Brit. J. Pharmacol. (1961), **17**, 278–285.

EFFECT OF 5-HYDROXYTRYPTAMINE ON THE RESPIRATION OF EXCISED LAMELLIBRANCH GILL

BY

K. E. MOORE, A. S. MILTON* AND R. E. GOSSELIN

From the Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, New Hampshire

(*Received August* 21, 1961)

5-Hydroxytryptamine, but not acetylcholine or catecholamines, stimulated the endogenous respiration of the excised gills of *Modiolus demissus* and *Mytilus edulis*. Respiratory stimulation by 5-hydroxytryptophan is presumed to have occurred only after it had been decarboxylated to 5-hydroxytryptamine. 2-Bromolysergic acid diethylamide inhibited the effect of 5-hydroxytryptamine, while lysergic acid diethylamide mimicked it. The glycogen that was degraded during incubation of the gill cannot account for all of the oxygen that was consumed, indicating that some other substrate within the gill was also oxidized. That the metabolic actions of 5-hydroxytryptamine may be related to its cilio-acceleratory activity is discussed.

There have been numerous observations to suggest that in many invertebrates 5-hydroxytryptamine acts as a neurohumoral agent (Welsh, 1955). Aiello (1957) and Gosselin & Ernst (1958) reported that 5-hydroxytryptamine caused a marked acceleration of the lateral cilia on the excised gill of *Mytilus edulis* and *Modiolus demissus*. Subsequent studies have revealed that the gill of *Mytilus* contains measurable quantities of 5-hydroxytryptamine and also the enzymes that are capable of producing (Milton & Gosselin, 1960) and of degrading (Blaschko & Milton, 1960) this amine. As a result of these findings Gosselin (1961) postulated that 5-hydroxytryptamine serves as a humoral regulator of the cilia on these gills.

Ciliary activity appears to be dependent upon the metabolism of the gill, since various metabolic inhibitors reduce ciliary activity (Gray, 1924 ; Weller & Ronkin, 1952 ; Aiello, 1960). The lack of information on the metabolism and the factors that control the metabolism of molluscan gill (Martin, 1961) prompted us to study the effects of 5-hydroxytryptamine on the metabolism of the excised gill in the hope of correlating the cilio-acceleratory actions of 5-hydroxytryptamine with some of its metabolic actions. In this paper we report the effects of 5-hydroxytryptamine and certain other neurohumoral agents on the respiration of the excised gills of two marine molluscs, *Mytilus edulis* and *Modiolus demissus*.

METHODS

Specimens of both *Modiolus demissus* from the Marine Biological Laboratories at Woods Hole and *Mytilus edulis* from Ipswich, Mass., were stored in moist containers at 4° C. Under

* Present address: Department of Pharmacology, University of Edinburgh, Scotland.

these conditions *Modiolus* remained viable for more than two weeks. *Mytilus*, which are less hardy, were used within a week of shipping.

The entire gill was excised and immersed in artificial sea water of the following composition: NaCl, 423 mM; KCl, 9 mM; $CaCl_2$, 9.27 mM; $MgCl_2$, 22.9 mM; $MgSO_4$, 25.5 mM; $NaHCO_3$, 2.15 mM; final *p*H 7.75 to 7.85. After 20 to 30 min secreted mucus was removed, and each gill was cut into 2 or 3 pieces of equal size. One piece (70 to 100 mg wet weight) was placed in the main compartment of each Warburg flask. With sea water in the main compartment and agents to be added in the side arms, the final total volume of the reaction mixture was 2.0 ml. The centre well contained 0.2 ml. 2.5 N NaOH. The gas phase was oxygen and the bath temperature was 25° C. After a 10-min period of equilibration the manometer was read every 30 min for as long as 6 hr. Except in Tables 1 and 5, measured rates of oxygen uptake are reported as % of the control respiration. Specifically the "% of control respiration" equals 100 times the rate of oxygen consumption during the first hour after tipping divided by the rate for the 1 hr period before tipping. Gill protein was determined by a modification of the method of Gornall, Bardowill & David (1949) and was expressed as crystalline bovine albumin equivalent. Glycogen was determined by the anthrone method of Seifter, Dayton, Novic & Muntwyler (1950).

Drug concentrations are reported as the initial extracellular concentrations in contact with the gill tissue. The following drugs were used: 5-hydroxytryptamine creatinine sulphate, 5-hydroxytryptophan, acetylcholine iodide, (−)-adrenaline bitartrate, (−)-noradrenaline bitartrate, (±)-isoprenaline hydrochloride, eserine sulphate, lysergic acid diethylamide and 2-bromolysergic acid diethylamide.

RESULTS

Under the conditions of laboratory storage *Mytilus edulis* did not survive as well as *Modiolus demissus*; the latter was therefore used for most of the experiments. Qualitatively similar results, however, were obtained with both species. When expressed as μl. of O_2 consumed per hr per mg of gill protein, the qO_2 in 50 separate determinations of *Modiolus* was 4.0 ± 0.09 (mean and standard error). Comparable values for 50 determinations in *Mytilus* were more than 50% higher (6.3 ± 0.17). Excised gill of both species continued to respire at a constant rate for at least 6 hr.

Effect of 5-hydroxytryptamine

As seen in Table 1, the rate of oxygen consumption by *Modiolus* gill in the presence of 5-hydroxytryptamine was significantly higher than the rate for the control

TABLE 1

EFFECT OF 5-HYDROXYTRYPTAMINE ON THE OXYGEN CONSUMPTION OF EXCISED GILL FROM *MODIOLUS DEMISSUS*

5-Hydroxytryptamine was added to the flasks at the start of the experiment, and the qO_2 was based upon a 2-hr incubation. qO_2 represents consumption of μl. O_2/hr/mg gill protein. Values are mean±standard error of mean. *=Significantly different from control at 1% level

5-Hydroxytryptamine concentration	No. of expts.	qO_2
None (control)	9	4·0±0·22
10^{-6} M	5	4·4±0·55
10^{-5} M	6	5·8±0·65*
10^{-4} M	12	7·8±0·33*

gill. Because the respiration of the control gill was somewhat variable, stimulation by 5-hydroxytryptamine was more evident when each gill served as its own control. When measured in this manner a significant increase in the oxygen consumption

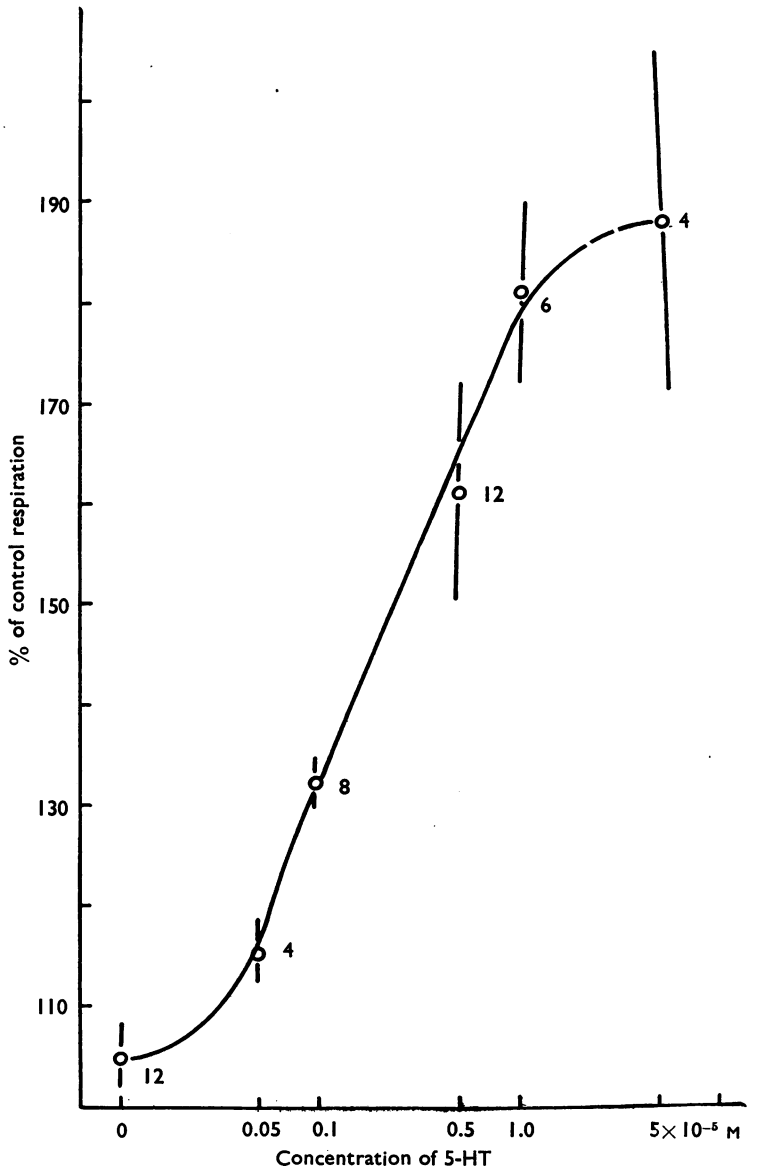

Fig. 1. Effect of 5-hydroxytryptamine on oxygen consumption of excised gill from *Modiolus demissus*. 5-Hydroxytryptamine was added after a 90-min control period. Each point represents the mean and the vertical line the standard error of the mean; the number beside each point denotes the number of experiments.

was observed at 10^{-6} M 5-hydroxytryptamine (Fig. 1). *Mytilus* gill was somewhat more sensitive to the action of 5-hydroxytryptamine; a significant rise in oxygen uptake was observed at 10^{-7} M. *Mytilus* is also more sensitive to the cilio-acceleratory actions of 5-hydroxytryptamine than is *Modiolus*; for dose-response curves on both

species see Gosselin (1961). The stimulatory effect of 5-hydroxytryptamine was rapid in onset; the maximal rate was noted in the first 30-min reading and was sustained for several hours. Neither control nor 5-hydroxytryptamine-stimulated respiration was affected by the addition of substrate amounts (0.01 M) of glutamate, glucose, succinate or α-ketoglutarate.

Effect of 5-hydroxytryptophan

It was reported previously (Milton & Gosselin, 1960) that 5-hydroxytryptophan produces prolonged stimulation of the beat frequency of lateral cilia on both *Mytilus* and *Modiolus* gill. When compared to the effects of 5-hydroxytryptamine, the response to 5-hydroxytryptophan was slower to develop and resistant to washing. Similar differences were encountered when the actions of 5-hydroxytryptophan on the respiration of the excised gill were studied. When 10^{-4} to 10^{-3} M 5-hydroxytryptophan was added, the oxygen uptake increased progressively with time; a maximal response was not reached until the second hour after addition of the compound (Table 2).

TABLE 2

EFFECT OF 5-HYDROXYTRYPTOPHAN ON OXYGEN CONSUMPTION OF EXCISED GILL FROM *MODIOLUS DEMISSUS*

Conc. of 5-hydroxy-tryptophan	No. of expts.	% of control respiration (mean±standard error)			
		1st hr	2nd hr	3rd hr	4th hr
None	7	102±2·5	106±2·1	105±3·1	107±4·5
10^{-5} M	8	106±3·3	111±2·8	109±3·8	112±7·5
10^{-4} M	8	142±13	162±12	161±11	152±15
10^{-3} M	8	149±4·8	169±10	165±13	146±33

Effect of other neurohormones: acetylcholine and catecholamines

Acetylcholine has been proposed as a cilio-regulatory hormone in the gill of *Mytilus edulis* (Bülbring, Burn & Shelley, 1953; Milton, 1959). The cilio-acceleratory action of this compound is much less pronounced and less sustained than that of 5-hydroxytryptamine (Gosselin, unpublished). When acetylcholine was added to the excised gill, either alone or in combination with eserine, there was no significant difference (at 1% level) from the control oxygen consumption (Table 3). Although data from only one concentration of acetylcholine are reported

TABLE 3

EFFECT OF ACETYLCHOLINE AND CATECHOLAMINES ON THE OXYGEN CONSUMPTION OF EXCISED GILL FROM *MODIOLUS DEMISSUS*

The concentration of each agent used was 10^{-4} M. Only two experiments were performed using isoprenaline; both results are given

Treatment	No. of expts.	% of control respiration (mean±standard error)
None	4	96±7·5
Eserine	4	100±4·2
Acetylcholine (ACh)	4	91±4·7
ACh+eserine	4	86±6·7
None	4	103±2·5
Adrenaline	4	107±6·4
Noradrenaline	4	116±4·5
Isoprenaline	2	107; 100

in Table 3, the results of experiments with other concentrations (10^{-7} to 10^{-4} M) were not different. Also summarized in Table 3 are the effects of three catecholamines. Although these compounds exert a pronounced calorigenic action and stimulate glycolysis in many vertebrate tissues, they had no prominent effect on the rate of oxygen uptake by these molluscan gills. The stimulatory effect of noradrenaline is significant at the 5% but not at the 1% level. However, even during the first experimental period, auto-oxidation (and perhaps enzymatic oxidation) of these amines occurred. If their oxidative destruction could have been inhibited by methods that did not interfere with tissue respiration, it is conceivable that different results might have been obtained.

Effect of 5-hydroxytryptamine antagonists

Attempts were made to block the respiratory stimulation seen after addition of 5-hydroxytryptamine with the 5-hydroxytryptamine antagonists, lysergic acid diethylamide and 2-bromolysergic acid diethylamide. Pronounced differences were observed between these two agents (Table 4). At 10^{-4} M lysergic acid diethylamide stimulated

TABLE 4

EFFECT OF 5-HYDROXYTRYPTAMINE ANTAGONISTS ON THE OXYGEN CONSUMPTION OF EXCISED GILL FROM *MODIOLUS DEMISSUS*

LSD=Lysergic acid diethylamide; BOL=2-bromolysergic acid diethylamide; 5-HT=5-hydroxytryptamine. *=Significantly different from control at 1% level

Treatment	No. of expts.	% of control respiration (mean±standard error)	
1. None	4	100±2·1	
2. LSD (10^{-4} M)	8	258±25*	
3. 5-HT (10^{-5} M)	4	209±28*	3 vs 4, $P=0·2$
4. LSD+5-HT	4	272±41*	
1. None	6	101±5·3	
2. BOL (10^{-4} M)	6	113±4·7	
3. 5-HT (10^{-5} M)	6	211±13*	3 vs 4, $P<0·01$
4. BOL+5-HT	6	141±6·3*	

markedly the oxygen consumption of gills; smaller but significant effects were seen even at 10^{-6} M (not shown). Since a combination of 5-hydroxytryptamine (10^{-5} M) and lysergic acid diethylamide (10^{-4} M) resulted in a somewhat higher (not significant at the 1% level) rate of respiration than with either agent alone, no antagonism could be demonstrated. On the other hand, 2-bromolysergic acid diethylamide enhanced only slightly the gill respiration. When added along with 5-hydroxytryptamine, 2-bromolysergic acid diethylamide significantly reduced the stimulation produced by the former.

Effect of 5-hydroxytryptamine on glycogen breakdown

Since no exogenous substrate was required to maintain the respiration of excised gill, it was of interest to establish the nature of the endogenous substrates. The glycogen content of gill was determined before and after incubation in sea water. A representative series of analyses is shown in Table 5. Because the glycogen content of freshly excised gills was somewhat variable, each experiment was performed with the gills from one specimen. The concentration of glycogen was usually lower in *Mytilus* than in *Modiolus*, at least after the molluscs had been stored in

TABLE 5

EFFECT OF 5-HYDROXYTRYPTAMINE ON ENDOGENOUS CARBO HYDRATE METABOLISM OF EXCISED LAMELLIBRANCH GILL

Each experiment was performed on the gill from one specimen at 25° C in an atmosphere of 100% oxygen. All values are based on a 3-hr period of incubation and are adjusted to 100 mg weight of gill

	Modiolus		*Mytilus*	
	μl. O_2 consumed	Glycogen content (μg)	μl. O_2 consumed	Glycogen content (μg)
Expt. 1				
Not incubated	—	660	—	410
Sea water only	134	617	192	288
10^{-5} M 5-hydroxy-tryptamine in sea water	239	577	370	240
Expt. 2				
Not incubated	—	430	—	414
Sea water only	94	366	226	356
10^{-5} M 5-hydroxy-tryptamine in sea water	196	346	391	219

the laboratory. During incubation of the excised gill the glycogen content decreased; this reduction was accelerated by the addition of 5-hydroxytryptamine. Glycogen breakdown was somewhat more rapid in *Mytilus* than in *Modiolus*. Barely detectable amounts of lactic acid were observed in the incubation media at the end of these experiments.

DISCUSSION

It appears that there is a parallel between the 5-hydroxytryptamine-induced stimulation of oxygen consumption of excised lamellibranch gill and the stimulatory effects of this agent on the cilia of these gills. The respiratory response is not as sensitive to 5-hydroxytryptamine as is the beat frequency of the lateral cilia (Gosselin, 1961). However, other types of cilia on the same gills (for example, frontal and abfrontal cilia) are probably less sensitive to 5-hydroxytryptamine (Gosselin & O'Hara, 1961). Since oxygen uptake reflects the metabolism of all cells in the excised gill, including many that are not ciliated, it is not surprising that the correspondence between ciliary activity and gill oxygen consumption is not precise. Other agents that produce an acceleration of ciliary beat frequency, such as 5-hydroxytryptophan, lysergic acid diethylamide, and, to lesser extent, 2-bromo-lysergic acid diethylamide (Gosselin & Ernst, 1958), also produce an increase in the rate of oxygen uptake. Veratrine, which stimulates the beat frequency of *Mytilus* gill cilia, is also reported to increase the oxygen consumption of the excised gill (Aiello, 1960). Conversely, the catecholamines, which have equivocal effects on beat frequency (Bülbring *et al.*, 1953; Gosselin, unpublished data), have no pronounced effect on gill respiration. On the other hand, acetylcholine, which has been reported to have some excitatory activity on ciliary beat frequency (Bülbring *et al.*, 1953), does not augment the oxygen consumption.

Data on the respiratory actions of 5-hydroxytryptophan are consistent with the hypothesis (Milton & Gosselin, 1960) that it is the 5-hydroxytryptamine formed from 5-hydroxytryptophan and not the 5-hydroxytryptophan itself that is

responsible for the stimulatory effects of this compound on gill cilia. In contrast to the prompt responses to all other cilio-excitatory substances, 5-hydroxytryptophan produces a gradual rise in the rate of oxygen consumption over a period of 2 hr. As reported elsewhere (Milton & Gosselin, 1960), an increase in the endogenous 5-hydroxytryptamine content occurs when gills are incubated in 5-hydroxytryptophan solutions.

The effects of the 5-hydroxytryptamine antagonists are difficult to explain. Both 2-bromolysergic acid diethylamide and lysergic acid diethylamide were reported to stimulate cilia in low concentrations and to prevent the stimulation of cilia induced by 5-hydroxytryptamine in higher concentrations (Gosselin & Ernst, 1958). Studies of gill oxygen consumption led to distinctly different conclusions. Although 2-bromolysergic acid diethylamide displayed only slight metabolic activity alone, it effectively prevented the respiratory effects of 5-hydroxytryptamine. On the other hand, lysergic acid diethylamide had marked stimulatory effects, but did not prevent the actions of 5-hydroxytryptamine (Table 4). No explanation of these differences can be offered, but it is perhaps relevant that studies of ciliary activity were carried out with 5-hydroxytryptamine and its antagonists at concentrations 40-fold less than those specified in Table 4. It is also interesting to note that lysergic acid diethylamide mimicked, but 2-bromolysergic acid diethylamide blocked, the effects of 5-hydroxytryptamine on the carbohydrate metabolism of *Fasciola hepatica* (Mansour, 1959).

As in former studies of marine molluscan tissue (Glaister & Kerly, 1936), the addition of exogenous substrates failed to elevate the oxygen consumption of the excised tissues. Similarly, negative results were also obtained by Martin (1961) with tissues of cephalopods and snails. Undoubtedly one of the endogenous substrates is glycogen. Although glycogen breakdown was shown during aerobic incubation with and without added 5-hydroxytryptamine, the amount of glucose liberated, if completely oxidized, could account for only 30 to 50% of the oxygen consumed. Apparently the oxidation of some other substrates accounts for the remainder of the oxygen uptake. Further studies are required to establish the nature of these substrates.

The question of whether the effect of 5-hydroxytryptamine on the carbohydrate metabolism of the gills is due to a direct action on some metabolic enzyme system or whether it is secondary to increased ciliary activity is an important one. This report does not shed any light on this question. Efforts to stimulate the respiration of gill homogenates by the addition of 5-hydroxytryptamine have so far been unsuccessful. It would appear that the integrity of the cell must be preserved in order to show the metabolic effects of 5-hydroxytryptamine. Further work needs to be done on this point. In recent studies, Moore & Gosselin (1961) have found that 5-hydroxytryptamine markedly stimulates the anaerobic glycolysis of the excised gill. Since it has been reported that gill cilia do not beat under anaerobic conditions (Aiello, 1960), it would appear that 5-hydroxytryptamine exerts a direct effect on carbohydrate metabolism. We feel that the effect of 5-hydroxytryptamine on the carbohydrate metabolism may be responsible for both the increased oxygen consumption and accelerated ciliary activity that is observed after the addition of 5-hydroxytryptamine.

This work was supported by NSF grant G 14248 and PHS grant RG 5145. The lysergic acid diethylamide and 2-bromolysergic acid diethylamide were generously supplied by Sandoz Pharmaceuticals, Hanover, New Jersey. The technical assistance of Mrs J. Sawdy is gratefully acknowledged. One of us (A. S. M.) was a visiting Fulbright lecturer.

REFERENCES

AIELLO, E. L. (1957). Influence of the branchial nerve and of 5-hydroxytryptamine on the ciliary activity of *Mytilus* gill. *Biol. Bull.*, **113**, 325.

AIELLO, E. L. (1960). Factors affecting ciliary activity on the gill of the mussel *Mytilus edulis*. *Physiol. Zool.*, **33**, 120–135.

BLASCHKO, H. & MILTON, A. (1960). Oxidation of 5-hydroxytryptamine and related compounds by *Mytilus* gill plates. *Brit. J. Pharmacol.*, **15**, 42–46.

BÜLBRING, E., BURN, J. H. & SHELLEY, H. J. (1953). Acetylcholine and ciliary movement in the gill plates of *Mytilus edulis*. *Proc. roy. Soc. (Lond.), B*, **141**, 445–466.

GLAISTER, D. & KERLY, M. (1936). Oxygen consumption and carbohydrate metabolism of the retractor muscle of the foot of *Mytilus edulis*. *J. Physiol. (Lond.)*, **87**, 56–66.

GORNALL, A. G., BARDOWILL, C. J. & DAVID, M. M. (1949). Determination of serum proteins by means of the Biuret reaction. *J. biol. Chem.*, **177**, 751–766.

GOSSELIN, R. E. (1961). The cilioexcitatory activity of serotonin. *J. cell. comp. Physiol.*, **58**, in press.

GOSSELIN, R. E. & ERNST, M. M. (1958). Action of serotonin on the gill cilia of lamellibranchiates. *Fall Meeting of American Society for Pharmacology and Experimental Therapeutics*. Abstracts, Ann Arbor, Michigan.

GOSSELIN, R. E. & O'HARA, G. (1961). An unsuspected source of error in studies of particle transport by lamellibranch gill cilia. *J. cell. comp. Physiol.*, **58**, in press.

GRAY, J. (1924). The mechanism of ciliary movements. IV, Relation of ciliary activity to oxygen consumption. *Proc. roy. Soc. (Lond.), B.*, **96**, 95–114.

MANSOUR, T. E. (1959). The effect of serotonin and related compounds on the carbohydrate metabolism of the liver fluke *Fasciola hepatica*. *J. Pharmacol. exp. Ther.*, **126**, 212–216.

MARTIN, A. W. (1961). *Comparative Physiology of Carbohydrate Metabolism in Heterothermic Animals*, ed. A. W. MARTIN, p. 48. Seattle: University of Washington Press.

MILTON, A. S. (1959). Choline acetylase in the gill plates of *Mytilus edulis*. *Proc. roy. Soc. (Lond.), B.*, **150**, 240–244.

MILTON, A. S. & GOSSELIN, R. E. (1960). Metabolism and cilio-accelerator action of 5-hydroxytryptophan (5-HTP) in gill plates of *Mytilus* and *Modiolus*. *Fed. Proc.*, **19**, 126.

MOORE, K. E. & GOSSELIN, R. E. (1961). Effect of 5-hydroxytryptamine (5-HT) on glycolysis in lamellibranch gill. *The Pharmacologist*, **3**, 77.

SEIFTER, S., DAYTON, S., NOVIC, B. & MUNTWYLER, E. (1950). The estimation of glycogen with the anthrone reagent. *Arch. Biochem.*, **25**, 191–200.

WELLER, H. & RONKIN, R. R. (1952). Effects of 2,4-dinitrophenol upon oxygen consumption and ciliary activity in ctenidia of *Mytilus*. *Proc. Soc. exp. Biol., N.Y.*, **81**, 65–66.

WELSH, J. H. (1955). *The Hormone, Physiology, Chemistry, and Applications*, ed. PINCUS, G. & THIMANN, K. V., p. 107. New York: Academic Press.