J. mar. biol. Ass. U.K. (1960) 39, 51–61
Printed in Great Britain

STEROID SULPHATASE, ARYLSULPHATASE AND β-GLUCURONIDASE IN MARINE INVERTEBRATES

By E. D. S. CORNER
The Plymouth Laboratory

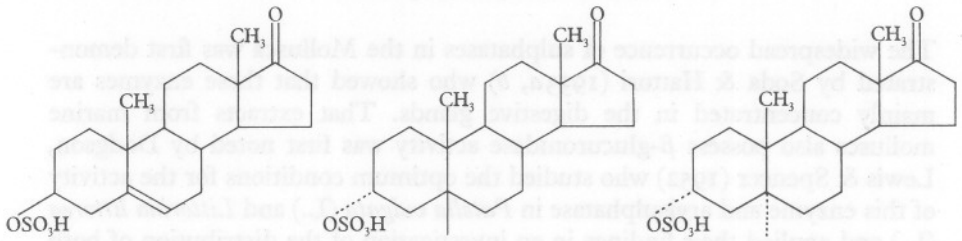
Y. A. LEON AND R. D. BULBROOK
Division of Chemistry and Biochemistry, Imperial Cancer Research Fund, London, W.C. 2

The widespread occurrence of sulphatases in the Mollusca was first demonstrated by Soda & Hattori (1933*a*, *b*) who showed that these enzymes are mainly concentrated in the digestive glands. That extracts from marine molluscs also possess β-glucuronidase activity was first noted by Dodgson, Lewis & Spencer (1952) who studied the optimum conditions for the activity of this enzyme and arylsulphatase in *Patella vulgata* (L.) and *Littorina littorea* (L.) and applied their findings in an investigation of the distribution of both enzymes among various other marine molluscs (Dodgson, Lewis & Spencer, 1953).

Molluscan tissues have also been investigated as a possible source of a sulphatase capable of hydrolysing the steroid conjugates normally encountered in human urine (Henry & Thevenet, 1952; Stitch & Halkerston, 1953*a*, *b*; 1956; Jayle & Baulieu, 1954; Savard, Bagnoli & Dorfman, 1954; Roy, 1954, 1956*a*; Leon, Bulbrook & Corner, 1960). The main reason for such studies has been the need to find an alternative to acid hydrolysis of the steroid conjugates, a method which causes much destruction and alteration of some of the liberated steroids. However, although many of the gastropods studied possess an enzyme capable of hydrolysing dehydro*epi*androsterone sulphate (I), and some species belonging to this class possess an enzyme which will also hydrolyse aetiocholanolone sulphate (II), it has not been possible to detect an enzyme which would effect the hydrolysis of androsterone sulphate (III). This means that complete enzymic hydrolysis of all the steroid conjugates present in human urine cannot be achieved with any of the molluscan enzymes studied so far. Moreover, sulphatases from other sources, such as mammalian liver (Gibian & Bratfisch, 1956; Roy, 1957), fungi (Cohen & Bates, 1949; Stitch & Halkerston, 1953*b*), and bacteria (Buehler, Katzman & Doisy, 1950) have also proved to be unsuitable for this purpose.

The present work is an extension of an earlier study (Leon *et al.*, 1960) in which a search was made for a convenient source of a 'steroid sulphatase'

that would effect the hydrolysis of androsterone sulphate. In this paper the term 'steroid sulphatase' is used to describe any enzyme which will effect the hydrolysis of a steroid sulphate. However, since the role of these enzymes in mammalian and molluscan tissues is not known, the use of this term is not intended to imply physiological function. In the earlier investigation all the animals used belonged to the mollusca, but in the present study species representative of eight additional marine invertebrate phyla have been examined, and the activities of arylsulphatase and β-glucuronidase in these animals have been examined in relation to their diets, habitats and methods of feeding. No steroid sulphatase has been detected in any marine animal tested other than certain molluscs of the class gastropoda, and there appears to be no correlation between the activity of this enzyme and the diet of the



animal in which it is present. Moreover, even the most active preparations of steroid sulphatase so far studied have no measurable influence on the hydrolysis of androsterone sulphate, and an enzyme capable of effecting this change has therefore still to be found.

MATERIALS AND METHODS

Animals. Test species were collected from the intertidal zone or from the off-shore waters in the vicinity of Plymouth, with the exception of *Helix pomatia* L., specimens of which were gathered in a Sussex garden. This terrestrial species was included for comparison because enzyme preparations from *Helix* have been used for the hydrolysis of steroid conjugates. Full names of all the test species are given in Tables 1 and 2, but are not hereafter used in any part of the text other than the summary.

Extraction of enzymes. Acetone-dried powders were prepared from the tissues of approximately twenty animals of each test species. Exceptions to this were *Marinogammarus*, *Ligia* and *Procerodes* where much larger numbers of test animals were used; and *Maia*, *Cancer*, *Clionacelata*, *Ciona* and *Sepia* where the number of test animals was only two or three. Whenever possible the digestive gland was the tissue used in preparing the enzymes, but in many instances dissection of this organ was prohibited by the smallness of the test species, and the whole animal was then used.

Estimations of enzyme activity. Arylsulphatase was assayed using dipotassium 2-hydroxy-5-nitrophenylsulphate (NCS) and potassium nitrophenylsulphate (NPS) as substrates; the method employed was that described by Dodgson *et al.* (1953).

Androsterone, dehydro*epi*androsterone and aetiocholanolone sulphates (AS, DHAS and AeS respectively), were used in estimations of steroid sulphatase activity by the method of Roy (1956*a*, *b*). β-Glucuronidase was assayed by the method of Dodgson *et al.* (1953), using *p*-chlorophenylglucuronide monohydrate as the substrate.

RESULTS

β-Glucuronidase and arylsulphatase

The experimental findings, summarized in Tables 1 (Mollusca) and 2 (other phyla), demonstrate the widespread occurrence of β-glucuronidase and arylsulphatase among marine invertebrates. Thus, each of the twenty-three species tested was found to possess some arylsulphatase activity, although two species with low activity towards NCS (*Ligia* and *Antedon*) were inactive when NPS was used as substrate. β-Glucuronidase activity was found in eighteen of the species tested, but was not detected in *Turritella* (Mollusca), *Marinogammarus* and *Ligia* (Arthropoda), *Clionacelata* (Porifera) and *Amphioxus* (Cephalochorda).

The number of species used in experiments with molluscs was sufficient to allow examination of the possibility of a correlation between enzyme activity on the one hand and diet and habitat on the other (Table 3). The figures in the first row of the table include all the species of molluscs tested and show that β-glucuronidase activity is much greater in the herbivorous than in the carnivorous or detritus-eating species. There is a similar but less marked difference in the mean values for arylsulphatase activity. These figures include the results obtained using acetone-dried powders prepared from two herbivorous and two detritus-eating species where the whole animal was used. If the enzymes are mainly concentrated in the digestive gland then only the results of experiments in which these glands were used are strictly comparable. The figures in the second row of the table are derived from the results on species where it was possible to dissect out the digestive glands, and they emphasize the difference in β-glucuronidase levels between the herbivorous, the carnivorous, and the detritus-eating species. The figures show again the raised arylsulphatase levels in the herbivores compared with the carnivores but the mean value for the arylsulphatase of the detritus-eating species is not significantly different from that of the herbivores. It is worth mentioning that the differences between the enzyme activities of herbivorous and carnivorous molluscs would be even greater were it not for the anomalous result obtained using *Nassarius*, which possesses a very high arylsulphatase and β-glucuronidase activity, but is described as carnivorous (see Table 1). However, although the animal is reported to feed largely on carrion, there is a possibility that it may inadvertently ingest a certain amount of plant material as well, and so, to some extent, be omnivorous (A. Graham, private communication).

TABLE 1. β-GLUCURONIDASE AND ARYLSULPHATASE ACTIVITY IN MOLLUSCS

Species	Test material: Habitat	Test material: Feeding and diet	Source of enzyme	Enzyme activities*: Arylsulphatase NCS	Enzyme activities*: Arylsulphatase NPS	Enzyme activities*: β-Glucuronidase
Patella vulgata (L.)	Intertidal	Browses on algal sporelings and diatoms (1)	Visceral hump	652,335	157,450	155,900
Littorina littorea (L.)	Intertidal	Browses on algal sporelings and diatoms (1) (3)	Whole animal without shell	585,860	168,920	69,070
Lepidochitona cinerea (L.)	Intertidal	Diatoms and algal detritus (1)	Whole animal	41,200	25,220	10,890
Patina pellucida (L.)	Intertidal	*Laminaria* and attached sporelings and diatoms (1) (2)	Whole animal	25,620	7,450	23,790
Aplysia punctata (Cuvier)	Intertidal	*Ulva*, *Fucus* (1) (4)	Digestive gland	287,390	55,030	28,520
Nucella lapillus (L.)	Intertidal	Carnivorous (barnacles and mussels) (1)	Digestive gland	96,260	39,550	15,480
Nassarius reticulatus (L.)	Offshore; mud deposits	Carnivorous (barnacles and mussels (1)	Digestive gland	435,930	235,610	75,800
Buccinum undatum L.	Offshore; gravel and mud deposits	Carnivorous (crabs, worms, etc.) (1)	Digestive gland	227,830	81,400	14,620
Turritella communis (Risso)	Offshore; gravel and mud deposits	Ciliary feeder on detritus (7)	Whole animal	181,440	37,070	0
Crepidula fornicata (L.)	Offshore; gravel and mud deposits	Ciliary feeder on detritus (1) (5)	Digestive gland	74,100	9,360	8,310
Pecten maximus (L.)	Offshore; gravel and mud deposits	Ciliary feeder on detritus (6)	Digestive gland	941,110	449,050	16,910
Sepia officinalis (L.)	Pelagic	Carnivorous (*Crangon*, etc.)	Digestive gland	58,860	19,490	9,740
Helix pomatia (L.)	Terrestrial	Herbivorous (green plants)	Digestive gland	759,670	367,260	230,700
			Crop fluid	2,551,170	823,190	58,460

Arylsulphatase activity tested at pH 5·5; β-glucuronidase activity at pH 4·0; Activities are expressed as μg. phenol liberated/g powder/h.
References: (1) Graham, 1955; (2) Graham & Fretter, 1947; (3) Graham (unpublished observations); (4) Eales, 1921; (5) Orton, 1912; (6) Hunt, 1925; (7) Yonge, 1947.

* These figures for enzymic activity have been previously reported by Leon *et al.* (1960) and are reproduced here to show the correlation between enzyme activity and ecological data.

TABLE 2. β-GLUCURONIDASE AND ARYLSULPHATASE ACTIVITY IN ANIMALS OF PHYLA OTHER THAN MOLLUSCA

Phylum	Test material: Class	Test material: Species	Habitat	Feeding and diet	Source of enzyme	Enzyme activities: Arylsulphatase NCS	Enzyme activities: Arylsulphatase NPS	Enzyme activities: β-Glucuronidase
Arthropoda	Malacostraca	*Cancer pagurus* L.	Intertidal	Carnivorous	Digestive gland	157,200	45,860	15,480
Arthropoda	Malacostraca	*Maia squinado* (Herbst)	Offshore; sandy or rocky localities	Carnivorous; also browses on algal tufts (1)	Digestive gland	280,460	77,580	7,450
Arthropoda	Malacostraca	*Marinogammarus marinus* (Leach)	Intertidal	Browses; chiefly vegetable detritus	Whole animal	38,780	9,940	0
Arthropoda	Malacostraca	*Ligia oceanica* (L.)	Intertidal	Browses; *Fucus* and small epiphytic algae on large sea weeds (3)	Whole animal	7,270	0	0
Coelenterata	Anthozoa	*Calliactis parasitica* (Couch)	Offshore; attached to shells inhabited by *Eupagurus*	Carnivorous	Whole animal	21,470	7,640	5,450
Echinodermata	Crinoidea	*Antedon bifida* (Pennant)	Offshore; mud deposits	Ciliary feeder; detritus and small living organisms	Whole animal	10,040	0	7,740
Porifera	Demospongiaria	*Clionacelata* (Grant)	Offshore; boring in rocks	Ciliary feeder; detritus and small living organisms	Whole animal	88,290	28,850	0
Platyhelminthes	Turbellaria	*Procerodes ulvae* (Oersted)	Intertidal	Browses; unicellular algae (2)	Whole animal	9,700	3,630	9,310
Tunicata*	Ascidiacea	*Ciona intestinalis* (L.)	Offshore; attached to submerged structures	Ciliary feeder; detritus (4)	Whole animal	—	—	—
Cephalochorda*	—	*Amphioxus lanceolatus* (Pallas)	Offshore; shell gravel	Ciliary feeder; detritus (5)	Digestive system	109,420	22,550	0
Annelida	Polychaeta	*Chaetopterus variopedatus* (Renier)	Offshore; muddy gravel	Ciliary feeder; detritus (6)	Whole animal	36,010	14,140	24,500

Experimental conditions as in Table 1.
References: (1) Carlisle (1957); (2) Spooner (unpublished observations); (3) Nicholls (1931); (4) MacGinitie (1939*b*); (5) Hunt (1925); (6) MacGinitie (1939*a*).
* Sub-phyla of the Chordata.

Although Dodgson *et al.* (1953) state that enzymic activity cannot be correlated with feeding habits, re-examination of their results shows the same trend as that in Table 3—namely, that the herbivorous molluscs have greater levels of β-glucuronidase and arylsulphatase activity than the carnivorous species.

Reference to both Tables 1 and 2 shows that of the eight species examined which are thought to feed on detritus, four were without β-glucuronidase-activity; whereas, of a further fourteen species which appear to be definitely carnivorous or herbivorous, only one (*Ligia*) was found to lack the enzyme. Correlation between habitat and enzyme activity merely reflects the fact that the herbivorous species are intertidal, whereas the carnivorous animals may, in addition, be pelagic or live off-shore in gravel or muddy deposits. Tables 1 and 2 show that many of the species that contain sulphatase and β-glucuronidase dwell on a substratum of muddy composition and the question arises of whether their enzymes are of bacterial origin, not being extracted from the tissues of the animals but from the bacterial flora that inhabit them. However, this does not seem likely, for Dodgson, Melville, Spencer & Williams (1954) have shown that the bacteria present in the digestive organs of certain marine molluscs have only a very low arylsulphatase activity; and it has been found in the present work that acetone-dried powders, prepared from samples of mud collected from the habitats of the various test animals, possessed only a slight enzymic activity, far less than would account for the activities of the preparations obtained using the tissues of the animals concerned.

'*Steroid sulphatase*'

'Steroid sulphatase' activity appears to be confined to the phylum mollusca; it was not detected in any of the other invertebrate phyla. It was only found in seven of the mollusca tested, each of these belonging to the subclass Prosobranchia of the class Gastropoda. Moreover, the enzyme was not found in all members of the Prosobranchia; and it was absent from the only species representing a different subclass of the gastropods, *Aplysia* from the Opisthobranchia (Table 4). The gastropods can be divided into two groups, according to the specificity of their steroid sulphatase. Thus, enzymes from four of the species tested would only effect the hydrolysis of DHAS, whereas enzymes from three species were active when both DHAS and AeS were used as substrates. In Table 4 the species are listed in order of maximum DHAS sulphatase activity, and it is of interest to note that the species with the highest activity are those which will also hydrolyse AeS. Two of these species belong to the carnivorous order, Stenoglossa; the third is the herbivorous land snail, *Helix*. It was not possible to detect an enzyme that would effect the hydrolysis of AS in any of the species tested. There is no apparent correlation between steroid sulphatase activity and diet and habitat because, of the marine molluscs that possess this enzyme, three are carnivores and three are herbivores.

TABLE 3. THE RELATIONSHIP BETWEEN ENZYMIC ACTIVITY AND FEEDING HABITS IN THE MOLLUSCA

The enzyme levels found for the digestive gland of *Helix pomatia* are included but not those found for the crop fluid.
The figures in the table are mean levels, calculated from the data in Table 1.
The figures in brackets in the columns headed NCS refer to the number of results from which the mean was derived. Units of enzyme activity are those defined under Table 1.

	NCS			NPS			β-Glucuronidase		
	Herbivores	Carnivores	Detritus	Herbivores	Carnivores	Detritus	Herbivores	Carnivores	Detritus
All preparations	462,175 (5)	204,720 (4)	309,463 (4)	151,222	94,013	130,175	101,596	29,810	9,027
Digestive gland preparations only	566,465 (3)	204,720 (4)	507,600 (2)	193,247	94,013	229,205	138,373	28,910	12,610

TABLE 4. STEROID SULPHATASE ACTIVITY OF VARIOUS MOLLUSCS

Activities are expressed as μg steroid liberated/g Powder A

Species	Order	Subclass	Class	Steroid sulphatase AeS	Steroid sulphatase DHAS
Nassarius reticulatus	Stenoglossa	Prosobranchia	Gastropoda	10,409	10,650
Helix pomatia	—	—	Gastropoda	8,138	8,147
Buccinum undatum	Stenoglossa	Prosobranchia	Gastropoda	12,056	3,592
Nucella lapillus	Stenoglossa	Prosobranchia	Gastropoda	0	2,900
Patella vulgata	Archaeogastropoda	Prosobranchia	Gastropoda	0	2,012
Patina pellucida	Archaeogastropoda	Prosobranchia	Gastropoda	0	900
Littorina littorea	Mesogastropoda	Prosobranchia	Gastropoda	0	891
Turritella communis	Mesogastropoda	Prosobranchia	Gastropoda	0	0
Crepidula fornicata	Mesogastropoda	Prosobranchia	Gastropoda	0	0
Aplysia punctata	Aplysiomorpha	Opisthobranchia	Gastropoda	0	0
Pecten maximus	Pseudo-lamellibranchia	—	Lamellibranchia	0	0
Lepidochitona cinerea	—	—	Polyplacophora	0	0
Sepia officinalis	Decacera	—	Cephalopoda	0	0

The very active preparation of steroid sulphatase obtained from the digestive glands of *Nassarius* has been used in studies of the enzyme under different environmental conditions. The findings have shown that when DHAS is used as the substrate enzymic activity at 37° C. is four times as great as that at 12° C., and that when the test medium consists of 50% sea water, all enzymic activity disappears.

DISCUSSION

β-Glucuronidase and arylsulphatase

Vertebrates possess enzyme systems that effect the conjugation of phenolic substrates with sulphuric and glucuronic acids. Many phenolic substances, both of endogenous and exogenous origin, are excreted in the urine after conjugation as sulphates or glucuronides. In contrast, the physiological significance of β-glucuronidase, and various sulphatases which are concerned in the hydrolysis of glucuronic and sulphuric acid conjugates and are of widespread occurrence, has not yet been established. The possible function of these enzymes in the marine invertebrates is the hydrolysis of glucuronides and sulphates present in the diet. Thus, the finding that so many species of marine invertebrates possess arylsulphatase and a β-glucuronidase implies that these animals, although they show marked differences of phyla, habitat and method of feeding, must all live on diets containing sulphuric acid and glucuronic acids in conjugated form. These substances occur as structural units in certain polysaccharides and mucopolysaccharides. Thus, the hemicelluloses of plant origin contain glucuronic acid, and other polysaccharides such as hyaluronic acid and chondroitin sulphate, found in animal tissues, contain sulphuric acid as well. While the polysaccharide commonly found in marine plants (alginic acid) and animals (chitin) possesses uronic acids other than glucuronic as the structural unit, it is possible that polysaccharides other than alginic acid and chitin occur in the diets of marine animals. However, the exact substrates on which the invertebrate enzymes act are still unknown (Roy, 1956*a*).

The view that β-glucuronidase and arylsulphatase are involved in digestion is supported to some extent by the finding that, compared with carnivorous molluscs, herbivores possess a much higher enzyme activity; for it seems likely that this is because the latter species have to deal with a diet consisting of plants with thick cell walls.

'Steroid sulphatase'

Unlike arylsulphatase and β-glucuronidase, steroid sulphatase appears to have no obvious role as a digestive enzyme. The animals that possess it have very varied diets and feeding habits, which also closely resemble those of certain members of other classes and phyla from which the enzyme is absent.

The distribution of steroid sulphatase is interesting because the enzyme has been detected in only two classes in the ten phyla so far examined. These are the mammalia (Gibian & Bratfisch, 1956; Roy, 1957); and certain molluscs of the class Gastropoda, subclass Prosobranchia. Moreover, among marine gastropods, only those of the order Stenoglossa were found to possess an enzyme capable of hydrolysing AeS. However, the significance of the distribution of steroid sulphatase remains difficult to assess until a more comprehensive study has been made using a larger number of specimens from each phylum.

Preliminary results which show that a steroid sulphatase of use in the hydrolysis of AeS is exclusively confined to the order Stenoglossa (among marine invertebrates) are sufficiently interesting to make further investigation worthwhile. In addition, the discovery that *Nassarius* provides a rich source of sulphatase for use in steroid analysis warrants a more detailed investigation of the nature of the enzyme found in this animal. However, the specificity of the steroid sulphatase, present in the few available species that have been found to possess it, is such that the enzyme cannot be used to achieve complete hydrolysis of all the urinary steroid conjugates; the sulphates of 3α:5α steroids can still only be hydrolysed by chemical methods.

The writers are indebted to Mrs Ann Peace for valuable technical assistance; to the captains and crews of R.V.s 'Sula' and 'Sarsia' for collecting most of the test animals; to Mr N. A. Holme for many helpful discussions, and to Prof. A. Graham for his advice and interest.

SUMMARY

A study has been made of the distribution of β-glucuronidase, arylsulphatase and 'steroid sulphatase' in nine marine invertebrate phyla.

Species representative of all the phyla examined possess β-glucuronidase and arylsulphatase, but only certain gastropod molluscs, subclass Prosobranchia have a 'steroid sulphatase'. Two marine species, *Buccinum undatum* and *Nassarius reticulatus*, possess an enzyme of specificity similar to that of the sulphatase obtained from the land snail, *Helix pomatia*, in that it assists the hydrolysis of both aetiocholanolone and dehydro*epi*androsterone sulphates. However, the 'steroid sulphatase' prepared from three other marine species, *Patella vulgata*, *Patina pellucida* and *Littorina littorea*, has a higher specificity, assisting the hydrolysis of only the latter substrate. An enzyme that will effect the hydrolysis of androsterone sulphate has yet to be found.

Experiments with molluscs have shown that the β-glucuronidase and arylsulphatase activities of herbivorous species are, in general, greater than those of carnivores. Further experiments, using members of all the phyla examined, have shown that, compared with herbivorous and carnivorous species, animals

which feed on detritus are more often lacking in β-glucuronidase activity. However, this correlation between diets and enzymic activity is not found in experiments with 'steroid sulphatase' and there is evidence consistent with the view that this enzyme may not perform any physiological function related to the hydrolysis of steroid sulphates in the few species in which it has been found.

REFERENCES

BUEHLER, H. J., KATZMAN, P. A. & DOISY, E. A., 1950. Hydrolysis of 17-ketosteroid conjugates. *Fed. Proc.*, Vol. 9, p. 157.

CARLISLE, D. B., 1957. On the hormonal inhibition of moulting in decapod crustacea. *J. mar. biol. Ass. U.K.*, Vol. 36, pp. 291–307.

COHEN, H. & BATES, R. W., 1949. Hydrolysis of conjugated sulfates of oestrogens by commercial enzyme preparations of *Aspergillus oryzea*. *Endocrinology*, Vol. 44, pp. 317–24.

DODGSON, K. S., LEWIS, J. I. M. & SPENCER, B., 1952. Arylsulphatase activity in marine molluscs. *Biochem. J.*, Vol. 51, xiii.

—— —— —— 1953. The arylsulphatase and β-glucuronidase of marine molluscs. *Biochem. J.*, Vol. 55, pp. 253–59.

DODGSON, K. S., MELVILLE, T. H., SPENCER, B. & WILLIAMS, K., 1954. The aryl-sulphatase of a strain of *Alcaligines metalcaligines* isolated from intertidal mud. *Biochem. J.*, Vol. 58, pp. 182–7.

EALES, N. B., 1921. *Aplysia*. *L.M.B.C. Mem.*, No. 24, 92 pp.

GIBIAN, H. & BRATFISCH, G., 1956. Eine Steroidsulfatase aus Säugetierleber. *Hoppe-Seyl. Z.*, Vol. 305, pp. 265–8.

GRAHAM, A., 1955. Molluscan diets. *Proc. malac. Soc. Lond.*, Vol. 31, pp. 144–59.

GRAHAM, A. & FRETTER, V., 1947. The life history of *Patina pellucida* (L.). *J. mar. biol. Ass. U.K.*, Vol. 26, pp. 590–601.

HENRY, R. & THEVENET, M., 1952. Action du suc digestif d'*Helix pomatia* L. sur les différents stéroids conjugés urinaires. *Bull. Soc. Chim. biol., Paris*, Vol. 34, pp. 886–96.

HUNT, O. D., 1925. The food of the bottom fauna of the Plymouth fishing grounds. *J. mar. biol. Ass. U.K.*, Vol. 13, pp. 560–99.

JAYLE, M. F. & BAULIEU, E. E., 1954. Données expérimentales sur l'hydrolyse des 17-cétostéroids conjugés urinaires par les diastases du suc digestif d'*Helix pomatia* L. *Bull. Soc. Chim. Biol., Paris*, Vol. 36, pp. 1391–1406.

LEON, Y. A., BULBROOK, R. D. & CORNER, E. D. S., 1960. In the Press.

MACGINITIE, G. E., 1939*a*. The method of feeding of *Chaetopterus*. *Biol. Bull., Woods Hole*, Vol. 77, pp. 115–18.

—— 1939*b*. The method of feeding of tunicates. *Biol. Bull., Woods Hole*, Vol. 77, pp. 443–47.

NICHOLLS, A. G., 1931. Studies on *Ligia oceanica*. Part. II. The process of feeding, digestion and absorption, with a description of the structure of the foregut. *J. mar. biol. Ass. U.K.*, Vol. 17, pp. 675–705.

ORTON, J. H., 1912. The mode of feeding of Crepidula, with an account of the current-producing mechanism in the mantle cavity, and some remarks on the mode of feeding in Gastropods and Lamellibranchs. *J. mar. biol. Ass. U.K.*, Vol. 9, pp. 444–78.

ROY, A. B., 1954. The steroid sulphatase of *Patella vulgata*. *Biochim. Biophys. Acta*, Vol. 15, pp. 300–1.

ROY, A. B., 1956*a*. The steroid sulphatase of *Patella vulgata*. *Biochem. J.*, Vol. 62, pp. 41–50.

—— 1956*b*. The enzymic synthesis of steroid sulphates. *Biochem. J.*, Vol. 63, pp. 294–300.

—— 1957. Steroid sulphatase. *Biochem. J.*, Vol. 66, pp. 700–3.

SAVARD, K., BAGNOLI, E. & DORFMAN, T. I., 1954. Neutral steroid sulfatase. *Fed. Proc.*, Vol. 13, p. 289.

SODA, T. & HATTORI, C., 1933*a*. Gluco-sulfatase. III. *Bull. chem. Soc. Japan*, Vol. 8, pp. 65–9.

—— —— 1933*b*. Gluco-sulfatase. IV. *J. chem. Soc. Japan*, Vol. 54, pp. 377–8.

STITCH, S. R. & HALKERSTON, I. D. K., 1953*a*. Enzymatic hydrolysis of urinary neutral 17-ketosteroid conjugates. *Nature, Lond.*, Vol. 172, pp. 398–9.

—— —— 1953*b*. The enzymatic hydrolysis of neutral 17-ketosteroid conjugates. *J. Endocrin.*, Vol. 9, p. xxxvi.

—— —— 1956. Hydrolysis of steroid conjugates in urine. *Biochem. J.*, Vol. 63, pp. 710–15.

YONGE, C. M., 1947. On the habits of *Turritella communis* (Risso). *J. mar. biol. Ass. U.K.*, Vol. 26, pp. 377–80.