to papaverine (Ernster, Dallner and Azzone, 1963; Santi, Contessa and Ferrari, 1963). This similarity of biochemical properties prompted a pharmacological comparison between rotenone and papaverine. The purpose was to examine the reliability of the previously proposed hypothesis on the mechanism of action of papaverine.

In the present investigations on rotenone and papaverine we have studied the spasmolytic activity on isolated gut (guinea-pig ileum and rabbit duodenum); the vasodilator effect on the hind-limb of the dog, by recording the arterial femoral flow with a Shipley and Wilson rotameter; the effects on respiration and arterial blood pressure in rabbits and dogs.

Rotenone at final concentrations ranging from 10^{-8} to 10^{-9} (w/v) inhibits selectively the tonic response of intestinal smooth muscle to acetylcholine and histamine without preventing the immediate short-lasting contraction. Thus rotenone mimics the spasmolytic effect of papaverine, but its degree of activity is