CHAPTER 10

Nitrogen Metabolism

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I. NITROGENOUS CONSTITUENTS OF MOLLUSCAN ORGANISMS

A. *Amino Acids*

A large proportion of the dry matter of molluscan bodies is made up of amino acids, either free or combined to form macromolecules among which the proteins are predominant. The pattern of the amino acid composition of the bulk of the proteins of molluscan tissues is similar to the pattern generally observed in animal tissues (Fig. 1 in Duchâteau *et al.*; 1954). On the other hand, no common pattern is observed in the free amino acid pools of molluscan tissues (Tables I–V and VIII–XI).

TABLE I

DISTRIBUTION OF AMINO ACIDS IN THE PROTEIN AND NONPROTEIN COMPONENTS OF MUSCLES IN BIVALVIA[a]

Amino acid	*Mytilus edulis* Adductor muscle[b]			*Ostrea edulis* Total adductor muscles[b]			*Anodonta cygnea* Total adductor muscles[b]		
	Total hydrolyzed	Nonprotein	Protein	Total hydrolyzed	Nonprotein	Protein	Total hydrolyzed	Non-protein	Protein
Alanine	2300	163	2137	1350	364	986	720	3.2	716.8
Arginine	2770	199	2571	920	37.5	882.5	1040	13.3	1026.7
Aspartic acid	3160	45.4	3114.6	1110	14.7	1095.3	1290	1.6	1288.4
Glutamic acid	5020	152	4868	2230	149	2081	1730	10.7	1719.3
Glycine	1350	191	1159	1450	140	1310	700	4.8	695.2
Histidine	1420	5.8	1414.2	250	12.9	237.1	200	0.9	199.1
Isoleucine	1550	11.9	1538.1	860	10.8	849.2	620	2.3	617.7
Leucine	2380	7.4	2373.6	1380	7.3	1372.7	1040	1.3	1038.7
Lysine	2210	18.9	2191.1	1520	12.4	1507.6	1060	3	1057.0
Methionine	710	4.7	705.3	420	4.8	415.2	290	0.1	289.9
Phenylalanine	770	4.6	751.5	540	4.8	535.2	370	0.6	369.4
Proline	790	13.9	776.1	630	93.7	536.3	450	0.4	449.6
Threonine	1280	14.6	1265.4	590	5.5	584.5	510	1.3	508.7
Tyrosine	900	6.1	893.9	430	5.8	424.2	350	0.8	349.2
Valine	1210	6.9	1203.1	730	6.1	723.9	500	1.2	498.8
	27820	845.2	26964.9	14410	869.3	13540.7	10870	45.5	10824.5

[a] Determination by microbiological assay. Values stated in milligrams per 100 gm of fresh tissue.
[b] Duchâteau and Florkin, unpublished (1951).

TABLE II

Distribution of Amino Acids in the Protein and Nonprotein Components of Tissues in Gastropods[a]

Amino acid	*Buccinum undatum* Foot[b]			*Buccinum undatum* Foot[b]			*Buccinum undatum* Penis[c]			*Haliotis tuberculata* Foot[c]			*Patella vulgata* Foot[c]			*Lymnaea stagnalis* Foot[c]		
	Total hydrolyzed	Non-protein	Protein	Total hydrolyzed	Non-protein	Protein	Total hydrolyzed	Non-protein	Protein	Total hydrolyzed	Non-protein	Protein	Total hydrolyzed	Non-protein	Protein	Total hydrolyzed	Non-protein	Protein
Alanine	1170	153	1017	940	79.4	860.6	1150	52.2	1097.8	560	19.9	540.1	1130	101	1029	1050	61.4	988.6
Arginine	1130	174	956	1310	184	1126	1800	199	1601	940	607.9	322.1	1410	320	1090	880	54.2	825.8
Aspartic acid	1230	9.9	1220.1	1360	14.4	1345.6	1830	21.5	1808.5	530	21.1	508.9	1150	53.5	1096.5	1860	84.2	1775.8
Glutamic acid	2020	72.2	1947.8	2370	88	2282	3050	74.5	2975.5	1030	51.9	978.1	2200	94.2	2105.8	1870	167	1703
Glycine	1380	24.5	1355.5	1640	27	1613	1580	14.7	1565.3	1230	73.7	1156.3	1280	27.7	1252.3	1440	50.2	1389.8
Histidine	230	9.7	220.3	260	9.5	250.5	320	3.7	316.3	94	7.4	86.6	170	3.1	166.9	210	7.8	202.2
Isoleucine	590	16.9	573.1	770	5.9	764.1	1010	5.8	1004.2	330	16.8	313.2	—	13	—	810	20.7	789.3
Leucine	1090	15.3	1074.7	1360	7.3	1352.7	1680	5.1	1674.9	510	8.8	501.2	1270	6.2	1263.8	1020	27.3	992.7
Lysine	910	13.6	896.4	1270	4.2	1265.8	1480	5.5	1474.5	510	24.8	485.2	840	7	833	880	31.2	848.8
Methionine	380	5.6	374.4	450	7.6	442.4	540	2.4	537.6	—	—	—	—	5.3	—	170	3.9	166.1
Phenylalanine	430	6.3	423.7	450	4.8	445.2	580	3.1	576.9	210	7.3	203.7	420	3.5	416.5	490	18.8	471.2
Proline	690	36	654	780	41	739	800	3.6	796.4	390	9.5	380.5	780	491	289	640	29.1	610.9
Threonine	590	24.1	565.9	650	16.2	633.8	890	7.4	882.6	320	26.6	293.4	580	21.2	557.8	680	26	654
Tyrosine	430	6.5	423.5	500	10.2	489.8	660	2.9	657.1	190	9.1	180.9	440	10.7	429.3	330	3.9	326.1
Valine	630	16.8	613.2	790	7.3	782.7	980	4.8	975.2	310	8.6	301.4	620	17.2	602.8	660	21.9	638.1
	12900	584.4	12315.6	14900	506.8	14393.2	18350	406.2	17943.8							12990	607.6	12382.4

[a] Determination by microbiological assay. Values stated in milligrams per 100 gm of fresh tissue.

[b] Duchâteau *et al.* (1954).

[c] Duchâteau-Bosson and Florkin, unpublished (1951–1952).

TABLE III

DISTRIBUTION OF AMINO ACIDS IN THE PROTEIN AND NONPROTEIN COMPONENTS OF MUSCLES IN *Octopus vulgaris*[a]

Amino acid	Sucker[b]			Sucker[b]			Mantle[b]			Mantle[b]		
	Total hydrolyzed	Nonprotein	Protein	Total hydrolyzed	Nonprotein	Protein	Total hydrolyzed	Nonprotein	Protein	Total hydrolyzed	Nonprotein	Protein
Alanine	530	6.3	522.7	940	13.2	926.8	620	82	538	520	27.7	492.3
Arginine	760	179	581	1330	218	1112	760	305	455	740	136.3	603.7
Aspartic acid	780	5.7	774.3	1410	5.9	1404.1	800	66.5	733.5	680	13.5	666.5
Glutamic acid	1100	21.3	1078.7	2030	30.7	1999.3	1250	147	1103	1030	74.3	955.7
Glycine	1040	7.6	1032.4	1790	15.2	1774.8	1050	110.9	939.1	1350	34.8	1315.2
Histidine	150	0.5	149.5	280	1.3	278.7	170	9.6	161.4	140	1.4	138.6
Isoleucine	590	5.5	584.5	—	6	—	500	35.2	464.8	—	11.7	—
Leucine	610	3.5	606.5	1210	3.1	1206.9	680	38.1	641.9	580	4.7	575.3
Lysine	680	4.2	675.8	1290	4.1	1285.9	750	53.9	696.1	570	8.9	561.1
Methionine	—	1	—	—	—	—	—	—	—	—	—	—
Phenylalanine	290	1.1	288.9	550	1.8	548.2	330	16.8	313.2	270	2.5	267.5
Proline	460	2	458	710	0.5	709.5	490	58	432	490	11.8	478.2
Threonine	400	2.7	397.3	700	4	696	430	29.9	400.1	340	5.5	334.5
Tyrosine	270	1.2	268.8	500	3.4	496.6	310	15.9	294.1	250	3.1	246.9
Valine	350	2.3	347.7	670	2.5	667.5	400	21.5	378.5	310	4.9	305.1

[a] Determination by microbiological assay. Values stated in milligrams per 100 gm of fresh tissue.
[b] Duchâteau and Florkin, unpublished (1951).

1. Free Amino Acids

Data have been gathered concerning the composition of the proteins of a given tissue and of the free amino acid pool of the same tissues (Tables I–III). These compositions are different, a conclusion which is not unexpected. Each cell contains a large number of proteins, enzymes or others, and they differ with respect to their composition and amino acid sequences. The concentrations of these proteins are different, and each has a different speed of turnover. In the muscle tissue of the rabbit, for instance, the turnover is twice as rapid for aldolase as it is for glyceraldehyde-3-phosphate dehydrogenase (M. V. Simpson and Velick, 1954; Heimberg and Velick, 1954). There is nothing astonishing, therefore, in the fact that the amino acid pattern of the proteins of a tissue differs from the free amino acid pool in the same tissue.

Chemical isolation and characterization of free amino acids from molluscan tissues goes back to the nineteenth century. Chittenden (1875) isolated glycine from the muscles of *Pecten irradians,* Ackermann (1922) obtained arginine from *Mytilus edulis,* and Ackermann *et al.* (1922) from *Eledone moschata.* Owing to analytical progress, and particularly to the advent of the microbiological assay method for the determination of amino acids, more complete analyses were made possible. Noland (1949) used this method for determinations of some amino acids in molluscs. In the laboratory of the present author the microbiological method was used in a number of analyses (Tables I–IV) and later replaced by the more reliable method of automatic analyses by column chromatography according to Spackman *et al.* (1958) (Tables VIII–XI). While these analyses have been mostly concerned with the amino acids found in proteins, free amino acids also have been detected. J. W. Simpson *et al.* (1959) found β-alanine in several species of molluscs. Awapara and Allen (1959) isolated β-aminoisobutyric acid from *Mytilus edulis.* As β-alanine and β-aminoisobutyric acid are the products of pyrimidine metabolism in vertebrate tissues, their study is of importance.

Rather large proportions of the nitrogen present in the excreta of molluscs may be represented by the nitrogen of free amino acids (see Albritton, 1955; Table 112, p. 200).

2. Proteins

Apart from hemoglobins and hemocyanins (see Chapter 6 by Read and Chapter 7 by Ghiretti) very few data exist in the literature on the composition of specific proteins of molluscs. Analyses have been made on the conchiolins of the prismatic layer (Roche *et al.,* 1951) and the nacreous layer of shell (Grégoire *et al.,* 1955; Florkin *et al.,* 1961; see also Vol. I, Chapter 8). Data on the submicroscopic structure of nacreous conchiolin and on its amino acid composition have also been reported for fossil shells (Florkin *et al.,* 1961).

TABLE IV

FREE AMINO ACIDS FOR THREE GASTROPODS[a]

Amino acid	*Ampullaria glauca*		*Helix pomatia* Hepatopancreas[c]		*Telescopium telescopium* L. Crystalline style[d]
	Hepato-pancreas[b]	Shell gland[b]	Nonhydrolyzed	Hydrolyzed	
Alanine	—	—	19.9	18.5	2.9
Arginine	15.9	3.7	—	—	—
Aspartic acid	27.6	2.0	11.5	29.9	4.2
Glutamic acid	67.7	12.9	110.1	113.6	2.6
Glycine	45.2	2.4	17.8	26.2	7.4
Histidine	4.6	0.2	2.9	2.1	—
Isoleucine	19.2	1.2	8.6	9.1	0.2
Leucine	26.0	1.9	6.8	8.6	
Lysine	8.1	2.4	4.7	6.7	—
Methionine	14.7	0.0	2.6	2.6	—
Phenylalanine	9.3	1.1	2.8	4.4	—
Proline	14.9	1.2	9.8	11.5	—
Threonine	18.5	2.7	8.4	10.8	6.7
Tyrosine	4.4	0.6	—	—	6.3
Valine	19.0	1.5	6.3	7.7	5.5

[a] Determination by microbiological assay. Values stated as milligrams per 100 gm of fresh tissue.

[b] Duchâteau and Florkin, in Florkin (1954).

[c] Duchâteau and Florkin, unpublished (1957).

[d] Swaminathan (1958).

TABLE V

FREE AMINO ACIDS IN THREE SPECIES OF CEPHALOPODS[a]

Amino acid	*Ommastrephes sloani pacificus*[b]		*Sepia esculenta*[c]	*Octopus ochellatus*[c]
	NH	H		
Alanine	75	83	179	15
Arginine	99	98	280	146
Aspartic acid	Trace	12	29	22
Glutamic acid	26	61	44	29
Glycine	42	82	104	23
Histidine	140	139	12	1.8
Isoleucine	15	25	12	6.3
Leucine	18	28	23	6.3
Lysine	9	24	32	7.6
Methionine	12	11	32	3.6
Phenylalanine	6	8	9.3	4.3
Proline	479	553	379	8.5
Threonine	23	28	53	7.3
Tyrosine	4	3	8.4	1.7
Valine	20	31	26	9.2

[a] Determination by microbiological assay. Values stated as milligrams per 100 gm of fresh muscle. [b] Konosu *et al.* (1958). [c] Ito (1957–1958).

B. *Taurine*

This sulfonic amino acid (2-aminoethanesulfonic acid; $NH_2CH_2CH_2SO_3H$) has been reported for several classes of molluscs (Cephalopoda: Krukenberg and Wagner, 1885; Henze, 1905, 1913, 1914; Suzuki and

TABLE VI
TAURINE CONTENT OF VARIOUS MOLLUSCS[a]

Mollusc	Habitat	Taurine present
Gastropoda		
Lymnaea palustris	Fresh water	−
Marisa cornuarietis	Fresh water	−
Pomacea bridgesi	Fresh water	−
Rumina decollata	Terrestrial	−
Otala lactea	Terrestrial	−
Mesodon thyroidus	Terrestrial	−
Bulimulus alternatus	Terrestrial	−
Murex fulvescens	Marine	+
Littorina irrorata	Marine	+
Oliva sayana	Marine	+
Polinices duplicata	Marine	+
Busycon perversum	Marine	+
Siphonaria lineolata	Marine	+
Fasciolaria distans	Marine	+
Thais haemastoma haysae	Marine	+
Bivalvia		
Anodonta grandis	Fresh water	−
Quadrula quadrula	Fresh water	−
Lampsilis sp.	Fresh water	−
Elliptio sp.	Fresh water	−
Rangia cuneata	Brackish-fresh water	+
Brachyodontes recurvus	Brackish-marine	+
Crassostrea virginica	Brackish-marine	+
Donax variabilis	Marine	+
Venus mercenaria	Marine	+
Dosinia discus	Marine	+
Arca incongrua	Marine	+
Arca campechiensis	Marine	+
Noetia ponderosa	Marine	+
Cephalopoda		
Lolliguncula brevis	Marine	+

[a] J. W. Simpson *et al.* (1959).

Joshimura, 1909; Okuda, 1929; Ackermann *et al.*, 1924. Gastropoda: Mendel, 1904; Mendel and Bradley, 1906; Schmidt and Watson, 1917. *Mytilus edulis:* Kelley, 1904). J. W. Simpson *et al.* (1959) have studied the distribution of taurine in molluscs (Table VI) and have found it in high concentration in all the marine species studied, but not in fresh-water and terrestrial forms (or less than 0.1 μmole/gm). This fact should be correlated with the role of taurine as one of the effectors of intracellular osmolar concen-

tration (Bricteux-Grégoire *et al.*, 1964a,b) which is decreased in marine bivalves transferred into brackish water. It may be postulated that the adaptation to life in fresh water is accompanied by an even more pronounced lowering of the level of the intracellular concentration of taurine.

C. *Ammonia*

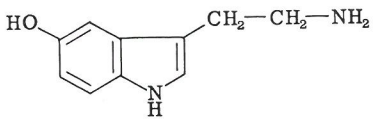
Older data on blood ammonia in invertebrates, and in molluscs in particular, have been collected in tables by Delaunay (1931, 1934). In this research, no attention had been given to the eventual variations the ammonia could show *in vitro* during the period of time before analysis. Measuring the amount of ammonia in the blood of *Anodonta cygnea* collected under paraffin, Florkin and Houet (1938) detected the presence of 51–71 μg of ammonia per 100 ml. In the blood of the snail *Helix pomatia,* Florkin and Renwart (1939) found much larger values, between 0.7 and 2 mg of ammonia per 100 ml. Contrary to what occurs in the blood of mammals and of birds, there is no increase in the concentration of ammonia more than an hour after the collection of the blood.

Ammonia has been reported present in the excreta of a number of molluscs (for references, see Albritton, 1955).

D. *Amines*

The decarboxylation of amino acids results in the formation of amines which can be considered as substitution products of ammonia and have the following structures: RNH_2, RR_1NH, and RR_1R_2N.

1. 5-Hydroxytryptamine (5-HT, Enteramine, Serotonin)



This is the specific secretion product of chromaffin cells and is therefore found in the tissues in which such cells are present, such as the posterior salivary glands of *Octopus vulgaris* and *Eledone moschata* (Vialli and Erspamer, 1940; Erspamer, 1940; Erspamer and Boretti, 1951). It is lacking in the anterior salivary glands of Octopoda as well as the posterior salivary glands of *Sepia officinalis* (Ottaviano, 1942), of *Loligo vulgaris,* and of *Octopus macropus,* these tissues having no chromaffin cells (Erspamer, 1952). According to Florey and Florey (1954), 5-HT could represent the nerve transmitter in the heart of cephalopods (see Chapter 4, Section V).

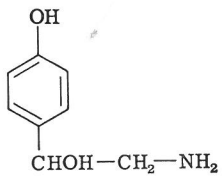
2. Tyramine (4-Hydroxyphenylethylamine): $HOC_6H_4CH_2CH_2NH_2$

Tyramine has been isolated by Henze (1913) in the form of a dibenzoyl derivative from extracts of the posterior salivary glands of *Octopus macropus,*

and by Erspamer (1952) in the form of a dibenzoyl derivative and a picrate from the posterior salivary gland of *Octopus vulgaris.*

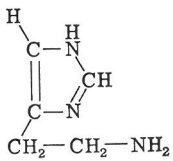
Tyramine has been considered by several authors to be the poison of cephalopod saliva (Henze, 1913; Baglioni, 1909). Fredericq (1947) has considered tyramine as playing a role in the humoral transmission of the impulses of the cardiomotor nerves in cephalopods. It appears, however, that tyramine is not necessarily present in the toxic secretions of octopods and even less in the toxic secretions of cephalopods (Erspamer and Boretti, 1951). Tyramine is found in large amounts in the posterior glands of *Octopus macropus* (1000–1500 μg per gram of fresh tissue) and of *Octopus vulgaris* (500–800 μg per gram of fresh tissue), but is lacking in the glands of *Eledone moschata* and *Sepia officinalis* (Erspamer, 1952). It has also been found in the urine of *Octopus hongkongiensis* (Emmanuel, 1957).

3. OCTOPAMINE (*p*-HYDROXYPHENYLETHANOLAMINE)



Octopamine is found in large amounts in the salivary glands of *Octopus vulgaris* (700–1200 μg per gram of fresh tissue). It is less abundant in the glands of *Eledone moschata,* and is present only in small amounts in the gland of *Octopus macropus* (Erspamer, 1952).

4. HISTAMINE (4-IMIDAZOLETHYLAMINE)



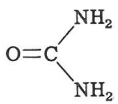
This compound has been found in the extracts of the posterior gland of *Octopus macropus* and of *Eledone moschata,* and in varying and smaller amounts in *Octopus vulgaris* (Ungar *et al.,* 1937; Bacq and Ghiretti, 1951; Erspamer, 1952). It has also been isolated from the salivary gland of the marine gastropod *Neptunea arthritica* Bernardi (Asano and Itoh, 1960).

5. TETRAMINE: $C_4H_{13}ON$ OR $(CH_3)_4NOH$

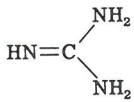
Tetramine has been isolated from the salivary glands of the marine gastropods *Neptunea arthritica* Bernardi, *Neptunea intersculpta, Fusitriton oregonensis, Buccinum leucostoma* (Asano and Itoh, 1959, 1960) and *Neptunea antiqua* (Fänge, 1958).

E. *Urea*

Urea H_2NCONH_2 has been found in small amounts in the tissues of *Helix pomatia* (Albrecht, 1923; Delaunay, 1931). It has been reported to be present in the excreta of a number of molluscs (see Albritton, 1955, Table 112, pp. 206 and 207).



Urea



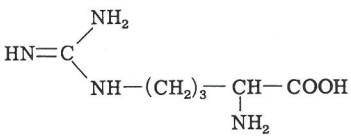
Guanidine

F. *Guanidic Derivatives*

Guanidine is an imino derivative of urea.

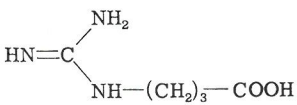
A number of derivatives of guanidine have been found in molluscs:

1. An Amino Acid

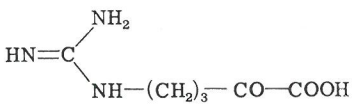


Arginine (α-amino-δ-guanidinovaleric acid)

2. ω-Guanidic Acids

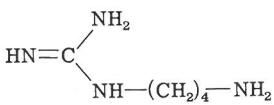


γ-Guanidinobutyric acid

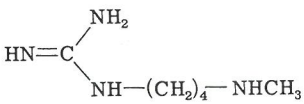


α-Keto-δ-guanidinovaleric acid

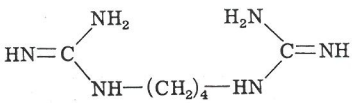
3. Bases



Agmatine

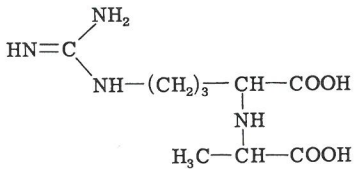


Methylagmatine



Arcaine

4. CONDENSATION PRODUCTS OF ARGININE



Octopine

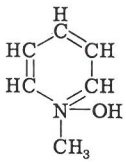
In many animal species, arginine is the basis of the phosphagen phosphoarginine. This is the case for all molluscs so far examined (Meyerhof, 1928; Lohmann, 1936; Robin *et al.*, 1959a). γ-Guanidinobutyric acid has been detected in a number of species, notably, *Pecten maximus, Mytilus edulis,* and *Sepia officinalis* (Thoai *et al.*, 1953). Agmatine is present in *Octopus vulgaris* (Irvin and Wilson, 1939), in *Eledone moschata* (Ackermann and Mohr, 1937) as well as in the bivalve *Arca noae* (Robin *et al.*, 1959b). There is no example, so far, of the presence of creatine, phosphocreatine, methylguanidine, glycocyamine, or taurocyamine in a mollusc (Roche *et al.*, 1957). Arcaine was found in *Arca noae* (Ackermann, 1931; Kutscher *et al.*, 1931).

A number of other guanidine derivatives have been found in molluscs: methylagmatine in the muscle of *Octopus* (Iseki, 1931); octopine (Okuda, 1929; Ackermann and Mohr, 1937; Mayeda, 1936; Moore and Wilson, 1936, 1937; Irvin, 1938; Roche *et al.*, 1952b; Humoto, 1954); and α-keto-δ-guanidinovaleric acid, along with γ-guanidinobutyric acids in many species (Thoai *et al.*, 1952, 1953; Roche *et al.*, 1952b; Robin and Thoai, 1957).

G. *Methylated Bases*

1. N-METHYLPYRIDINIUMHYDROXIDE: C_6H_9ON

This compound has been isolated from *Mytilus edulis* by Ackermann (1922).



2. TRIMETHYLAMINO OXIDE: $(CH_3)_3NO$

Henze (1914), Kojima and Kusakabe (1956), Asano and Sato (1954), and Konosu *et al.* (1958) have reported the isolation of this compound from muscles of cephalopods. Other authors failed to identify it, or

trimethylamine, in the urine of *Octopus vulgaris* (Hoppe-Seyler and Linneweh, 1931), in muscles of cephalopods (Ackermann *et al.*, 1922, 1924), or in the adductor muscles of *Mytilus edulis* (Bricteux-Grégoire *et al.*, 1964a) or of *Ostrea edulis* (Bricteux-Grégoire *et al.*, 1964b).

3. GLYCINE-BETAINE: $(CH_3)_3—N^+—CH_2—COOH$

This has been found in a number of molluscs: *Pecten irradians, Sycotypus canaliculatus, Mytilus edulis, Octopus vulgaris, Eledone moschata, Arca noae* (see Ackermann, 1962), *Ostrea edulis* (Bricteux-Grégoire *et al.*, 1964b), *Octopus honkongiensis* (urine) (Emmanuel, 1957), *Patella* (Ackermann and Janka, 1954), *Arion empiricorum* (Ackermann and Menssen, 1960), *Polypus punctatus* (Takahashi, 1915).

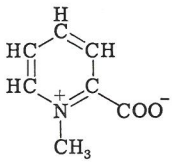
4. CARNITINE (β-oxy-γ-butyrobetaine):
$C_7H_{15}O_3N$ OR $(CH_3)_3—N^+—CH_2—CHOH—CH_2COO^-$

This betaine has been isolated from *Octopus octopodia* (Morizawa, 1927) and from *Arca noae* (Kutscher and Ackermann, 1933).

5. STACHYDRINE (*N*-methyl-D,L-proline-methylbetaine):
$C_7H_{13}O_2N + H_2O$

Stachydrine has been found in *Arca noae* (Kutscher and Ackermann, 1933).

6. HOMARINE (methylbetaine of α-pyridine-2-carboxylic acid or methylpicolinic acid)



Isolated from *Arca noae* by Hoppe-Seyler (1933), homarine has since been found in the tissues of a number of marine molluscs belonging to the genera *Patella* (Ackermann and Janka, 1954), *Arion* (Ackermann and Menssen, 1960), *Pecten, Venus, Nassa, Loligo,* and *Busycon* (Gasteiger *et al.*, 1955). It does not appear to exist in fresh water invertebrates or in vertebrates.

H. *Purines and Uric Acid*

Besides their presence as constituents of nucleic acids, the existence of purines in the free state has been demonstrated in the bodies of molluscs. Thiele (1963) was able to extract 6.5 mg of a mixture of purines from the body of one snail. The proportion of xanthine in the mixture amounts to

one-third and that of arginine to one-fifth. The presence of hypoxanthine in the urine of cephalopods was demonstrated by von Fürth (1900) (confirmed by Hoppe-Seyler and Linneweh, 1931). Uric acid has been found in the excreta of a number of molluscs (for references, see Table 112 in Albritton, 1955). Guanine and hypoxanthine have been found in the urine of *Octopus hongkongiensis* (Emmanuel, 1957) and in *Arion empiricorum* (Menssen, 1960).

II. THE METABOLISM OF NITROGENOUS COMPOUNDS

A. *Amino Acid Oxidases and Deamination*

Blaschko and Hawkins (1951) have shown the presence of D-amino acid oxidase in the extracts of the hepatopancreas of *Octopus vulgaris* and *Sepia officinalis*. A specific D-amino acid oxidase for D-glutamic and D-aspartic acids has also been found in the hepatopancreas of *Eusepia officinalis, Loligo forbesi, Octopus vulgaris,* and *Eledone cirrosa* (Blaschko and Himms, 1955). Blaschko and Hawkins (1952) have confirmed the existence of D-amino acid oxidase in the hepatopancreas of *Octopus* and of *Sepia* and have shown its existence in the hepatopancreas of *Helix aspersa* (confirmed by Blaschko and Hope, 1956). Sarlet *et al.* (1950) have not found a D-amino acid oxidase in the hepatopancreas of *Anodonta cygnea* (confirmed by Blaschko and Hawkins, 1952). Further Villee (1947) has not found the enzyme in the hepatopancreas of *Buccinum* or in the adductor muscle of the scallop; nor have Blaschko and Hope (1956) observed this enzyme in the hepatopancreas of *Mytilus edulis*.

The first demonstration of the presence of an L-amino acid oxidase in a mollusc is due to Roche *et al.* (1952a). These authors noticed that, in the extracts of the organs of many invertebrates, arginine is present, as well as α-keto-δ-guanidinovaleric acid and γ-guanidinobutyric acid. This led them to the hypothesis that, as opposed to vertebrates which transfer arginine through the action of arginase, these invertebrates oxidize arginine in the presence of their own L-amino acid oxidase which would act on arginine, while the corresponding enzyme of vertebrates does not. In fact, they found that a L-amino acid oxidase is present in the hepatopancreas of a number of molluscs (*Mytilus edulis, Sepia officinalis, Aplysia* sp.) which oxidizes L-arginine, L-ornithine, L-lysine, and L-citrulline and which is without action on proline. [The mammalian L-amino acid oxidase acts on proline, but not on arginine, ornithine, and lysine.]

Blaschko and Hope (1956) have confirmed the existence in the hepatopancreas of *Mytilus edulis* of the enzyme discovered by Roche *et al.* (1952a). Glahn *et al.* (1955) have isolated from the hepatopancreas of *Cardium tuberculatum,* a L-amino acid oxidase which oxidizes L-histidine. The hepatopancreas of neither *Pecten maximus* nor *Ostrea edulis* appears capable of

effecting this oxidation, while in *Solen ensis* and *Mya arenaria* the hepatopancreas does it as well as that of *Cardium tuberculatum*. The two types of L-amino acid oxidases (type *Mytilus* and type *Cardium*) act on L-citrulline, L-ornithine, and L-histidine, whereas the amino acid oxidase of snake venoms is without action on these substrates. They differ in their optimum pH (*Cardium*, 9.2; *Mytilus* 7.0), and the *Cardium* enzyme appears to be less active on basic amino acids. Neither of the enzymes acts on the aliphatic amino acids alanine, glycine, isoleucine, valine, aspartic acid, or glutamic acid. They differ from the mammalian enzyme, which does not act on the basic amino acids.

Robin and Thoai (1957) have shown that the hepatopancreas and the muscles of *Lymnaea stagnalis* contain a L-amino acid oxidase which acts on L-arginine with the formation of α-keto-δ-guanidinovaleric acid and γ-guanidinobutyric acid. This enzyme also acts on lysine and ornithine (Olomucki *et al.*, 1960).

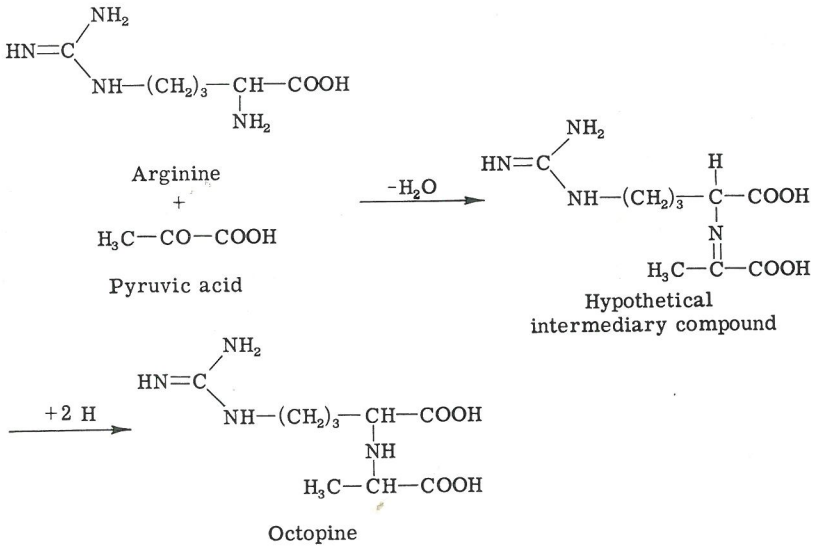
B. Conversion of Arginine to Guanidic Derivatives

The conditions of biogenesis which explain the distribution of guanidic derivatives have recently been reviewed by Thoai (1959). Some of the derivatives are direct or indirect products of arginine degradation. Molluscs as well as other phyla of invertebrates possess, as stated above, a L-amino acid oxidase acting on arginine (contrary to the case in vertebrates). In many molluscs the simultaneous presence of arginine and L-amino acid oxidase in muscles and in hepatopancreas, demonstrated in a number of cases, could explain the presence of α-keto-δ-guanidinovaleric acid and of γ-guanidinobutyric acid as a result of an oxidative deamination of arginine *in situ*.

It is assumed that agmatine results from the decarboxylation of arginine and that methylagmatine results from the methylation of agmatine.

It is possible to consider arcaine as resulting from the amidination of agmatine. In favor of this scheme is the finding that arcaine accompanies agmatine in animals living in polluted water, for instance in *Arca noae* found on rocky bottoms (Kutscher *et al.*, 1931; Robin *et al.*, 1959b). However, the reaction itself has not been observed thus far in any mollusc, and other hypotheses have been proposed to explain the origin of arcaine (Zervas and Bergmann, 1931; Kutscher and Ackermann, 1931).

Octopine was first isolated from the muscle of *Octopus* by Morizawa (1927). Moore and Wilson (1937), Irvin (1938), and Irvin and Wilson (1939) have demonstrated that arginine is the natural precursor of octopine. Knoop and Martius (1939), who chemically synthetized octopine by reductive condensation of arginine and pyruvic acid, proposed that the biosynthesis of octopine involves a condensation of arginine with pyruvic acid to form a Schiff's base which is then reduced to octopine as shown in the accompanying scheme.



That this scheme is the biosynthetic one acting *in vivo* has been proved by Thoai and Robin (1959a,b; 1960, 1961) using extracts of mantle of *Sepia officinalis,* adductor muscles of *Pecten maximus* and *Cardium edule,* and foot muscle of *Cardium edule* and of *Lymnaea stagnalis* to which NADH, arginine, and pyruvic acid (not lactic acid) were added. Octopine is thus derived from the arginine liberated by the hydrolysis of the phosphagen phosphoarginine, and the pyruvic acid resulting from glycolysis, which is not reduced to lactic acid.

The over-all reaction of octopine synthesis is

$$\text{arginine} + \text{pyruvic acid} + \text{NADH} + \text{H}^+ \rightarrow \text{octopine} + \text{NAD}^+ + \text{H}_2\text{O}$$

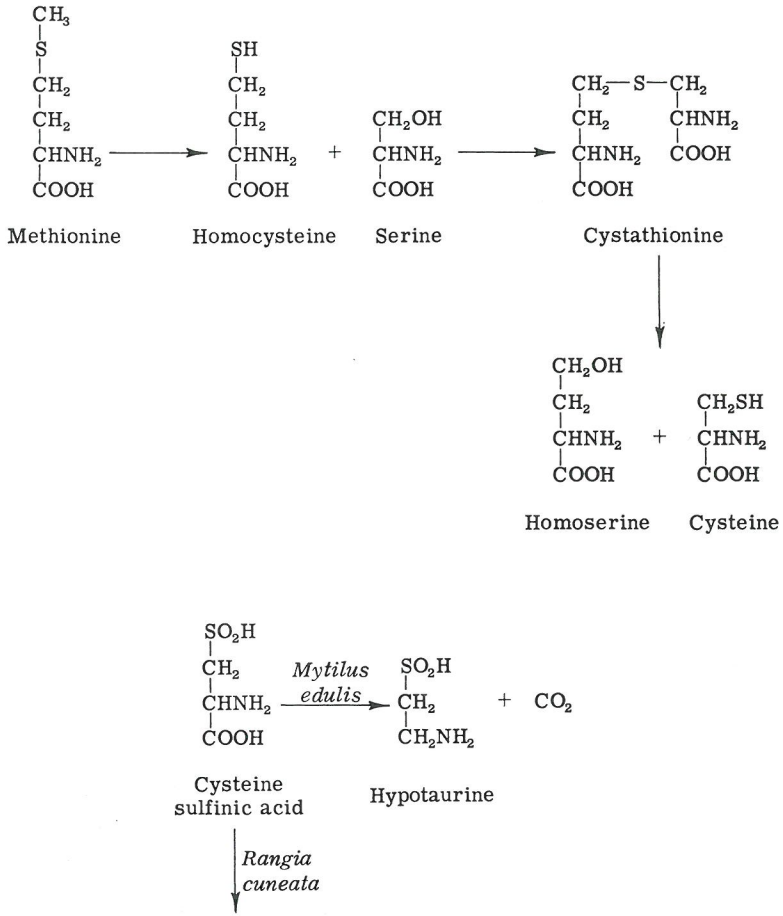
The enzyme system is not found in the hepatopancreas, but only in muscle. Some of the properties of octopine synthetase have been described by Thoai and Robin (1961).

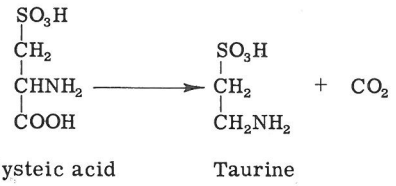
In *Lymnaea stagnalis,* as in other molluscs and other invertebrates, the tissues (and muscle more than the hepatopancreas) contain a L-amino acid oxidase acting on arginine with the formation of α-keto-δ-guanidinovaleric acid and γ-guanidinobutyric acid. In spite of the presence of an active arginase, and a transformation in ornithine and urea, the metabolism of arginine follows, partially at least, the pathway of oxidative deamination (Robin and Thoai, 1957). Another, but less important, metabolic pathway in *Lymnaea* is an oxidative decarboxylation with formation of guanidobutyramide (Thoai *et al.,* 1957). The enzyme responsible for this, arginine decarboxyoxidase, was first observed by Thoai *et al.* (1955, 1956) in *Streptomyces griseus* Waksman. Three mechanisms are involved in the metabolism of arginine in *Lymnaea:* (1) the action of arginase with the formation of

ornithine and urea; (2) oxidative decarboxylation; and (3) oxidative deamination.

C. *The Metabolism of Sulfur Amino Acids*

We have seen (Section I,B) that taurine has been detected in marine molluscs, but not in fresh-water or terrestrial species. Allen and Awapara (1960) have studied comparatively two bivalves, *Mytilus edulis,* a marine form, and *Rangia cuneata,* a fresh-water species of the same group. They have shown that both convert methionine to cysteine and oxidize it to products that can give taurine by decarboxylation. The pathway of the sulfur-containing amino acids is not different from that found in mammals. Cysteine sulfinic acid is oxidized to cysteic acid and later decarboxylated to taurine in *Rangia cuneata,* but in *Mytilus edulis,* cysteic acid is not formed and hypotaurine, the product of the decarboxylation of cysteine sulfinic acid, is the intermediate. The various reactions are as follows:



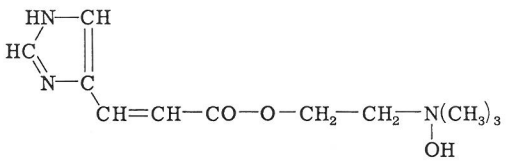


Hypotaurine (2-aminoethanesulfinic acid) has been found in tissues of molluscs by Shibuya and Shunji (1957) and by Ouchi (1959). According to J. W. Simpson *et al.* (1959), the absence of taurine in fresh-water and terrestrial forms is due to the rapid metabolizing of taurine in the tissues, whereas it is retained in the tissues of marine forms and plays a role in intracellular osmotic pressure.

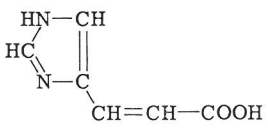
D. *The Metabolism of Histidine*

The general pathway of histidine degradation appears to follow the urocanic pathway (the histidine reaction).

Urocanic acid is a constituent of urocanylcholine or murexine.



Murexine

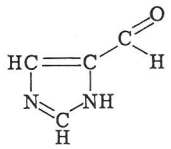


Urocanic acid

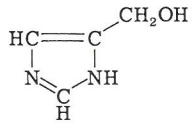
Murexine was first isolated from the hypobranchial extracts of *Murex trunculus, Murex brandaris,* and *Tritonalia erinacea* (Erspamer and Dordoni, 1947; Erspamer, 1948). It appears as a characteristic of species producing indigoids and has not been found in the hypobranchial extracts of the following species: *Vulsocerithium vulgatum, Turritella communis, Euthria cornea, Dolium galea,* nor in extracts of different organs of *Helix pomatia, Mytilus galloprovincialis,* or *Ostrea edulis.* In the case of the indigoid-

producing species, the metabolism of histidine is specialized, at least partly, toward the synthesis of a poisonous secretion.

Another pathway of histidine metabolism, which does not follow the urocanic pathway, has been found in other molluscs. It consists of a conversion into imidazole compounds and has been described by Thoai *et al.* (1954) in the hepatopancreas of *Mytilus edulis* L., an organ rich in L-amino acid oxidase. The same authors have shown that the action of the preparations of hepatopancreas on L-histidine leads to the formation of two groups of substances: (1) imidazolepyruvic acid and imidazole acetic acid and (2) imidazolemethanal (I) and imidazolemethanol (II).



(I)



(II)

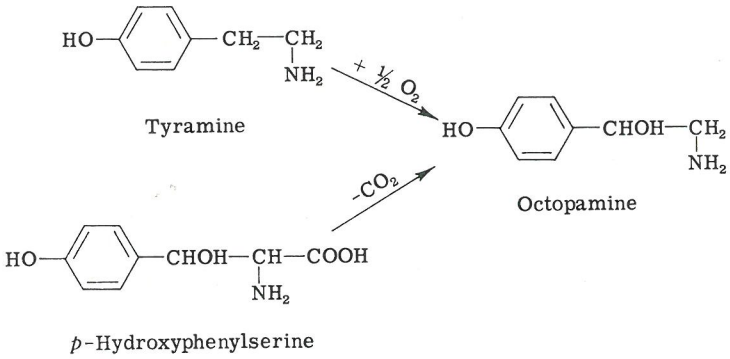
The formation of these compounds cannot be explained by the same pathway, and they point to the existence of another pathway still unknown. The vertebrates appear to open the imidazole ring without shortening the side chain of histidine (through urocanic acid and α-formamido-L-glutamic acid). At least in some molluscs apparently the side chain is shortened before the cycle is opened.

Roche *et al.* (1955) have observed that there are several pathways of enzymic degradation leaving the imidazole nucleus intact, while liberating the α-amino group in the form of ammonia.

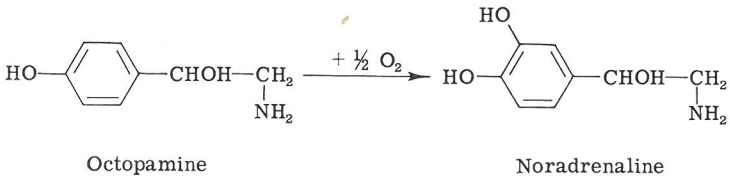
E. *Decarboxylases and the Genesis of Amines*

The amines found in the posterior salivary glands of some cephalopods (5-hydroxytryptamine, tyramine, octopamine, histamine, tryptamine) appear as the products of a decarboxylation of the corresponding amino acids, 5-hydroxytryptophan, tyrosine, *p*-hydroxyphenylserine, histidine and tryptophan, respectively (see Table VII). The decarboxylases in the posterior glands of *Octopus apollyon* can be shown by adding various substrates, as Table VII illustrates. It will be seen that more decarboxylases are found than are required for the actual number of amines present in the gland.

Octopamine may result from the decarboxylation of *p*-hydroxyphenylserine (Table VII), but it is known that octopamine may also result from the oxidation of tyramine. The reactions involved are shown in the accompanying diagram.



Octopamine itself can be oxidized into hydroxyoctopamine or noradrenaline (norepinephrine).



F. *Conversion of Arginine to Urea*

The enzyme arginase brings about the formation of ornithine and urea from arginine. In 1918 Clementi found this enzyme in a number of invertebrates, including *Helix pomatia,* and Baldwin (1935a) confirmed the presence of arginase in *Helix.* At that time, it was commonly held that the existence of arginase in an organism was linked with the ureotelic nature of its nitrogen metabolism, and that the snail *Helix pomatia* excreted a part of the nitrogen in the form of urea. Baldwin and Needham (1934) brought arguments supporting the interpretation according to which the small amounts of urea eventually produced in the metabolism of *Helix* may be derived from exogenous arginine. Bricteux-Grégoire and Florkin (1962a,b) have provided direct evidence in support of this notion. They injected guanido-C^{14}-arginine into the hepatopancreas of a snail and 2 hours later recovered 5% of the activity in the form of urea.

Arginase has been shown to exist not only in *Helix pomatia,* but also in *Viviparus fasciatus, Planorbis corneus, Lymnaea stagnalis, Helix aspersa, Arion ater* (Baldwin, 1935a), and *Achatina fulica* (Saxena, 1953).

G. *Enzymes of the "System of Ureogenesis"*

One may ask whether the arginine from which urea is formed by the action of arginase is derived from the system of urea synthesis, such as

TABLE VII

AMINO ACIDS DECARBOXYLATED BY THE POSTERIOR SALIVARY GLANDS OF *Octopus apollyon*[a]

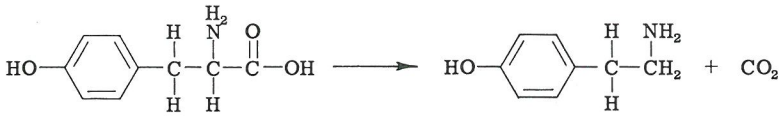
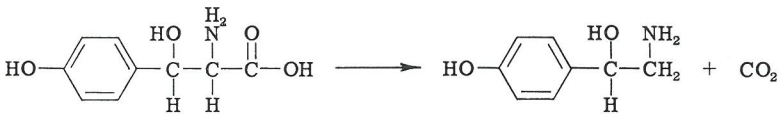
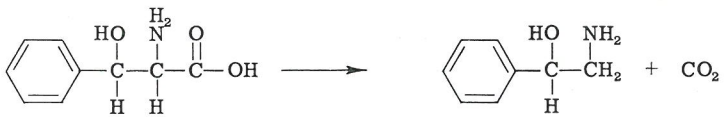
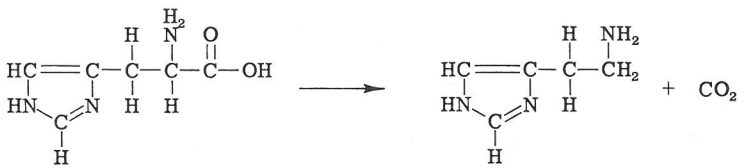
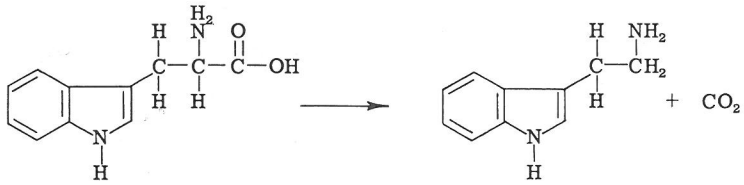
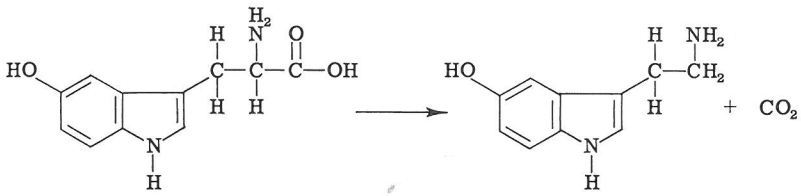
	
Tyrosine	Tyramine
	
m-Tyrosine	*m*-Hydroxyphenyl-ethylamine
	
p-Hydroxy-phenylserine	*p*-Hydroxyphenyl-ethanolamine (octopamine)
	
Phenylserine	Phenylethanolamine
	
Histidine	Histamine

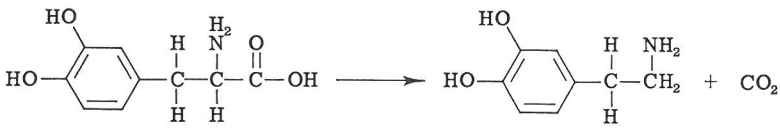
TABLE VII (*Continued*)



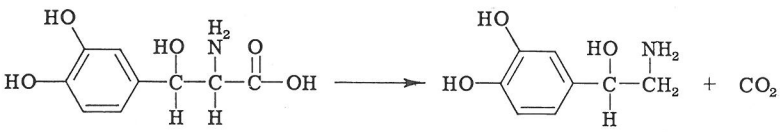
Tryptophan — Tryptamine



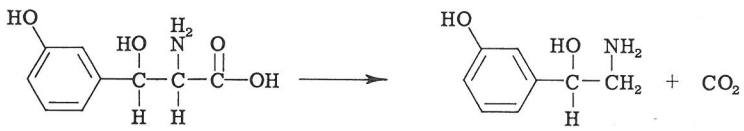
5-Hydroxytryptophan — 5-Hydroxytryptamine (enteramine, serotonin)



3, 4-Dihydroxyphenylalanine (Dopa) — 3, 4-Dihydroxyphenylethylamine (Dopamine, hydroxytyramine)



3, 4-Dihydroxyphenylserine — 3, 4-Dihydroxyphenylethanolamine (Arterenol, noradrenaline, norepinephrine)



m-Hydroxyphenylserine — *m*-Hydroxyphenylethanolamine

[a]After Hartman *et al.* (1960).

that found in ureotelic vertebrates. The sequence of steps of ureogenesis in ureotelic vertebrates is formulated as follows:

$CO_2 + NH_3 + ATP$ (+ glutamyl derivative) $\rightarrow$ carbamyl phosphate (1)
carbamyl phosphate + ornithine $\rightarrow$ citrulline (2)
citrulline + aspartate + ATP $\rightarrow$ arginosuccinate (3)
arginosuccinate $\rightarrow$ arginine + fumarate (4)
arginine $\rightarrow$ ornithine + urea (5)

The enzymes catalyzing the individual steps are the following:

carbamyl phosphate synthetase (1)
ornithine transcarbamylase (2)
arginosuccinate synthetase (3)
arginine synthetase (4)
arginase (5)

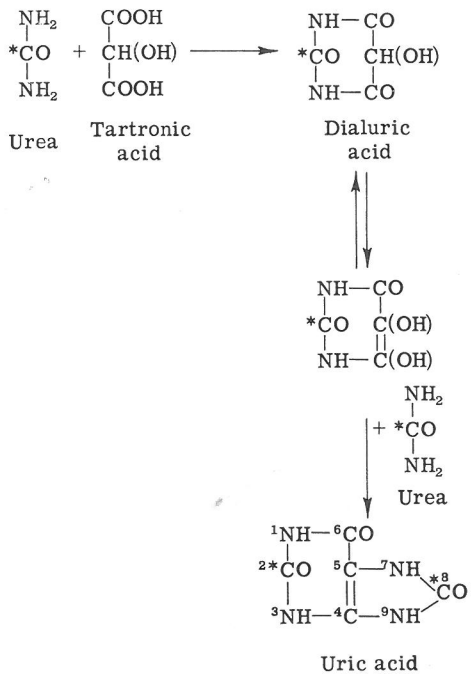
Linton and Campbell (1962) have studied the hepatopancreas of the land snail *Otala lactea* with respect to the enzymes of the system of ureogenesis (see Cohen and Brown, 1960) and have found enzymes (2) to (5), but no carbamyl phosphate synthetase. In *Helix pomatia* hepatopancreas, Bricteux-Grégoire and Florkin (1964) have been unable to detect the presence of enzymes (1) and (2). *In vitro,* however, when carbamyl phosphate and C^{14}-ornithine are incubated with a homogenate of hepatopancreas, labeled urea is isolated.

In conclusion, it can be said that the hepatopancreas of the land snails does not contain the complete system of ureogenesis.

H. *Biogenesis of the Purine Ring*

Several authors have concluded that uric acid, which is the main terminal nitrogenous product of amino acid metabolism in the snail, is synthesized in the hepatopancreas from urea (Wolf, 1933; Baldwin, 1935b; Grah, 1937). According to this view, which was hinted at again recently by Linton and Campbell (1962), the biosynthesis of uric acid should follow different pathways in molluscs and in birds. *Helix pomatia* would accomplish the biosynthesis of the purine ring along the pathway proposed by Wiener (1902), by which two molecules of urea are combined with a molecule of tartronic acid to form a molecule of uric acid. With respect to the birds, this scheme is obsolete since Buchanan and his collaborators (1948) have demonstrated that in these animals uric acid biosynthesis is accomplished from simple molecules: CO_2, acetate, formate, glycine, aspartic acid, and glutamic acid. Heller and Jezewska (1959) have shown that these precursors are also utilized by the moth *Antheraea pernyi* for the synthesis of purines.

According to Wiener's scheme for the synthesis of the purine ring, the labeled carbon of C^{14}-urea should be found in the C-2 and C-8 of uric acid, as shown in the accompanying scheme.



However, when C^{14}-urea was injected into the snail *Helix pomatia* (Bricteux-Grégoire and Florkin, 1962a), the greater part of the activity was found to be localized in the C-6 and C-4, i.e., in the positions also found to be chiefly labeled following the administration of C^{14}-bicarbonate to the pigeon (Buchanan *et al.*, 1948). The interpretation of these results is that the urea injected into the snail is decomposed by urease in the kidney (Baldwin and Needham, 1934; Heidermanns and Kirchner-Kühn, 1952) and that the CO_2 resulting from this action takes part in the biosynthesis of uric acid according to the pathway discovered by Buchanan *et al.* (1948).

I. *Purinolytic Enzymes and Purinolysis (Including Urease)*

The complete course of purinolysis leads from the purine nucleus to ammonia through the successive actions of uricase, allantoinase, allantoicase, and urease, as shown in Fig. 1.

This series of enzymes is found, for instance, in *Mytilus edulis* (Przylecki, 1922; Brunel, 1938; Florkin and Duchâteau, 1943). That the successive steps of the purinolysis pathways can take place in different organs is shown in the case of *Meretrix meretrix,* in which guanine appears to be deaminated to xanthine in the gills, where the guanine deaminase is present. The xanthine could then be converted to uric acid in the foot and hepatopancreas where xanthine dehydrogenase predominates. The resulting uric

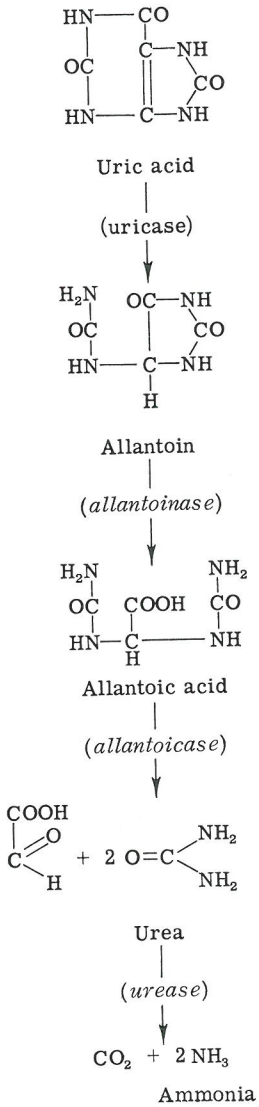


FIG. 1. The uricolytic enzyme system.

acid is degraded mainly in the hepatopancreas, while the breakdown of urea to ammonia takes place in the gills and in the hepatopancreas (Ishida, 1955b).

Data on the distribution of the enzymes of purinolysis have been published by a number of authors. Adenine deaminase (adenase) has been reported by Mendel and Wells (1909) in *Sycotypus* and by Truszkowski

(1928), as well as by Duchâteau *et al.* (1941), in *Anodonta*. Working with more up-to-date methods, Ishida (1954) found no adenase in *Meretrix meretrix lusoria*. Guanine deaminase (guanase) has been found in *Anodonta* (Truszkowski, 1928; Duchâteau *et al.*, 1941) and in the gills of *Meretrix* (Ishida, 1954). Duchâteau *et al.* (1941) were not able to demonstrate the presence of adenosine deaminase, or of guanosine deaminase in *Anodonta*, but Aikawa (1959) observed the presence of adenosine deaminase in *Meretrix*. Therefore, the view proposed by Duchâteau *et al.* (1941) according to which the system of the deamination of aminopurines acts not on nucleosides but on free purines in invertebrates must be abandoned.

Xanthine oxidase has been found in all molluscs examined: *Helix pomatia* (Baldwin and Needham, 1934), *Anodonta* and *Planorbis* (Florkin and Duchâteau, 1941), and *Anadara inflata, Meretrix meretrix, Venerupis philippinarum, Crassostrea gigas,* and *Mytilus edulis* (Tsuzuki, 1957). Whereas the xanthine oxidase is present in the hepatopancreas of *Anodonta* (Florkin and Duchâteau, 1941), Ishida (1955b) found the main concentration in the foot of *Meretrix*.

Uricase has been detected in all bivalves studied: *Mytilus edulis* (Brunel, 1938; Ishida *et al.*, 1956), *Meretrix meretrix lusoria* and *Venerupis philippinarum* (Ishida and Tsuzuki, 1955), *Anodonta* (Przylecki, 1926; Truszkowski and Chajkinowna, 1935; Truszkowski and Goldmanowna, 1933; Florkin and Duchâteau, 1943), *Anadara inflata, Mactra sulcataria,* and *Crassostrea gigas* (mainly in the hepatopancreas) (Ishida *et al.*, 1956).

Allantoinase and allantoicase are found in *Mytilus edulis* (Brunel, 1938) as well as in the hepatopancreas of *Anodonta cygnea* (Florkin and Duchâteau, 1943). The latter does not contain urease (Truszkowski and Chajkinowna, 1935). In marine bivalves studied so far, urease has been detected in *Mytilus edulis* (Przylecki, 1922), *Mactra sulcataria* (mainly in the hepatopancreas) (Ishida, 1955a), *Venerupis philippinarum* (Ishida, 1955a) *Anadara inflata* (mainly in the gills) (Ishida, 1955a).

In gastropods, uricase, but not allantoinase or allantoicase, is found in *Planorbis* (Florkin and Duchâteau, 1943). On the other hand, no uricase could be detected in the hepatopancreas of *Sycotypus* (Mendel and Wells, 1909) or of *Helix pomatia* (Truszkowski and Chajkinowna, 1935) in spite of indirect arguments to the contrary (Spitzer, 1937; Grah, 1937; Plum, 1935). The kidney of the snail contains urease (Baldwin and Needham, 1934; Heidermanns and Kirchner-Kühn, 1952).

III. NITROGENOUS COMPOUNDS IN OSMOTIC REGULATION

As pointed out by Duchâteau *et al.* (1952), a comparison of the concentration of the free amino acid pool in *Mytilus* and *Ostrea* on one hand and in *Anodonta* on the other, suggests a participation of the free amino acids in the osmolar intracellular concentration under the conditions met by species

living in sea water or in fresh water. The importance of amino acids and taurine as osmotically effective compounds is confirmed by a study of the axoplasm of *Loligo forbesi* and of *Sepia officinalis* (Lewis, 1952). In the latter, aspartic acid and taurine are the nitrogenous components found in highest concentrations. In squid axoplasm, a large proportion of the organic anions is made up of a hydroxy derivative of taurine, isethionic acid (2-hydroxyethane sulfonic acid) (Koechlin, 1954, 1955; Deffner and Hafter, 1959a,b) (see also Volume I, Chapter 9).

The concept of isosmotic intracellular concentration was proposed in 1956 by Duchâteau and Florkin. We found that when the Chinese crab, *Eriocheir sinensis,* a very euryhaline crustacean, was kept in fresh water, the amino acid component in the muscles was smaller than in animals adapted to sea water (Duchâteau and Florkin, 1955). As the hydration of the muscles is approximately the same in sea water as in fresh water (Scholles, 1933), the reversible variation of the amino acid component could only depend on active modification. I have emphasized this point and its intracellular regulatory aspect in a report presented in 1955 to the meeting of the Gesellschaft für physiologische Chemie in Mosbach (Florkin, 1956). We found a similar difference in the concentration of free amino acids in *Carcinus maenas* living in sea water and in brackish water. In view of these findings we proposed that the variation of the amino acid component resulting from the change of concentration in the medium accomplished an "intracellular regulation acting against the water movement between cells and body fluids as a consequence of changes of concentration in the latter" (Duchâteau and Florkin, 1956).

In 1958, Shaw studied the phenomenon in *Carcinus maenas,* using fibers of the carpopodite extensor and flexor muscles of the chela. He observed that when the concentration of the external medium is modified (sea water → diluted sea water), the osmotic pressure in the fibers varies proportionally with changes in the concentration of the body fluids, resulting from the change in the fluid medium outside the body. He showed that when *Carcinus* is transferred from sea water to brackish water, the change in the concentration of the amino acids in the cells is much larger than is accounted for by the slight change in hydration. Shaw therefore also has adopted the view first proposed by Duchâteau and Florkin (1956) according to which the muscle fibers of a euryhaline marine crab are able to limit, by a change in intracellular osmotic pressure, the discrepancy between the intracellular osmotic pressure and the osmotic pressure of the internal medium, the latter being modified by the transfer into diluted sea water.

Some marine invertebrates, when transferred to diluted sea water, exhibit osmotic regulation of the blood. This regulation is a form of anisosmotic extracellular regulation, as it keeps the concentration of the blood at a value differing from the external concentration. In the case of the marine inverte-

brates endowed with a certain degree of euryhalinity, the anisosmotic regulation is of the hyperosmotic type. There are other types of anisosmotic regulation of the blood, for instance in marine bony fishes, in which the blood is kept at a concentration lower than that of sea water (hypoosmotic regulation). In marine invertebrates not provided with an anisosmotic extracellular regulation, blood is in concentration equilibrium with the fluid medium and follows its variations.

When a stenohaline marine invertebrate is transferred into diluted sea water, the blood is diluted and reaches an equilibrium with the external medium. Water passes into the cells, which now have a greater osmotic pressure than the blood, producing a disruption of the cells and death of the animal. This does not take place in a euryhaline marine form transferred to brackish water, owing to the fact that the intracellular concentration is lowered by the intracellular isosmotic regulation (bringing the cells into equilibrium with the new concentration of the blood). The blood's concentration is changed either in the absence of anisosmotic regulation or in the presence of the latter, which reduces the lowering of the intracellular concentration but does not prevent it entirely. Free amino acids play an important role in intracellular isosmotic regulation, which takes place in all euryhaline marine invertebrates studied so far, whether or not they possess an anisosmotic extracellular regulation (for the mechanism of isosmotic intracellular regulation, see Florkin and Schoffeniels, 1964).

The contribution of nitrogenous compounds to intracellular isosmotic regulation in molluscs has been studied in two euryhaline forms, *Mytilus edulis* and *Ostrea edulis*. Both *Mytilus* and *Ostrea,* though euryhaline, do not possess anisosmotic extracellular regulation. In *Mytilus edulis,* Potts (1958) has shown that a transfer from sea water to diluted sea water results in a lowering of the free amino acid pool inside the fibers of the adductor muscles. There is also a lowering of taurine concentration. These changes are, to a small extent, a result of the slight change of hydration of the tissue, but are for the greatest part due to a regulation resulting in a lowering of the concentration of sodium, chloride, free amino acids, and taurine within the muscle fibers. Bricteux-Grégoire *et al.* (1964a) have performed a more detailed analysis of the adductor muscle fibers of *Mytilus* in sea water and in 50% sea water and have shown that among the identified nitrogenous compounds taking part in the active intracellular isosmotic regulation the most efficient are glycine, taurine, and glycine-betaine (Tables VIII and IX).

In *Ostrea edulis* (Bricteux-Grégoire *et al.,* 1964b), the adductor muscles are made up of two distinct parts: a white and a yellow muscle. (This contrasts with *Mytilus* in which the adductors are not divided into a fast and a slow part.) The dialyzable nitrogenous constituents account for a greater part of the osmotically active compounds in the yellow muscle, while inorganic ions predominate in the white muscle. There is little difference

(except in the case of alanine) with respect to free amino acids, but the glycine-betaine, taurine, and undetermined nitrogen compounds predominate in the yellow muscle. When *Ostrea edulis* is transferred from sea water to 50% sea water, the extracellular fluid is decreased and the hydration of the muscle fibers becomes greater. The resulting dilution influences the concen-

TABLE VIII

DIALYZABLE NITROGENOUS CONSTITUENTS OF THE ADDUCTOR MUSCLES[a] OF *Mytilus edulis*, KEPT IN SEA WATER (S) AND IN 50% SEA WATER (S/2)[b]

	mg per 100 gm of fresh tissue		mOsm per liter of total water		mOsm per kg of fiber water	
Amino acids	S	S/2	S	S/2	S	S/2
Alanine	164	116	24.6	16.2	31.5	19.7
Arginine	208	146	15.9	10.4	20.4	12.6
Aspartic acid (total)	130	23	13.1	2.1	16.8	2.6
Glutamic acid (total)	144	156	13.1	13.1	16.8	15.9
Glycine	464	138	82.7	22.8	105.9	27.7
Histidine	28	19	2.4	1.5	3.1	1.8
Isoleucine	4.0	5.1	0.41	0.48	0.5	0.6
Leucine	6.1	7.8	0.63	0.74	0.8	0.9
Lysine	41	25	3.8	2.1	4.9	2.6
Phenylalanine	8.4	4.7	0.68	0.35	0.9	0.4
Proline	36	16	4.1	1.7	5.2	2.1
Serine	63	32	8.0	3.8	10.2	4.6
Threonine	52	23	5.9	2.4	7.6	2.9
Tyrosine	14	8.7	1.0	0.6	1.3	0.7
Valine	11	5.9	1.2	0.62	1.5	0.7
Total	1373.5	726.2	177.5	78.9	227.4	95.8
Taurine	727	557	77.7	55.2	99.5	67.1
Trimethylaminoxide	0	0	0	0	0	0
Glycine-betaine	737	475.7	84.32	50.37	108	61.2
Total dialyzable N	541	325	518.0	288.0	663.2	349.9
Dialyzable amino N (ninhydrin)	353	196	337.9	173.7	432.7	211.1
Water (%)	74.6	80.6	—	—	—	—

[a] Contrary to what is observed in such bivalves as *Ostrea* or *Anodonta*, there is no distinction between a slow and a fast part in the adductors of *Mytilus*.

[b] Determination by column chromatography (Bricteux-Grégoire *et al.*, 1964a).

tration of intracellular osmotically active substances in a much more pronounced fashion than in *Mytilus*. But part of the adjustment results from an intracellular isosmotic regulation which is mainly due to changes of the concentrations of intracellular potassium, sodium, glycine-betaine, alanine, glycine, and undetermined nitrogen compounds. The variation of taurine concentration is entirely accounted for by the dilution (Tables X and XI).

IV. TERMINAL PRODUCTS OF NITROGEN METABOLISM IN MOLLUSCS

It has long been known that in the frog and man 80–85% of the total nitrogen of urine is in the form of urea, whereas in birds and snakes, the uric acid of the excreta accounts for approximately the same proportion. On the other hand, in the urine of *Sepia officinalis* the ammonia nitrogen repre-

TABLE IX

OSMOTICALLY ACTIVE CONSTITUENTS (MILLIOSMOLES PER KILOGRAM OF FIBER WATER) IN THE ADDUCTOR MUSCLES OF *Mytilus edulis* KEPT IN SEA WATER (S) AND IN 50% SEA WATER (S/2)[a]

Constituent	S	S/2	Difference
Cl[b]	280.0	75.0	
K[b]	188.0	108.4	
Na[b]	208.4	98.3	
Ca[b]	7.8	2.3	
Sum of inorganic constituents	684.2	284.0	400
Total dialyzable N	663.2	349.9	313
Sum	1347.4	633.9	
Δ[c] calculated (from the concentrations)	2.52	1.19	
Δ of the external water (measured)	2.27	1.15	
Total dialyzable N	663.2	349.9	313
1. Trimethylamino oxide	0	0	0
2. Glycine-betaine	108	61	47
3. Taurine	99	67	32
4. Dialyzable amino acids determined	227	96	131
5. Glycine	106	28	78
6. Amino N (ninhydrin)	433	211	222
Total of 2, 3, and 4	434	224	210
Total of 2, 3, and 6	640	339	301

[a] Amino acid determination by column chromatography (Bricteux-Grégoire *et al.*, 1964a).

[b] Means of two determinations.

[c] Δ = lowering of freezing point (°C).

sents 65% of the total nonprotein nitrogen. These are examples of clear-cut cases of ureotelic, uricotelic, or ammoniotelic nitrogen metabolism, respectively. The term in each case means that a terminal product predominates. Such a conclusion is justified when it can safely be assumed that the nitrogen in the excretion analyzed represents the total nitrogen actually excreted. It would be quite misleading, for instance, to take the distribution of the nitrogenous substances present in the urine of the carp *Cyprinus carpio* as indicative of the relative proportions of the different nitrogenous

excretion products that are partly eliminated through the gills (see Florkin, 1945).

Some years ago we raised doubt about the presumed terminal products of nitrogen metabolism as a result of analyses of the water in which animals

TABLE X

DIALYZABLE NITROGENOUS CONSTITUENTS IN MUSCLE FIBERS OF *Ostrea edulis* KEPT IN SEA WATER (S) OR IN 50% SEA WATER (S/2)[a]

	mg per 100 gm of fresh tissue				mOsm per kg of fiber water			
	Yellow muscle		White muscle		Yellow muscle		White muscle	
Constituent	S	S/2	S	S/2	S	S/2	S	S/2
1. Amino acids								
Alanine	299	226	163	130	54.3	33.0	34.2	20.3
Arginine	30.6	25.9	24.9	21.2	2.8	1.9	2.7	1.8
Total aspartic acid	23.4	33.5	62.9	80.7	2.8	3.3	8.8	8.4
Total glutamic acid	89.7	87.3	76.7	87.2	9.9	7.7	9.8	8.2
Glycine	245	187	217	172	52.8	32.4	54.2	31.7
Histidine	—	—	—	—	—	—	—	—
Isoleucine	Trace	Trace	Trace	1.7	—	—	—	—
Leucine	Trace	Trace	Trace	3.0	—	—	—	—
Lysine	37.4	24.8	39.3	30.1	4.1	2.2	5.0	2.9
Phenylalanine	Trace	Trace	Trace	Trace	—	—	—	—
Proline	58.6	40.2	36.3	33.8	8.2	4.6	5.9	4.1
Serine	8.1	5.6	7.8	6.1	1.2	0.7	1.4	0.8
Threonine	4.1	2.5	4.1	2.4	0.6	0.3	0.6	0.3
Tyrosine	Trace	Trace	Trace	Trace	—	—	—	—
Valine	Trace	Trace	Trace	2.7	—	—	—	—
Total	795.9	627.2	632.0	570.9	136.7	86.1	122.6	78.5
2. Taurine	581	497	426	466	75.1	51.9	63.8	51.7
3. Betaine	741.1	619.6	517.6	422.3	102.3	69.1	82.8	51.2
4. Trimethylamine oxide	0	—	—	—	—	—	—	—
5. Dialyzable amino N (ninhydrin)	291	230	175	149	336.1	213.8	233.8	147.1
6. Total dialyzable N	550	409	316	262	635.1	380.7	422.7	259.5

[a] Determination by column chromatography (Bricteux-Grégoire *et al.*, 1964b).

were kept, and into which they eliminate not only the products of their kidneys but also their feces (Florkin, 1945). Some excretion products may be retained in the kidneys in an insoluble form. We must, therefore, be very cautious in the interpretation of such data as those collected in Table 112 of Albritton's "Standard Values in Nutrition and Metabolism" (1955)

TABLE XI: OSMOTICALLY ACTIVE CONSTITUENTS (MILLIOSMOLES PER KILOGRAM OF FIBER WATER) IN MUSCLE FIBERS OF THE ADDUCTOR MUSCLES OF *Ostrea edulis* KEPT IN SEA WATER (S) AND IN 50% SEA WATER (S/2)[a]

Constituent	Yellow muscle		White muscle		Difference	
	S	S/2	S	S/2	Yellow muscle	White muscle
Cl	106.9	99.8	243.4	198.5		
K	165.0	78.3	111.3	58.4		
Na	179.9	111.4	299.6	197.6		
Ca	9.0	6.8	19.9	11.5		
Sum of inorganic constituents	460.8	296.3	674.2	466.0	164.5	208.2
Total dialyzable N	635.1	380.7	422.7	259.5	254.4	163.2
Sum	1095.9	677.0	1096.9	725.5	418.9	371.4
Δ^b calculated (from concentrations)	−2.05	−1.27	−2.05	−1.36	—	—
Δ of external water (measured)	−2.26	−1.20	−2.26	−1.20	−1.06 (=567 mOsm)	−1.06 (=567 mOsm)
Total dialyzable N	635.1	380.7	422.7	259.5	254.4	163.2
1. Trimethylamine oxide	0	0	0	0	—	—
2. Betaine	102	69	83	51	33	32
3. Taurine	75	52	64	52	23	12
4. Dialyzable amino acids determined	137	86	123	79	51	44
5. Glycine	53	32	54	32	21	22
6. Amino N (ninhydrin)	336	214	234	147	122	87
7. Total of 2, 3, and 4	314	207	270	182	107	88
8. Total of 2 and 6	438	238	317	198	155	119
9. Total dialyzable N minus amino N (ninhydrin)	299	167	189	113	132	76
10. Total dialyzable N minus N determined (2 + 6)	197	98	106	62	99	44

[a] Amino acid determination by column chromatography (Bricteux-Grégoire *et al.*, 1964b).

[b] Δ = lowering of freezing point (°C).

or in Table 22 of Prosser and Brown's "Comparative Animal Physiology" (1961).

Let us consider the case of the snail *Helix pomatia*. According to Hesse (1910) *Helix pomatia* excretes 3.85 mg of nitrogen per kilogram. When this observation was performed, it was known that the whitish content of the kidneys was largely uric acid, as Jacobson had established in 1820, and as many authors had confirmed. Marchal (1889) had isolated the uric acid contained in the kidneys of 150 snails, and after its purification had concluded that a kidney contains more than 7 mg of uric acid. In fact, at the end of hibernation, as Baldwin and Needham (1934) have shown, a snail's kidney contains a mean weight of 32 mg of uric acid, i.e., about three-quarters of the dry weight of the organ. Uric acid is mostly excreted in its acid form, a small portion being in the form of urates (Heidermanns, 1953).

In the tables referred to above, the data concerning *Helix pomatia* are those first published by Delaunay (1927) and made generally known later by the publications of Needham (1935) and by Baldwin (1947). Delaunay analyzed a "water extract" of the kidneys prepared in such a way that, about 80% of the purine compounds were left unextracted as Jezewska *et al.* (1963) have pointed out. Delaunay considered that the snail has two kinds of excreta: solids composed mainly of purines, and liquid consisting mainly of ammonia and urea.

To collect the "liquid excreta," Delaunay kept the snails partially immersed in distilled water and determined the nitrogenous compounds given out in this water. This he called "liquid excretion," but in the tables referred to above they are considered as representing the excreta of *Helix*. Jezewska *et al.* (1963) have proceeded in a different way in their study of the nitrogen compounds in snail's excretion. The hibernating operculated snails were stored in a refrigerator at 4°C. In the spring, they were transferred to room temperature and after breaking hibernation, were fed with lettuce and cauliflower leaves in glass beakers, the bottom of which was covered with a layer of 2–4 mm of water. The snails remained on the walls of the beakers, where they deposited their feces as well as their renal excreta. These were easily distinguished. The yellowish renal excreta were collected during April, May, and June, and air dried.

During hibernation as well as during the active period, snails were dissected and their kidneys were isolated. The content of each kidney was rinsed out with distilled water into a 100 ml flask. A saturated solution of lithium carbonate was added under conditions of shaking and moderate heating until all concretions were dissolved. The air-dried renal excreta were treated in the same way. The results of the analyses performed on the dissolved excreta are given in Table XII. They show that 90% of the total nitrogen in the excreta and in the content of the kidneys consists of uric acid,

TABLE XII

NITROGEN COMPOUNDS IN KIDNEY AND EXCRETA FROM KIDNEY IN THE SNAIL *Helix pomatia*[a,b]

Material	Expt. no.	N total (mg)	Nitrogen: Uric acid mg	Uric acid %	Xanthine mg	Xanthine %	Guanine mg	Guanine %	Total purines mg	Total purines %	Unknown compound mg	Unknown compound %	N accounted for mg	N accounted for %
Kidney														
During hibernation	1	43.9	25.0	56.9	8.6	19.5	5.8	13.2	39.5	90.0	0.0	0.0	39.4	90.0
	2	38.1	24.3	63.7	4.8	12.5	5.0	13.1	34.1	89.5	0.0	0.0	34.1	89.5
	3	59.4	44.6	75.0	4.8	8.0	5.9	9.9	55.3	93.0	0.0	0.0	55.3	93.0
	Average	47.1	31.3	66.4	6.0	12.7	5.6	11.8	42.9	91.0	0.0	0.0	42.9	91.0
During the feeding period	4	21.9	6.0	27.3	8.1	36.9	4.2	19.1	18.3	83.5	3.3	15.0	21.6	98.6
	5	22.1	5.0	22.6	8.7	39.3	5.3	24.0	19.0	85.9	3.2	14.0	22.2	100.4
	6	26.3	11.1	42.2	8.4	31.9	3.6	13.6	23.2	88.1	1.4	5.3	24.6	93.5
	7	15.1	7.8	51.6	3.6	23.8	3.6	23.8	15.0	99.3	0.0	0.0	15.0	99.3
	Average	21.3	7.5	35.2	7.2	33.9	4.2	19.7	18.9	88.7	1.9	8.6	20.8	97.6
Excreta														
First after hibernation	8	33.6	21.7	64.6	12.6	37.5	0.0	0.0	34.3	102.1	0.0	0.0	34.3	102.1
During the feeding period	9	33.1	12.8	38.7	12.0	36.3	3.8	11.5	28.6	86.4	2.1	6.3	30.7	92.7
	10	34.7	15.8	45.5	8.8	25.3	7.4	21.3	32.0	92.2	2.1	6.1	34.1	98.3
	11	31.1	14.1	45.3	9.0	28.9	6.9	22.2	30.0	96.5	0.5	1.6	30.5	98.0
	Average	33.0	14.2	43.0	9.9	30.0	6.0	18.2	30.2	91.5	1.5	4.5	31.7	96.1

[a] The average values of two determinations are given, and the results are presented in milligrams N per kidney or per 100 mg of excreta, and as percentage of total N. The difference between the determinations did not exceed 0.5 mg per kidney or per 100 mg of excreta.

[b] Jezewska *et al.* (1963).

xanthine, and guanine. Urea, ammonia and allantoin were not found. During the feeding period, the total N content in the kidneys was only 50% of that in hibernating snails.

These data allow us to explain the nature of the erroneous data still often printed. Delaunay's "water extract" represented only 522 mg N per 100 gm of the kidneys of feeding snails, whereas the figures of Jezewska *et al.* (1963) recalculated on the same basis represent about 10,000 mg. Therefore the evaluation of urea, ammonia, and amino acids given by Delaunay would amount to only 1% of the total nitrogen of the excreta analyzed by Jezewska *et al.* (1963). Such small amounts are very likely overlooked in Table XII, the data of which were obtained on small samples. The purinotelic character of nitrogen metabolism in the snail is obvious and the proportions in the excreta of end products other than uric acid, guanine, and xanthine amount to mere traces.

TABLE XIII

DISTRIBUTION OF NONPROTEIN NITROGEN IN THE URINE OF *Sepia officinalis*[a]

Parameter	Nonprotein nitrogen	N of ammonia	N of urea	N of amino acid	N of purines		Unidentified nitrogen
					Total	Uric acid	
Mg per 100 ml	142	92	3	12	7	3.2	28
Per cent of non-protein nitrogen	—	64.4	2.1	8.4	4.9	2.2	20.2
Mg per 100 ml	125	87	1.8	9	4	2.6	23.2
Per cent of non-protein nitrogen	—	69.6	1.4	7.2	3.2	2.1	18.6

[a] Delaunay (1925, 1927).

In the case of the cephalopod *Sepia officinalis,* it is astonishing that no information concerning the nitrogenous end products of metabolism has been obtained for forty years, i.e., since Delaunay's determinations published in 1925. In this case, Delaunay correctly used the urine taken from the urinary bladder. His results are shown in Table XIII. Here again, the data clearly indicate the nature of the main terminal product of nitrogen metabolism, in this case ammonia.

With the reservation that our knowledge of the nitrogenous excretion of molluscs is shamefully limited, we have two clear-cut cases, one showing the existence of ammoniotelic metabolism in *Sepia,* and the other purinotelic metabolism in *Helix.*

As we have seen (Section II,G), the data in the literature that pertain to the existence of a ureotelic metabolism or of the enzymic system of "ureogenesis" in molluscs have not been confirmed by further study. This, of

course, does not mean that small amounts of urea cannot be formed by other metabolic pathways.

It appears from all of our present knowledge of nitrogen metabolism in molluscs, that the ammonia resulting from amino acid deamination, under the catalysis of an enzymic system differing from the one active in vertebrates, is partly used in purine synthesis, through the same pathway as the one described in birds, and partly excreted as such. When communication with the aquatic external medium is direct, the animal simply drains a large part of the ammonia into the medium, and the nitrogen used in purine synthesis also finally appears in the form of eliminated ammonia resulting from the action of a very extended purinolytic system. When the ecology of the animal involves a water shortage, as is the case in the gastropods that have become terrestrial, more ammonia is diverted toward purine synthesis, and the enzymic purinolytic system is reduced in length and extension, the result being that the terminal products of amino acid metabolism, as well as of purine metabolism, are excreted in the form of purines and uric acid.

Another important feature of molluscan metabolism is the extended use of amino acids as substrates for decarboxylation, for example, in cephalopods, with production of a number of amines the physiological uses of which are still conjectural.

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