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COMPARATIVE BIOCHEMICAL STUDIES ON THE LIPIDS OF MARINE INVERTEBRATES, WITH SPECIAL REFERENCE TO THE STEROLS

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INTRODUCTION

For a number of years the author and his collaborators have been engaged in a systematic comparative study of the fatty constituents of marine invertebrates. So far special attention has been devoted mainly to the unsaponifiable fractions of such fats, in particular to the sterols which they contain. Work on the phosphatide fractions of invertebrate fats and their saponifiable components is now in progress and will be discussed in a future publication.

The British biochemist Dorée must receive credit for the first significant comparative study on the occurrence of sterols in animals. The report of his observations, published in 1909 (36), represents an

1 Contributions to the study of Marine Products, XXV.
outstanding contribution to the general subject of comparative biochemistry, but it has not yet received the attention it justly deserves. The object of his studies has been expressed with admirable clarity in the introductory paragraph of his report.

If cholesterol is a body which is one of the primary constituents of animal protoplasm, we should expect to find it not only in the highly organized animals, but throughout the series from Chordata to Protozoa; or if cholesterol itself were not present its place should be filled by other and closely related forms. In the latter case it might be found that each of the great classes of the animal kingdom was characterized by the presence of a different member of the cholesterol family. On the other hand, if cholesterol is not of primary importance to all forms of life, it is not impossible that animals might be found into the composition of whose protoplasm it did not enter.

Dorée isolated and characterized the sterols from one or two representatives of most of the important phyla. In establishing the presence of sterols in all the animals under investigation he therefore inferred that sterols were typical constituents of all animals. Cholesterol was shown to be present not only in all warm-blooded animals but also in several of the invertebrates. Although Dorée did not find a typical sterol for each of the phyla, he supplied convincing evidence that at least two marine invertebrates, the sponge, Cliona celata, and the starfish, Asterias rubens, contain sterols distinctly different from cholesterol. About the same time the occurrence of sterols other than cholesterol had been demonstrated in sponges by Henze (46) and in insects by Menozzi and Moreschi (70).

For almost 25 years following the publication of Dorée's paper comparative studies on the occurrence of sterols in animals remained dormant. Numerous articles have appeared during that period which deal with the isolation of sterols from vertebrates, their principal value resting on the demonstration of the ubiquity of cholesterol in this phylum. However, little if any attention was paid to the sterols of marine invertebrates, which, on the basis of earlier observations, appeared to be the most interesting. With the elucidation of the structure of cholesterol in 1932 and the subsequent phenomenal development of steroid chemistry, the general interest in this group of compounds underwent substantial expansion. The time had become ripe for a continuation and expansion of Dorée's work which might lead eventually to an understanding of the biochemical evolution of the sterols. It is because of their great abundance and diversity that the marine invertebrates have so far become the almost exclusive subjects for such a comparative study.
Only a few of the various types of naturally occurring organic compounds are as well suited for a comparative investigation as the sterols. They are apparently present in all animals and in a diversity adequate for comparative purposes. In addition they enjoy great stability and they may be isolated readily and determined quantitatively. The same holds true in a somewhat lesser degree for other components of the unsaponifiable fraction of fats.

With the exception of 7-dehydrosterols of the type of Provitamin-D, the sterols are remarkably resistant against the action of heat and air, particularly when mixed with other organic matter. Such stability permits the investigator to accumulate and store substantial quantities of biological material, air-dried or otherwise preserved, without fear of significant alterations of the sterol present in it. It also makes possible the utilization of old, dried museum specimens. In several instances it has been shown that samples of dried sponges more than 50 years old did not differ noticeably in their sterol content from freshly collected specimens.

METHODS

The isolation of the sterols and other unsaponifiable matter is easily accomplished as a rule, and it is only in rare instances that complications may be expected. When air- or vacuum-dried material is available, it is best broken into small pieces and then thoroughly extracted with acetone in a suitable Soxhlet extractor. For the extraction of quantities exceeding several hundred grams, the type of extractor shown in Fig. 1 has been found to be most effective. It forces the vapors of the solvent to pass through the center of the extraction bulb, thereby heating the solvent in contact with the material and accomplishing a hot extraction. As the extraction proceeds the sterol occasionally begins to separate from the solvent in the lower flask. After from one to two days of continuous extraction, depending on the amount of material, the extract is evaporated to dryness.

The residue is then refluxed with benzene in an apparatus (Fig. 2) which permits, by codistillation, the steady removal of any water contained in the residue. Once this process is completed, the benzene solution is decanted off some brown, smeary material which adheres to the wall of the flask. The flask is then rinsed several times with fresh benzene. The combined benzene extracts are evaporated to dryness, and the residue is dried to constant weight at about 100° C.

In this communication it is a residue thus obtained which will be referred to as the fat.
When fresh wet material is to be extracted it is best minced and then refluxed with benzene in a round bottom flask of suitable size. As before, the water is removed continuously by codistillation with benzene. In this manner several liters of water may be removed from wet material within 24 hours. As soon as no more water is carried over by the benzene, the extract is decanted from the residue, which is then thoroughly extracted with benzene in a Soxhlet apparatus.
All benzene extracts are eventually combined and evaporated to dryness as before.

In order to obtain the unsaponifiable material, one part by weight of fat is refluxed for one hour with ten parts by volume of a 10% solution of potassium hydroxide in 75% ethanol. The solution is then cooled and mixed with two volumes of water. The mixture is extracted with several portions of ether until the last remains colorless, or practically so. The ether extracts are combined, washed several times with water and evaporated to dryness. The water adhering to the residue is removed by codistillation with benzene. The consistency of the residue at room temperature ranges from a viscous oil to a high melting solid, depending on the amount of sterol present in the mixture.

In many instances the bulk of the sterol may be obtained from the total unsaponifiable fraction by simple recrystallization. This is best accomplished by refluxing the unsaponifiable material with successive portions of methanol until all but a small amount of brown material has become dissolved. Cooling of the combined methanol extracts leads to the separation of the crystalline sterol or sterol mixture. The amount of sterol remaining in the mother liquor is then determined quantitatively by carrying out a precipitation with digitonin on an aliquot part.

The unsaponifiable fraction of certain coelenterates contains the sterols mixed with such substantial quantities of higher aliphatic alcohols and hydrocarbons as to make separation by recrystallization difficult. In such cases, and particularly where only small amounts are available, it is advisable to precipitate the total sterol with digitonin and to recover it from the digitonide by Bergmann’s method (10). The separation of nonsteroid alcohols and of hydrocarbons is best accomplished by way of the alcohol sulfates (9).

STEROLS FROM ANIMALS

Prior to the beginning of the present studies the only known major animal sterol was the well studied cholesterol. This sterol is frequently accompanied by small amounts of its 5, 6-dihydro- and 7-dehydro-derivative. In addition, the existence of other animal sterols had been indicated in certain sponges, echinoderms and insects. At present the structures of eight new animal sterols have been established and about six more have been isolated, the structures of which remain uncertain thus far.

The animal sterols known at present may be divided into three groups according to the number of carbon atoms they possess. The most prominent member of the group, with 27 carbon atoms, is the
well known cholesterol, \( \text{C}_{27}\text{H}_{46}\text{O} \). Other sterols are of the order \( \text{C}_{28} \) and \( \text{C}_{29} \). These may be regarded as derivatives of corresponding \( \text{C}_{27} \) sterols with a methyl or an ethyl group attached to the C-24-atom of the side chain. The presence of such groups at this carbon atom confers asymmetry upon it and hence makes possible the existence of two isomers, epimeric at C-24. To clarify this point it is convenient to write the structure of one such C-24-methyl epimer, with the methyl group above the side chain as in formula I, and to refer to it as a C-24-a-methyl sterol. Correspondingly, the C-24-b-methyl epimer would be represented as shown by formula II. In the case of

![Diagram I: Campestanol](image1)

![Diagram II: Ergostanol](image2)

the saturated C-24-methyl sterols, campestanol has been named the a- and ergostanol the b-methyl derivative of cholestanol; these two compounds are now being used as reference substances. By a series of deductions it has been shown that stigmastanol is the corresponding C-24-b-ethyl and poriferastanol the corresponding C-24-a-ethyl derivative\(^2\) (25).

Table I presents a survey of the known types of sterols which have been isolated so far from marine invertebrates and certain other animals. In addition it shows which sterols, or their C-24-epimers, have also been found to occur in plants. In the case of the C-24-methyl and ethyl derivatives it is of interest to note that the representatives of the a-series predominate in marine animals and those of the

\(^2\) The numbering system originally proposed by Bergmann and Low (25) has been changed to conform with that suggested by Fieser and Fieser (39).
### TABLE I.—NATURALLY OCCURRING STEROLS

#### A. Cholestanne-Series

\[ R = - \text{CH}_2\text{CH}(	ext{CH}_3)_2 \]

1. **Cholestane (Dihydrocholesterol)**
   - Sponges

2. **Cholesterol**
   - Animals

3. **7-Dehydrocholesterol**
   - (animals, small amounts)

#### B. 24-Methyl-Cholestanne Series

1. **a-series:** \[ R = - \text{CH}_.\text{CH}(	ext{CH}_3)_2 \]
2. **b-series:** \[ R = - \text{CH}.\text{CH}(	ext{CH}_3)_2 \]

- **a-series:** Campestanol
- **b-series:** Ergostanol
TABLE I.—Continued

\[
\text{CH}_3 \\
\text{CH.CH}_2\text{CH}_2\text{R}
\]

a-series: Campesterol  
(Plants, Sponges, Mollusks?)

b-series: Dihydrobrassicasterol  
(Plants, Sponges?)

\[
\text{HO-CH.CH=CH.R}
\]

a-series: Chalinasterol (Ostreasterol)  
(Mollusks, Sponges, Coelenterates)

b-series: Brassicasterol (Shakosterol)  
(Plants, Mollusks)

\[
\text{CH}_3 \\
\text{CH.CH=CH.CH}_2\text{R}
\]

a-series: Unknown

b-series: Ergosterol  
(Fungi, Annelids)

\[
\text{HO-CH.CH=CH.CH}_2\text{CH}_2\text{R}
\]

a-series: Stellastenol  
(Echinoderms)

b-series: \(\gamma\)-Ergostenol  
(Fungi)

\[
\text{CH}_3 \\
\text{CH-CH=CH.CH}_2\text{R}
\]

a-series: Stellasterol  
(Echinoderms)

b-series: Dihydroergosterol  
(Fungi)
TABLE I.—Continued

\[
\begin{align*}
\text{CH}_3 & \quad \text{CH.CH = CH.R} \\
& \quad \text{a-series: Neospongosterol (Sponges)} \\
& \quad \text{b-series: Unknown}
\end{align*}
\]

C. 24-Ethyl-Cholestan e Series.

\[
\begin{align*}
\text{C}_2\text{H}_6 & \quad \text{a-series: } R = -\text{CH.CH(CH}_3)_2; \quad \text{b-series: } R = -\text{CH.CH(CH}_3)_2 \\
& \quad \text{C}_2\text{H}_4
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3 & \quad \text{CH.CH}_2\text{.CH}_2\text{.CH}_2\text{.R} \\
& \quad \text{a-series: Poriferastanol (\gamma-Sitostanol)} \\
& \quad \text{(Plants, Sponges)} \\
& \quad \text{b-series: Stigmasterol (Plants)}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3 & \quad \text{CH.CH}_2\text{.CH}_3\text{.CH}_2\text{.R} \\
& \quad \text{a-series: Clionasterol (\gamma-Sitosterol)} \\
& \quad \text{(Marine Invertebrates, Plants)} \\
& \quad \text{b-series: Dihydrostigmasterol (Plants)}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3 & \quad \text{CH.CH = CH.R} \\
& \quad \text{a-series: Poriferasterol (Sponges)} \\
& \quad \text{b-series: Stigmasterol (Plants)}
\end{align*}
\]
b-series in plants. It is not to be inferred, however, that the presence of one epimer excludes that of another. There exist sufficient indications that in certain plants and marine invertebrates both epimers are present. A possible clue to this interesting dualism of these epimers may be seen in the structure of fucosterol (63), a compound found in many algae (31). This C29-sterol possesses no center of asymmetry at C-24 because of the presence of a double bond at this point. Hydrogenation of this double bond may lead to a C-24-a- or b-ethyl derivative, or both. If one assumes that fucosterol and its C-24-methylene...
homolog are intermediates in the biochemical formation of other sterols, one can envisage the hydrogenation of this critical double bond to proceed predominantly in the direction of the a-configuration in marine invertebrates and in the opposite direction in the case of plants.

The sterols represent the major part in the unsaponifiable fractions of most of the marine invertebrates which have been investigated thus far. As mentioned before, this is not always true for the fraction obtained from coelenterates. Here the amounts of higher aliphatic alcohols, such as myristyl- (C_{14}H_{30}O), cetyl- (C_{16}H_{34}O), and octadecyl alcohol (C_{18}H_{38}O), exceed those of the sterols as a rule. In addition, butyl alcohol (16) may be present in significant quantities. This alcohol also forms a substantial part of the unsaponifiable fraction of starfish (19). Finally, in all fractions there are present varying amounts of aliphatic hydrocarbons ranging in consistency from light liquids to solids. More recently the presence of "naphthenic" hydrocarbons and ketones with pronounced optical activity has been indicated. As yet, however, the available data on these constituents of marine invertebrates are too meagre to permit their evaluation on a comparative basis.

**PROTOZOA**

Dorée envisaged the possibility that sterols are not of primary importance to all forms of life and that animals might be found in whose protoplasm they are absent. As yet no animal devoid of sterols has been discovered; however, it would be premature to interpret such observations as indicating the presence of sterols in all animals. In connection with his significant studies of the lipids of bacteria, Anderson et al. (1) demonstrated convincingly that sterols are absent in tubercle bacilli. In addition Carter et al. (31) have made observations which suggest the same to be true for certain primitive asexual algae, of the class Myxophyceae. On the basis of such information it is reasonable to assume that sterol-free animals, if at all capable of existence, might be found among the most primitive forms, the Protozoa.

Unfortunately, however, our present knowledge of the chemical nature of the lipids of Protozoa is far too limited to permit the drawing of any significant conclusions. The paucity of available information is due primarily to the extraordinary difficulties encountered in securing uniform species of Protozoa in quantities adequate for chemical studies. The two Protozoa about which some reliable data have been made available are listed in Table II. The figures for the
familiar *Noctiluca miliaris* are based on Pratje’s (76) studies, for which about 1.5 g. of dry material was available. The author called the sterol, isolated from *Noctiluca*, cholesterol, following the then prevalent custom of assigning this name to any inadequately identified animal sterol. However, until sufficient physical and chemical data are provided for this sterol, its identity will remain uncertain.

**TABLE II. LIPID COMPOSITION OF PROTOZOA**

<table>
<thead>
<tr>
<th>Class and Species</th>
<th>% of</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fat</td>
<td>Unsapon.</td>
</tr>
<tr>
<td>Flagellata <em>Noctiluca miliaris</em></td>
<td>12</td>
<td>34.4</td>
</tr>
<tr>
<td>Sporozoa <em>Eimeria gadi</em></td>
<td>22</td>
<td>36.0</td>
</tr>
</tbody>
</table>

More definite data are available for *Eimeria gadi*. Panzer (74) secured a relatively substantial quantity of this coccidium, representing about 80 g. of dry matter, from the air-bladder of the cod, *Gadus virens*. His identification of the sterol as cholesterol is based on sound and adequate evidence.

It must be re-emphasized that the few facts listed above should not be interpreted as indicating the presence of sterols in general, or of cholesterol in particular, in all Protozoa. It is dangerous and frequently misleading to base significant conclusions concerning comparative biochemistry on data derived from the study of but few representatives of a given phylum. This point will become apparent when the great diversity of sterols in Porifera is to be considered in the subsequent chapter.

The cholesterol of the parasitic *Eimeria* may well be of exogenous origin, having been assimilated from the host, in which this sterol is a typical component. Such dependency on host sterols, whatever their nature, may be characteristic for other parasitic protozoa. In nonparasitic forms, however, such as *Noctiluca*, the sterols may well be of endogenous origin and hence of types different from cholesterol. It is to be hoped that more data on the fats of these forms can be accumulated in the future, for without them the general picture of the comparative chemistry of lipids in animals will necessarily remain incomplete.

**PORIFERA**

In 1904, Henze (46) described the isolation of a sterol from the Mediterranean sponge, *Suberites domuncula*, which was quite different from cholesterol and which he named spongosterol. He therewith demonstrated convincingly the occurrence in animals of sterols other than cholesterol. Dorée, in connection with his systematic studies mentioned previously, discovered a sterol in the cosmopolitan sponge,
Cliona celata (36), which he named clionasterol, having demonstrated not only its difference from cholesterol but also from spongosterol. In 1933 the present author (11) isolated another sterol, microcionasterol, from the abundant New England sponge, Microciona prolifera, which again was quite different from the other sponge sterols; a few years later a fourth quite distinct sterol, chalinasterol, was isolated from Haliclona (Chalina) oculata (24). The fact that four different sponges had yielded four different and seemingly new sterols at once indicated a most unusual diversity of sterols in this phylum, and it appeared for a while that every one of the different families, if not genera, might possess a characteristic sterol. Further investigations, however, to be discussed below, showed that such is not the case.

Nevertheless the preliminary observations encouraged the initiation of a systematic study of the sterols and subsequently of the other lipids of sponges. The sponges are particularly well suited for chemical studies, since they may be obtained in adequate and occasionally very substantial quantities and in a sufficient number of different species. Their collection, preservation, storage and extraction in a wet or dry state is readily accomplished. In addition, they furnish sterols in such satisfactory yields as to make possible the elucidation of the structure of new sterols or the separation of complex mixtures.

Since its inception, the purpose of the study of spongesterols has been a dual one. It was anticipated that the study of new sterols would furnish data of interest in connection with the general chemistry of sterols, and in addition it was hoped that some sterol might be found in one of the more abundant sponges, the constitution of which would make possible its ready conversion into hormones or other compounds of pharmacological significance.

The other purpose was to collect as many data as possible concerning the occurrence of sterols in sponges in order to correlate them with the present taxonomy of sponges, which at times appears rather obscure to the uninitiated. The introduction of chemical features into the taxonomy of this phylum does not necessarily constitute a novelty, since the early division of sponges into Keratosa, Silicea and Calcarea has already taken cognizance of chemical differences in sponges.

At present the lipids of more than 50 species of sponges have been investigated. Most of the sponges have been collected personally by the author in the waters of the coasts of New England, of Bermuda, Bimini and Florida.3

3 In this work he has enjoyed the generous assistance of the Bingham Oceanographic Laboratory, the Bermuda Biological Station, the Lerner Marine Laboratory of the American Museum of Natural History, and the Marine Laboratory of the University of Miami. The results of these studies could not be reported had it not
The data concerning the lipids of the sponges which have been investigated thus far are shown in Table III. In accumulating these data it was soon recognized that quantitative determinations are of little significance unless the ash of the sponges is taken into consideration. Most of the sponges contain substantial quantities of foreign inorganic matter such as sand and shell fragments. But for the Keratosa, part of the ash also consists of the sponge spicules. In order to obtain reasonably accurate data relating to the fat content of true sponge material and of its organic fraction, a representative sample of dried ground sponge, weighing 3–10 g., was burnt and the ash determined. Another sample of about equal weight was boiled with concentrated nitric acid until all organic matter had disappeared. In the case of the sponges from Bermuda, Bimini, and to some extent from Florida, such treatment also dissolved the shell fragments and coral sand, leaving only the siliceous spicules. These were then filtered on a glass filter, washed with water, acetone and ether, dried and weighed. The sand present in other sponges may be siliceous and hence remain undissolved in the nitric acid. In order to effect its separation from the spicules, the total undissolved material was agitated with water in which only the spicules are readily, if only temporarily, suspended. Filtration of the suspension and repetition of the process eventually led to the isolation of the spicules in a high state of uniformity.

The spicules of calcareous sponges can not be isolated by this method. Here it is better to digest the sponge material with hot, rather concentrated, sodium hydroxide solution, which dissolves the organic matter. The separation and washing of the spicules is then carried out as described above.

The classification of the sponges listed in Table III is based on that proposed by de Laubenfels in his treatise on the Sponge Fauna of the Dry Tortugas (59). Representatives of the following classes and orders are included in the list.

A. Demospongiae Sollas
   I. Keratosa Bowerbank
   II. Haplosclerina Topsent
   III. Poecilosclerina Topsent
   IV. Halichondrina Vosmaer
   V. Hadromerina Topsent
   VI. Epipolasida Sollas

been for the enthusiastic co-operation of the eminent sponge taxonomist, Max W. de Laubenfels (Professor of Zoology, University of Hawaii, Honolulu, T. H.), who has identified practically all the sponges under consideration and whose company the author has enjoyed on collecting trips to the Bermudas and Bimini.
### TABLE III. THE LIPIDS OF PORIFERA

<table>
<thead>
<tr>
<th>Species</th>
<th>Total spic.</th>
<th>Org. fat</th>
<th>Org. unsap.</th>
<th>Fat sterol</th>
<th>Unsap. sterol</th>
<th>Type of sterol</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>AI Spongia obliqua</td>
<td>100</td>
<td>14.5</td>
<td>33.7</td>
<td>4.2</td>
<td>10</td>
<td>(27)</td>
<td></td>
</tr>
<tr>
<td>AI Oligoceras hemorrhages</td>
<td>100</td>
<td>8</td>
<td>35.5</td>
<td>3.1</td>
<td>10</td>
<td>(27)</td>
<td></td>
</tr>
<tr>
<td>AI Ircinia variabilis</td>
<td>100</td>
<td>4</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>(27)</td>
<td></td>
</tr>
<tr>
<td>AI Verongia fistularis</td>
<td>100</td>
<td>4.1</td>
<td>25.5</td>
<td>71</td>
<td>10</td>
<td>(27)</td>
<td></td>
</tr>
<tr>
<td>AI Verongia fulva</td>
<td>100</td>
<td>6</td>
<td>24.2</td>
<td>44</td>
<td>10</td>
<td>(27)</td>
<td></td>
</tr>
<tr>
<td>AI Dysidea crawshayi</td>
<td>100</td>
<td>15</td>
<td>33</td>
<td>60</td>
<td>10</td>
<td>(27)</td>
<td></td>
</tr>
<tr>
<td>AI Dysidea etheria</td>
<td>100</td>
<td>8</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>AI Ianthella sp.</td>
<td>100</td>
<td>2.5</td>
<td>31</td>
<td>73</td>
<td>10</td>
<td>(27)</td>
<td></td>
</tr>
<tr>
<td>AII Spongilla lacustris</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>AII Haliclona coerulescens</td>
<td>7</td>
<td>93</td>
<td>2.6</td>
<td>48</td>
<td>67</td>
<td>3, 4 (27)</td>
<td></td>
</tr>
<tr>
<td>AII Haliclona variabilis</td>
<td>9</td>
<td>91</td>
<td>5.8</td>
<td>32</td>
<td>63</td>
<td>4 (27)</td>
<td></td>
</tr>
<tr>
<td>AII Haliclona rubens</td>
<td>20</td>
<td>80</td>
<td>3.7</td>
<td>76</td>
<td>15</td>
<td>1 (27)</td>
<td></td>
</tr>
<tr>
<td>AII Haliclona viridis</td>
<td>26</td>
<td>74</td>
<td>5.8</td>
<td>30</td>
<td>68</td>
<td>1 (27)</td>
<td></td>
</tr>
<tr>
<td>AII Haliclona oculata</td>
<td>30</td>
<td>70</td>
<td>5</td>
<td>35</td>
<td>38</td>
<td>7 (24)</td>
<td></td>
</tr>
<tr>
<td>AII Haliclona permillis</td>
<td>20</td>
<td>80</td>
<td>14</td>
<td>30</td>
<td>44</td>
<td>3, 4 (27)</td>
<td></td>
</tr>
<tr>
<td>AII Haliclona longleyi</td>
<td>44</td>
<td>56</td>
<td>3.5</td>
<td>38</td>
<td>74</td>
<td>9 (27)</td>
<td></td>
</tr>
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<td>AV Suberites suberea</td>
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<td>AV Suberites compacta</td>
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<td>AV Terpios zeteki</td>
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<tr>
<td>AV Cliona celata</td>
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<td>AV Cliona cariboea</td>
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<td>AV Tethya gravida</td>
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<td>10</td>
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<td>AV Cinachyra cavernosa</td>
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<td>73</td>
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<td>AV Geodia gibberosa</td>
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<td>AV Cranella crania</td>
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<td>7 (24)</td>
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<td>3.5</td>
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<td>86</td>
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<tr>
<td>BV Euplectella sp.</td>
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<td>10 (27)</td>
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<tr>
<td>CII Leucetta floridana</td>
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<td>54</td>
<td>1.3</td>
<td>37</td>
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<td>10 (27)</td>
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</table>
The individual classes and orders are indicated by numbers in the first column of Table III. The figures in columns 2 and 3 show the considerable variations in spicule content between various species. They are rather typical for a given species and may be reproduced within reasonable limits. Because of such variations, however, it is advisable to compare the fat content of various sponges as calculated on the basis of organic matter rather than the total sponge. Such figures are shown in the fourth column.

So far more than ten different sterols have been isolated from sponges, of which all but two were shown to be new. These sterols are the following: (1) Cholesterol; (2) Cholestanol; (3) Clionasterol; (4) Poriferasterol; (5) Neospongosterol; (6) Chondrillasterol; (7) Chalinasterol; (8) Aaptostanol; (9) Haliclonasterol; (10) X-sterols.

The numbers given in the second to the last column of Table III correspond to the numbers of the sterols in this list. The structures of the first seven sterols have been definitely established and are included in Table I. Sterols 8 and 9 in Table III have been isolated in a high state of purity, but their structures have not been established definitely. In addition, a number of as yet poorly defined sterols have been observed, of which several should eventually turn out to be new. These unknown sterols will be referred to by the number 10 in Table III. The sterol isolated by Mazur (69) from a freshwater sponge, *Spongilla lacustris*, appears to be a mixture of cliona- and poriferasterol (15), and the sponge sterols originally named spongosterol and microcionasterol have been shown to be mixtures (22, 23).

As yet no sponge has been found which is devoid of sterols. However, in three sponges the sterol content of the fat or the unsaponifiable matter has been observed to be substantially below the average. They are *Spongia obliqua*, *Oligoceras hemorrhages* and *Hircinia (Ir-cinia) variabilis*, which belong to the order Keratosa.

About ten years ago the author received substantial quantities of commercial and semicommercial sponges, such as “Grass” and “Sheep-wool” sponges, from the Bahamas. These sponges had been sliced and air-dried but not prepared as for sale. Extraction of sponges so treated yielded only a very small quantity of fat, which afforded such
a minute amount of sterols as to be hardly noticeable. The impression was gained from these preliminary observations that sponges of the family Spongiidae are very low in fat content. Field studies on Bimini offered the opportunity to repeat the investigations on fresh material. When a freshly caught sponge of the species *Spongia* is injured, it discharges profusely a somewhat viscous liquid, the "gurry." In order to preserve this gurry and to compare its composition with that of the solid material, the following procedure was adopted.

Two freshly caught sponges were cut into small pieces over a dish in which the gurry was collected. The solid was then passed through a meat grinder and the minced material was placed on a large Buchner funnel. By means of heavy weights more gurry was then squeezed out of the solid, a process which took about 24 hours. Toluene was added to the gurry to prevent substantial decomposition. The various fractions of gurry were then combined and dehydrated by codistillation with benzene as described under Methods. The insoluble residue weighed 93 g., of which 22% was inorganic, mainly sodium chloride. The benzene extract afforded 19.4 g. of fat. The total amount of organic material in the gurry was therefore 92 g., of which the fat represented 21.1%.

The solid sponge material was then treated in a similar manner. It gave 261 g. of ash-free material, of which 12.25% was benzene soluble. The gurry therefore contained 26% of the total organic matter and 40% of the fat of the sponge. When the sponge is dried in the open air in the customary manner the gurry is drained off more or less completely, and unless special precautions are taken it is lost and with it the bulk of the sponge fat. Contrary to the original assumption, therefore, *Spongia* is rich in fat; in fact it is one of the richest so far discovered.

The amount of unsaponifiable matter in the fat is about average, but the sterol content is low, approximately 10% of that of the average sponge fat. The isolation of the sterol has been accomplished so far only by way of the digitonide. A similar condition prevails in *Oligoceras hemorrhages*, another member of the family Spongiidae. Various species of the genus *Hircinia* (*Hircinia*) show similar properties. Here also the gurry contains about one-fourth of the organic matter of the sponge and more than 50% of its fat. The sterol content of the unsaponifiable fraction is also substantially below the average figure. These similarities suggest close biochemical relationships between the three species.

No such complications are encountered in the cases of other *Keratosa*, but although the sterols of several of the more abundant species,
such as Verongia, may be obtained in substantial quantities, no definite sterol has yet been identified. As a rule the sterols occur in complex mixtures which are extremely difficult to separate.

Altogether the sterols of more than 50 species of sponges have been isolated, but nevertheless the data are inadequate to permit their correlation to the taxonomy of sponges in general. There appears little doubt, however, that such data will eventually prove their usefulness in supplementing the present taxonomy of the Porifera. Detailed studies on several species of one family of sponges (27) has revealed in them the presence of sterols of such peculiar character as to set this family apart from otherwise taxonomically closely related families. The families to be considered in this connection are the following:

Order: Halichondrina Vosmaer
   Family: Halichondriidae Gray
   Family: Hymeniacidonidae de Laubenfels

Order: Hadromerina Topsent
   Family: Choanitidae de Laubenfels
   Family: Suberitidae Schmidt
   Family: Clionidae Gray

Three species of the Choanitidae (Spheciospongia vesparia, Spheciospongia sp. and Anthosigmella varians) and two of the Clionidae (Cliona celata and C. carriboea) contain mixtures of clionasterol and poriferasterol. The proportions of the two sterols found in the mixtures of the various sponges are so much alike as to permit no distinction between the mixtures. The sterols or sterol mixtures isolated from nine different species of Suberitidae, however, are quite different. They have one important feature in common, namely the saturated nature of the principal sterol. The presence of a large amount of saturated sterol in a mixture is readily detected. In contrast to the levorotatory unsaturated sterols such as cholesterol, clionasterol, poriferasterol, etc., the saturated sterol shows a distinct positive optical rotation. Thus the observation of the optical rotation of a sterol mixture will at once give some information concerning the amount of saturated sterol present.

The principal saturated sterol of a number of Suberitidae is cholestanol; in others it may be a stanol of the order of C$_{28}$ or C$_{29}$, the complete identification of which is still lacking. The outstanding fact is that these sterols are saturated regardless of their molecular weight and that they are quite distinct from those found in species from other families of Hadromerina. Such predominance of saturated sterols suggests a sterol metabolism different from that taking place in other families.
The usefulness of sterol analysis in assisting the identification of certain sponges has been demonstrated already in several cases. One of these shall be mentioned as a particularly illustrative example.

A sponge which is rather common in the shallow waters of the southern east coast of the United States, and which is quite abundant in Biscayne Bay, Florida, has been described under a variety of names such as *Suberites distortus*, *S. tuberculosus*, and others. Substantial quantities of this sponge were collected by the author around Miami, Florida, in 1945 and in Bimini in 1948. At first this sponge was identified by de Laubenfels as *S. distortus* on the basis of Schmidt’s description (78). But a study of the sterols obtained from a large selection of specimens of this sponge revealed the presence of clionasterol and poriferasterol as well as the absence of any detectable amounts of saturated sterols. From this evidence it was concluded that the sponge in question did not belong to the family *Suberitidae* but rather to the *Choanitidae* or *Clionidae*. In the meantime de Laubenfels had had the opportunity of carrying out field studies on this sponge in Bimini and Florida, which led to the discovery in the sponges of certain microscleres, the presence of which proved the sponge to be of the genus *Anthosigmella* and hence of the family *Choanitidae*. In many specimens of this sponge the critical microscleres are so few in number that they may easily be overlooked, which may result in misidentification. On the other hand, the sterol analyses give such consistent and reliable results that they must be regarded as most valuable supplementary evidence in the identification of this sponge and of others superficially resembling it.

Another sponge of interest in this connection is *Hymeniacidon heliophila*. This species had been described as *Stylotella heliophila* by Parker (75) and later under the same name by George and Wilson (41). It is quite common near Beaufort, N. C. In 1932, de Laubenfels (58) referred it to *Hymeniacidon* because of its close resemblance to various European members of this genus. If this sponge were indeed a species of the genus *Stylotella* it would belong to the family *Suberitidae* of the Order *Hadromerida*, and hence one should expect it to contain a saturated sterol as the principal component of its sterol mixture. Otherwise it would belong to the family *Hymeniacidonidae* of the order *Halichondrina*.

Analysis of the sterol mixture that was obtained from a large amount of this sponge revealed the presence of a saturated sterol, cholestanol (23), as the principal sterol. On the basis of this evidence, therefore, it might be argued that the sponge under consideration is a species of *Suberitidae* and that it should be referred once more to this family.
Such a transfer will remain premature, however, until more is known about the sterol content of other species of *Hymeniacidon* and of the closely related *Halichondria*. Until such knowledge is available it is safe to state only that there appear to exist closer chemical relationships between *Hymeniacidon heliophila* and the Suberitidae than between the latter and Choanitidae and Clionidae.

At present the sterols of seven species of the genus *Halichona* (Order Haplosclerina) have been isolated and investigated. The results of these studies have revealed a surprising lack of uniformity of the sterols or sterol mixtures present in these species. Each of three species contains a different sterol in an astonishingly high degree of uniformity. Thus *Halichona oculata* has afforded chalinasterol (24), which is identical with the ostreasterol found in certain mollusks (5). Poriferasterol has been shown to be the principal sterol of *Halichona variabilis* (27). A new sterol, which has tentatively been named haliclonasterol, C$_{28}$H$_{48}$O, is present to the practical exclusion of other sterols in *Halichona longleyi* (27). The sterol mixtures isolated from *Halichona viridis* and *H. rubens* are of a similar composition, their most outstanding feature being the presence of more than 50% of cholesterol. Both *Halichona permollis* and *H. coerulescens* have yielded sterol mixtures in which poriferasterol and clionasterol appear to be the principal components.

At present the significance of these observations is difficult to evaluate. The necessity of a revision of this genus, into which approximately 100 species have been placed, is generally realized. The probability is envisaged here that in such a future revision sterol analyses will render valuable assistance. On the basis of the present, but unfortunately limited, evidence derived from sterol studies, there appear to exist at least four distinct groups within the genus *Halichona*. They are I. the *oculata* type, II. the *longleyi* type, III. the *rubens* and *viridis* type, and IV. the *permollis* and *coerulescens* type. The last group might possibly include *Halichona variabilis* also, if the difference between its sterol mixture and that of the two others is regarded as of a quantitative rather than a qualitative nature.

**COELENTERATA**

Table IV lists the coelenterates which at present have been subjected to more than a casual study and lipids obtained from them. All but two of the species belong to the Anthozoa which, for technical reasons, are best suited for chemical studies. The fat content of the coelenterates is rather high, particularly when calculated on the basis of the organic matter of the animal rather than its total weight. With the
exception of *Velella spirans*, the unsaponifiable fraction of coelenterate fats is almost as high as that of sponge fats. It has been pointed out in the previous chapter that the sterols form the most substantial part of the unsaponifiable fraction of sponge fats, but this is not the case in the corresponding fraction from coelenterates. Here the sterols are frequently present in such comparatively small amounts as to make difficult their isolation by means other than precipitation with digitonin. The bulk of the unsaponifiable matter consists of higher alcohols and hydrocarbons of the types which have been listed under METHODS. The fats of a number of coelenterates are rich in "wax-like" matter, such as cetyl palmitate (13), the saponification of which accounts for the presence of cetyl alcohol in the unsaponifiable fraction. The occurrence of other waxes, such as fatty acid esters of myristyl- and octadecylalcohol, is indicated by the presence of such alcohols in sea anemones (27) and gorgonids (16).

As yet little is known about the nature of the sterols of coelenterates. The only sterols which have been definitely identified so far are cholesterol and chalinasterol. Cholesterol has been found in certain sea anemones (36), in a coral, a gorgonid (18) and a jellyfish (45), chalinasterol in the colonial anemone, *Zoanthus proteus* (27). A new sterol, tentatively named palysterol, has been isolated from another colonial anemone, *Palythoa mammilosa* (27). Its structural relations to other sterols have not yet been established. The actiniasterol (55) and gorgoniasterol (18) which were regarded originally as new sterols have

### TABLE IV. LIPIDS OF COELENTERATA

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<th>Class and Species</th>
<th>Total org.</th>
<th>Total fat</th>
<th>Org. fat</th>
<th>Fat unsap.</th>
<th>Unsap. sterol</th>
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<tr>
<td>Mussa fragilis</td>
<td></td>
<td>0.5</td>
<td></td>
<td>41</td>
<td>5</td>
<td>(27)</td>
</tr>
<tr>
<td>Oculina diffusa</td>
<td></td>
<td>0.3</td>
<td></td>
<td>24</td>
<td>9</td>
<td>(27)</td>
</tr>
<tr>
<td>Siderastrea radians</td>
<td></td>
<td>0.3</td>
<td></td>
<td>26</td>
<td>11</td>
<td>(27)</td>
</tr>
<tr>
<td>Madrepore cervicornis</td>
<td></td>
<td>0.5</td>
<td></td>
<td>33</td>
<td></td>
<td>(18)</td>
</tr>
</tbody>
</table>
since been shown to be complex mixtures of ill-identified sterols (27, 35). The Provitamin-D content of the sterols of a number of coelenterates has been determined spectrographically (28, 42); it ranges from 2–10%. These data do not include any statements concerning the general nature of the sterols, and therefore they are of interest only in connection with the Vitamin-D problem.

Except for the lipids of the few species listed in Table IV, practically nothing is known about the organic constituents of stony corals. Contrary to popular belief, reef-building corals contain by no means inconsiderable quantities of organic matter. This was first pointed out in 1846 by Silliman (80), who reported in his classical paper on the composition of stony corals; he found that the organic matter represented from 4–8% of the total and that it was intimately united with the inorganic material throughout the structure of the coral. His observations were verified in 1922 by Clarke and Wheeler (32), who determined the organic matter of 28 species of corals and found that it amounted to from 2–7% of the total. Similar observations have been made quite recently by the present author (27).

Silliman was also the first to call attention to the fact that part of the organic matter of corals consists of some wax-like material which can be separated from its surroundings by boiling water. After coral fragments had been boiled, he found this wax floating on the surface of the water in transparent, jelly-like masses of yellowish color. It was insoluble in alcohol but readily soluble in cold ether, and the evaporation of its ethereal solution yielded a solid which resembled "wax." The average amount of such wax-like fats in stony corals is about 0.3% for species from Bermuda and probably 0.5% for those from more tropical regions. As Silliman had already pointed out, this lipid is not confined to the thin living layer of the coral but extends deep into the entire structure. The weight of the total fatty material of a massive coral of one cubic meter volume, such as a brain coral, may be calculated to be in excess of seven kilograms, and that of a coral reef to be of substantial magnitude.

Such considerations led the present author and Lester in 1940 (12) to call attention to coral reefs as one of the possible sources of petroleum. They maintained that, unless very unusual conditions prevail, the bulk of organic matter from dead marine animals and plants is probably brought back rapidly into circulation in one way or another. In the case of reef-building organisms, including bryozoa and coralline algae, a significant portion of the organic matter becomes trapped in the evergrowing inorganic mass and hence is removed from circulation. If this is the case, then reefs [bioherms (34)] must be regarded as vast
accumulators of materials which may be considered as potential precursors of petroleum. At some time comparatively mild changes in the physical conditions may loosen this material from its inorganic surroundings and bring it to the surface, as in the case of the wax-like material which Silliman found floating on the surface of the water in which corals had been boiled.

In this connection it is of interest to note that in February 1947 a commercial oil pool was discovered near the village of Leduc, Alberta. This pool has been described recently by Link (60), who holds that "the oil and gas accumulation is apparently due primarily to a stratigraphic trap created by coral-reef or bioherm development." He also points out that at present the majority of petroleum geologists would probably consider the surrounding shale and the underlying sediments as the source rock of the oil accumulated at Leduc. In his opinion, however, "the oil was not only stored in the bioherm but was also generated in the reef."

**PLATYHELMINTHES AND NEMATHELMINTHES**

With but few exceptions, nothing appears to be known about the lipids of animals belonging to phyla generally placed between the Coelenterata and Annelida. Substantial quantities of cholesterol have been isolated from the parasitic flatworms, *Diphyllobothrium latum* (*Bothriocephalus latus*) by Faust and Tallquist (38) and Welsch (95), *Fasciola hepatica* by Brand (30) and *Taenia taeniaformis* by Salisbury and Anderson (77). The nematodes, *Ascaris lumbricoides* and *A. megalocephala*, have been studied by Flury (40), who found that the dried worms contained 10.9% of fat, of which 24.7% was unsaponifiable.

**ANNELIDA**

For centuries an oil extracted from the earthworm *Perichaeta communissima* has been used in Japan as an antipyretic. Because of its alleged medicinal value the oil was investigated by Murayama and Aoyama (72), who found that it represents 2.3% of the dry animal and that it contains 31.2% of unsaponifiable matter. A similarly high amount of this fraction has been found in other earthworms and also in two marine species, as shown in Table V.

As yet only the sterols of *Lumbricus terrestris* have been adequately investigated. De Waele (94) has shown that the principal sterol is cholesterol, but Bock and Wetter (28) have demonstrated more recently that the original sterol mixture contains, in addition to cholesterol, more than 20% of a Provitamin-D, identified as ergosterol, and also some other poorly defined sterol or sterols.
Despite the fact that crustaceans are readily available in a profusion of species, practically no systematic studies have been carried out on the composition of their fatty constituents. There even exists a paucity of information concerning the amount of unsaponifiable matter present in the fats. The data shown in Table VI have been derived largely from planktonic forms, many of these being species that inhabit fresh water. Corresponding data for the more conspicuous marine forms, such as crabs, lobsters, etc. are still missing.

All available evidence indicates that cholesterol is the typical sterol of crustaceans. Dorée (36) first showed it to be the constituent of a crab, *Carcinus moenas*. Later cholesterol was isolated from *Portunus plicatus* and *Eriphia spinifrons* by Leulier and Poliarc (61), and quite recently from *Cancer magister* by Kind and Fasolino (52). The presence of substantial amounts of cholesterol in the oil of a *Cypridina* species has been shown by Kotake and Kimoto (57); Lovern (62) has
indicated its presence in the unsaponifiable matter of copepod oil, where it is accompanied by a large amount of alcohols, such as cetyl alcohol and higher homologs. Cholesterol has been isolated also from *Ligia exotica* (82). Of interest in this connection is the work by Vilbrandt and Abernethy (93) on the utilization of shrimp waste. These authors found that such waste contains 2.25% of an oil from which 19% of cholesterol can be extracted without taking recourse to saponification. They have pointed out that "a production of 80,000 pounds of cholesterol could have been accomplished by the extraction of the waste of the 1927 crop of shrimps."

**MYRIAPODA**

The fatty extract of the millipede, *Strongylosoma tambanum*, which has found some use in Japan under the name of "Aka-yusade" oil, contains 21.4% of unsaponifiable matter. Ueno and Yamasaki (89) have isolated a sterol from this fraction which they named yasudesterol in the belief that it was a new compound. However, the evidence presented by the authors is indicative of lack of uniformity in the sterol. Aside from this isolated fact, nothing appears to be known about the lipids of this class of animals.

**INSECTA**

The fats of insects have been the subject of numerous investigations, but systematic studies on the unsaponifiable fractions and the sterols they contain are still missing. Data have become available on the amounts of unsaponifiable matter in the fats of more than 30 species. They have been contributed principally by Mieller (71), Timon-David (81), Giral (43), Welsch (95), Ueno and Komori (90), Tsujimoto (84) and Bergmann (6). The lowest value is reported for *Ergates faber*, 0.75% (80), the highest for *Melolontha vulgaris*, 19.4% (95). The average of 31 values is about 7%.

The only insect fat of commercial value is the "chrysalis oil," a by-product of sericulture. Because it is readily available, it has served as the first starting material for the study of insect sterols. It was originally believed that the principal sterol of this oil from *Bombyx mori* was a new sterol, and consequently it had been named bombycysterol. More recent investigations by Bergmann (6) have shown, however, that this sterol is a mixture in which cholesterol is the principal component. The cholesterol is accompanied by other sterols, the nature of which has not yet been established. It appears quite certain that Kawasaki's inagosterol from locusts (51) is a mixture of similar composition. All available evidence indicates that analogous
mixtures are present in other herbivorous insects, and that cholesterol may be regarded as the typical sterol for all insects.

XIPHOSURA

The sterol of the horseshoe crab, *Limulus polyphemus*, is cholesterol, as has been shown by Bergmann et al. (18). Nothing appears to be known about the lipids of Arachnida.

MOLLUSCA

In 1934, Bergmann (4, 5) and Tsujimoto and Koyanagi (87) independently presented convincing evidence that the principal sterols of certain mollusks may be other than cholesterol. Since then a considerable amount of information on mollusk sterols has become available, the pertinent data of which have been summarized in Table VII. These data show a surprising constancy of the fat content of various fresh wet mollusks and of the amount of unsaponifiable matter present in the fats. The average fat content is somewhat higher in gastropods than in pelecypods. In disagreement with these figures are those shown in Table VIII which have been taken from Mieller's

### TABLE VII. Lipids of Mollusca

<table>
<thead>
<tr>
<th>Class and Species</th>
<th>% Fat of wet weight</th>
<th>% of Unsap.</th>
<th>Sterylacetate M. P. °C and sterol</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pelecypoda</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tapes philippinarum</td>
<td>0.8</td>
<td>15.3</td>
<td>137</td>
<td>(85)</td>
</tr>
<tr>
<td>Corbicula leana</td>
<td>0.7</td>
<td>14.8</td>
<td>126–7 corbi-, brassicasterol</td>
<td>(64, 67, 85)</td>
</tr>
<tr>
<td>Cristaria picata</td>
<td>1.3</td>
<td>13</td>
<td>137–8</td>
<td>(85)</td>
</tr>
<tr>
<td>Meretrix meretrix</td>
<td>0.7</td>
<td>12.6</td>
<td>137–8 meretristerol</td>
<td>(83)</td>
</tr>
<tr>
<td>Ostrea gigas</td>
<td>1.4</td>
<td>11.8</td>
<td>136–7 conchasterol</td>
<td>(88, 87)</td>
</tr>
<tr>
<td>Ostrea virginica</td>
<td>1.5</td>
<td>11</td>
<td>134–5 ostreasterol</td>
<td>(4)</td>
</tr>
<tr>
<td>Mya arenaria</td>
<td>1.6</td>
<td>—</td>
<td>131</td>
<td>(87)</td>
</tr>
<tr>
<td>Volsella modiolus</td>
<td>3 (?)</td>
<td>12</td>
<td>127–8</td>
<td>(87)</td>
</tr>
<tr>
<td>Tridacna gigas</td>
<td>0.9</td>
<td>11.5</td>
<td>156–7 shakosterol</td>
<td>(88)</td>
</tr>
<tr>
<td>Venus mercenaria</td>
<td>0.7</td>
<td>15</td>
<td>131</td>
<td>(4)</td>
</tr>
<tr>
<td>Modiolus modiolus</td>
<td>1.0</td>
<td>16</td>
<td>131</td>
<td>(4)</td>
</tr>
<tr>
<td>Modiolus demissus</td>
<td>—</td>
<td>—</td>
<td>156–7 brassicasterol</td>
<td>(27)</td>
</tr>
<tr>
<td><strong>Gastropoda</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haliotis gigantea</td>
<td>1.0</td>
<td>10</td>
<td>117 cholesterol</td>
<td>(85)</td>
</tr>
<tr>
<td>Turbo cornutus</td>
<td>1.0</td>
<td>14.6</td>
<td>116 cholesterol</td>
<td>(85)</td>
</tr>
<tr>
<td>Rapania thomaisiana</td>
<td>1.8</td>
<td>15</td>
<td>120 cholesterol</td>
<td>(87)</td>
</tr>
<tr>
<td>Callana nigrolineata</td>
<td>4.2</td>
<td>15</td>
<td>115 cholesterol</td>
<td>(87)</td>
</tr>
<tr>
<td>Tegula xanthostigma</td>
<td>2.8</td>
<td>11</td>
<td>118 cholesterol</td>
<td>(86)</td>
</tr>
<tr>
<td>Fulgur sp.</td>
<td>1.5</td>
<td>12.3</td>
<td>117 cholesterol</td>
<td>(4)</td>
</tr>
<tr>
<td>Buccinum undatum</td>
<td>—</td>
<td>—</td>
<td>cholesterol</td>
<td>(28, 36)</td>
</tr>
<tr>
<td>Littorina littorea</td>
<td>—</td>
<td>—</td>
<td>cholesterol</td>
<td>(53)</td>
</tr>
<tr>
<td>Nerita peleronta</td>
<td>—</td>
<td>—</td>
<td>cholesterol</td>
<td>(54)</td>
</tr>
<tr>
<td>Nassa obsoleta</td>
<td>—</td>
<td>—</td>
<td>cholesterol</td>
<td>(54)</td>
</tr>
<tr>
<td><strong>Cephalopoda</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sepia officinalis</td>
<td>—</td>
<td>—</td>
<td>cholesterol</td>
<td>(35, 48)</td>
</tr>
<tr>
<td>Octopus vulgaris</td>
<td>—</td>
<td>—</td>
<td>cholesterol</td>
<td>(95)</td>
</tr>
</tbody>
</table>

---

*Journal of Marine Research [VIII, 2]*
(71) little known report on the fats of invertebrates. The average of Mieller's figures for percentages of unsaponifiable matter is approximately twice that of those shown in Table VII. This discrepancy, which is well outside the limits of experimental error, may find an explanation in the fact that all species investigated by Mieller are inhabitants of fresh water, while those listed in Table VII are of marine origin with the exception of two terrestrial snails.

TABLE VIII. LIPIDS OF FRESHWATER MOLLUSKS (71)

<table>
<thead>
<tr>
<th>Species</th>
<th>% of Fat Unsaponifiable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limnaea stagnalis</td>
<td>29.8</td>
</tr>
<tr>
<td>Planorhics corneus</td>
<td>24.1</td>
</tr>
<tr>
<td>Paludina vivipara</td>
<td>35.1</td>
</tr>
<tr>
<td>Unio sp.</td>
<td>20.8</td>
</tr>
<tr>
<td>Anodonta sp.</td>
<td>24.1</td>
</tr>
<tr>
<td>Dreissena polymorpha</td>
<td>14.0</td>
</tr>
<tr>
<td>Sphaerias sp.</td>
<td>24.2</td>
</tr>
</tbody>
</table>

The first mollusk sterol to be recognized as different from cholesterol was ostreasterol (4). At first it was believed to be a typical constituent of all mollusks. Later investigations, however, revealed in a variety of species the presence of other sterols, among them cholesterol. The confusion resulting from such seemingly contradictory observations is cleared up in part when the species are arranged according to classes. It then becomes apparent that in the pelecypods the sterols other than cholesterol predominate or are present in substantial amounts. In contrast, the gastropods contain cholesterol as their principal sterol. It must be emphasized that these differences are of a quantitative rather than qualitative nature, since the presence of cholesterol has been detected in pelecypods (37), and that of sterols other than cholesterol in gastropods. Nevertheless, the over-all difference is quite distinct and merits special attention. Unfortunately nothing appears to be known as yet about the sterols of freshwater mollusks.

In most cases the melting point of the mollusk sterylacetate already affords a good indication of the nature of the principal sterol. A melting point between 110–120°C points to the presence of cholesterylacetate, m. p. 115–116°C, the melting points above 130°C to that of other mollusk steryl acetates (see Table VII). These new mollusk sterols have been described under the following names: ostreasterol, shakosterol, conchasterol, meretristerol, magakisterol and corbisterol. The structure of two of these sterols has been definitely established. Ostreasterol has been shown to be identical with chalinasterol (Table
I) (25), which in turn has been identified as the C-24-methyl epimer of brassicasterol. The properties of shakosterol (88) are so similar to those of brassicasterol as to leave little doubt about the identity of the two compounds. Moreover, the occurrence of brassicasterol in mollusks has been demonstrated convincingly, and it has been shown also that this sterol had once been mistaken for stigmasterol (8, 27). The suggestion has been made that meretristerol and conchasterol (67) are identical, but it appears doubtful that they are uniform compounds. The probability is strongly indicated that they are mixtures, difficult to separate, of the two epimers chalina- and brassicasterol. Too little is known about magakisterol (67) to permit any prediction concerning its constitution, but it is probably a mixture of substances. Corbisterol (64) appears to be a sterol of the Provitamin-D type, as has been suggested recently by the present author (27). The presence in mollusks of substantial amounts of sterols acquiring antirachitic activity upon irradiation has been known for some time. Thus Bock and Wetter (28) and Boer et al. (29) have shown that the Provitamin-D content (ergosterol) of the sterol mixture was 9.3–25% in the land snails Helix pomatia and Arion empiricorum and 5–26% in marine mollusks, Littorina littorea, Archidoris tuberculata and Mytilus edulis. The Provitamin-D content of the abundant mussels Mytilus and Modiolus has been found sufficiently high to make commercial use of these species in Vitamin-D preparations. The nature of the Provitamin-D from Mytilus has been thoroughly investigated recently by van der Vliet (91, 92), who believes the principal components to be ergosterol and 7-dehydrocholesterol.

ECHINODERMATA

At present reliable information on the lipids of echinoderms has become available for three of the five classes of this phylum. The pertinent data are given in Table IX. The figures given for the fat content of the various species are as yet of little comparative value. They are based on the total animal rather than its organic matter. The values for the amounts of unsaponifiable material present in the fat are more significant; their average is about 20%.

The sterols of Asteroidea were among the first animal sterols to be recognized as distinctly different from cholesterol. Doreé (36) called attention to their peculiar properties, and later Kossel and Edlbacher (56) described one of them in greater detail, naming it stellasterol. More recent investigators have assigned other names to these sterols, such as asteriasterol (73) and hitodesterol (66). The structure of these sterols was eventually elucidated by Bergmann (7, 20), who showed that the typical mixture contains a mono-unsaturated sterol,
stellastenol, and a di-unsaturated setrol, stellasterol. Both sterols are C-24-a-methyl sterols of the type shown in Table I. These sterols are easily differentiated from cholesterol by the high melting point of their acetate, by their optical rotations, which lie close to zero, and by the green color reaction which they give with bromine. Strong indications exist that the same sterol mixture, or one closely related to it, is also present in the Holothuroidea (18).

<table>
<thead>
<tr>
<th>Class and Species</th>
<th>% of Fat Unsaponifiable</th>
<th>Type of Principal Sterol</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Asteroidea</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asterias rubens</td>
<td>30</td>
<td>stellasterols</td>
<td>(56, 49)</td>
</tr>
<tr>
<td>Asterias forbesi</td>
<td>34</td>
<td>stellasterols</td>
<td>(7, 20)</td>
</tr>
<tr>
<td>Asterias rollestoni</td>
<td>10</td>
<td>hitodesterol</td>
<td>(66)</td>
</tr>
<tr>
<td>Astropecten scoparius</td>
<td>12</td>
<td>hitodesterol</td>
<td>(82)</td>
</tr>
<tr>
<td>Asterina pectinifera</td>
<td>20</td>
<td>hitodesterol</td>
<td>(82, 65)</td>
</tr>
<tr>
<td><em>Echinoidea</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tripneustes esculentus</td>
<td>26</td>
<td>cholesterol</td>
<td>(18)</td>
</tr>
<tr>
<td>Centrichinus antillarum</td>
<td>20</td>
<td>cholesterol</td>
<td>(18)</td>
</tr>
<tr>
<td>Lytechinus variegatus</td>
<td>—</td>
<td>cholesterol</td>
<td>(18)</td>
</tr>
<tr>
<td>Heliocidarus crassidus</td>
<td>—</td>
<td>cholesterol</td>
<td>(82)</td>
</tr>
<tr>
<td>Arbacia punctulata</td>
<td>22</td>
<td>cholesterol</td>
<td>(27)</td>
</tr>
<tr>
<td><em>Holothuroidea</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Holothuria princeps</td>
<td>11</td>
<td>stellasterols</td>
<td>(18)</td>
</tr>
<tr>
<td>Cucumaria chronjhelmi</td>
<td>11</td>
<td>stellasterols</td>
<td>(18)</td>
</tr>
</tbody>
</table>

In contrast, the principal sterol of all Echinoidea which have been studied so far is cholesterol (18, 52). Its presence has been demonstrated convincingly in four species and has been indicated in others. The striking difference in the nature of the sterols of *Asteroidea* and *Holothuroidea* on one side and that of *Echinoidea* on the other is to some extent paralleled by another constituent of the unsaponifiable fraction. As has been mentioned under METHODS, the glycerol ether, batyl alcohol, is a typical constituent of the unsaponifiable fraction of starfish fat. This compound (56, 19) may occur in such quantities as to obscure the presence of the sterol.

Recently Matsumota and Toyama (66) have shown that batyl alcohol, or a closely related compound, is also present in substantial amounts in the unsaponifiable fraction of the holothurian, *Cucumaria chronjhelmi*. In the corresponding fractions of *Echinoidea* the presence of such compounds has not yet been observed. If they are present at all, and it seems likely that they might be, it will be in quantities significantly smaller than those found in representatives of the other two classes.

In his discussion of the distribution of arginine and creatine in animals, Baldwin (2) has called attention to the fact that of all the invertebrate types studied prior to 1947 “the echinoderms are excep-
tional in that they alone include species in which the presence of creatine has been satisfactorily demonstrated.” In the echinoids, arginine and creatine occur side by side, while in the ophiuroids (brittle stars) only creatine could be detected.

TABLE X. DISTRIBUTION OF PHOSPHAGENS, STEROLS AND BATYLALCOHOL IN ECHINODERMATA

<table>
<thead>
<tr>
<th></th>
<th>Arginine</th>
<th>Creatine</th>
<th>Stellasterol</th>
<th>Cholesterol</th>
<th>Batylalcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asteroidea</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>abundant</td>
</tr>
<tr>
<td>Ophiuroidea</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+ (?)</td>
<td>?</td>
</tr>
<tr>
<td>Echinoidea</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>absent (?)</td>
</tr>
<tr>
<td>Holothuroidea</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>abundant</td>
</tr>
</tbody>
</table>

In Table X the data given by Baldwin (2) are compared with those based on the study of the sterols. The fact stands out that, on the basis of three quite different and independent sets of chemical analyses, the asteroids and holothurians may be regarded as a group set apart from the echinoids. It is of considerable interest that cholesterol is the principal sterol of the echinoids. The same compound is the typical sterol of the vertebrates, and on the basis of limited information it also appears to be characteristic for Protochordata. Since it has been shown that certain anatomical relationships exist between echinoids and Protochordata, the presence of cholesterol in the former appears to be of particular interest.

Unfortunately no data are available on the sterols of crinoids for a completion of the picture. Only one observation has been made so far on the sterols of the ophiuroids, Ophiopholis aculeata (27), and this will have to be accepted with some reservation until it has been verified. The total amount of available sterol was only about 40 mg. It was distinctly different from the starfish sterol, as shown particularly by its strongly negative rotation of \(-36^\circ\). The identity of this sterol with cholesterol has been indicated but not fully demonstrated because of lack of material. If further studies should prove the sterol of ophiuroids to be cholesterol, then another striking parallel between the occurrence of arginine-creatine and stellasterols-cholesterol will have been established for this phylum. It would indicate also that a closer biochemical relationship exists between the ophiuroids and echinoids than between the former and the asteroids.

In this connection a recent paper by Fell (38a), which deals with echinoderm embryology, is of particular interest. In it the author has

*See, however, a recent paper by Baldwin and Yudkin (3), in which the presence of creatine in annelids is indicated.

*The author is indebted to G. E. Hutchinson for having called his attention to this paper.
presented "a phylogenetic tree [Fig. 3] which the recapitulation theory would construct from embryological evidence, if larval forms are regarded as repeating ancestral conditions."

However, Fell arrives at the conclusion that "it is impossible to accept the result which implies that ophiuroids and echinoids are more closely related to each other than to other classes, and that the

![Phylogenetic tree of Echinoderms based on Embryological evidence](image)

holothurians and starfish are similarly connected," and that "the recapitulation theory as applied to larval forms leads to a reductio ad absurdum in the case of echinoderms." Looked at from the viewpoint of comparative biochemistry, this phylogenetic tree does not appear as absurd as indicated by Fell. On the contrary, the embryological evidence which favors close relationship between holothurians and starfish on the one side, and between echinoids and ophiuroids on the other, finds substantial support in biochemical evidence which indicates analogous relationships.

**TUNICATA**

Except for the observation by Bergmann et al. (18) that cholesterol is the principal sterol of the tunicate, *Styela plicata*, nothing appears to be known about the unsaponifiable matter of Protochordata.

**VERTEBRATA**

In contrast to the fats of invertebrates, those of vertebrates contain only small quantities of unsaponifiable matter. The average amount of this fraction in the fats of more than 50 species ranging from the lamprey to man is about 1.2%. In calculating this average value, the oils of a few species have not been included because they are known to contain exceptionally large amounts of unsaponifiable matter. These are, for example, the liver oils of sharks which may contain in excess of 80% of hydrocarbons and the head oils of the sperm whale and related species which consist principally of cetyl palmitate.
The sterols of vertebrates have been the subject of numerous investigations. In all species, cholesterol has been found to be the principal sterol, accompanied by only small amounts of cholestanol and 7-dehydrocholesterol. Hüttel and Behringer (50), however, have found by no means inconsiderable quantities of phytosterol-like compounds in fats extracted from the skins of certain toads. Thus the sterol mixtures from the skins of *I ufo vulgaris*, *B. vulgaris formosa* and *B. arenarium* contain 20, 5 and 17% of such sterols respectively. This observation emphasizes the peculiar character of the biochemistry of the toad in which the synthesis of "digitalis-like" heart poisons and the excretion of arginine are prominent features.

**DISCUSSION**

The most outstanding difference between the fats of vertebrates and invertebrates is the presence of substantial amounts of unsaponifiable material (see Table XI). In 1923, at a time when only a few data were known, Grün (44) had already called attention to this fact, and in addition he made certain predictions and drew certain conclusions which are of general interest. His comments appear to have been widely overlooked, principally because they form but a small fraction of a long review on the progress of fat chemistry, which has been published in a trade journal read only by a limited group of scientists. In order to make his comments better known, a complete translation of the pertinent paragraph is given herewith.

**TABLE XI. DISTRIBUTION OF UNSAPONIFIABLE MATTER IN ANIMALS**

<table>
<thead>
<tr>
<th>Phyla or Classes</th>
<th>% of Unsaponifiable Matter of Fat</th>
<th>Number of Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protozoa</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>Porifera</td>
<td>37</td>
<td>45</td>
</tr>
<tr>
<td>Coelenterata</td>
<td>35</td>
<td>16</td>
</tr>
<tr>
<td>Nemathelminthes</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>Annelida</td>
<td>22</td>
<td>7</td>
</tr>
<tr>
<td>Crustacea</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>Myriapoda</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>Insects</td>
<td>7</td>
<td>31</td>
</tr>
<tr>
<td>Mollusca (marine)</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>Echinodermata</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>Chordata</td>
<td>1.2</td>
<td>50</td>
</tr>
</tbody>
</table>

A systematic study of the fats of lower animals and plants might lead to the discovery of relationships which should prove to be of biological interest, in particular as far as the theory of evolution is concerned. In the lowest animals and plants, also in bacteria, one finds in contrast to the higher forms less glycerides than wax-alcohols and hydrocarbons. (There are, however, also vertebrates, whose liver fats consist up to 90% of hydrocarbons. They are interestingly enough
the Elasmobranchs which are most closely related to the Proselachii.) It is also to be taken into consideration that the fats of oil producing plants of higher order in their earlier processes of development consist mainly of wax-like compounds which make room for the glycerides as the plant matures until they have practically disappeared. (See for example the maturation of the "poppy-milk," hemp etc.) Since the biogenetic law teaches that the development of the individual is a rapid recapitulation of the development of the species, the composition of the fat of a youthful plant of higher orders may serve as an index to the composition of the fat of primitive precursors or the "Urform" in general.

The data which have been accumulated since the appearance of Grün's paper support his views in the difference between the fats of lower and higher animals. In addition, they indicate that among the invertebrates the lower forms have the higher amounts of unsaponifiable matter in their fats. It appears, therefore, that evolution has been accompanied by a decrease in the relative amounts of this fraction. It is to be hoped that future systematic studies will contribute more data needed to complete this fragmentary picture.

The relatively low figure for the amount of unsaponifiable matter in insect fats should not be interpreted as typical for terrestrial invertebrates in general. The fats of other terrestrial invertebrates may contain such material at the usual high level, such as 21.5% in the case of a millepede and 30% in that of earthworms.

### TABLE XII. DISTRIBUTION OF STEROLS IN ANIMALS

<table>
<thead>
<tr>
<th>Phyla and Classes</th>
<th>Principal Sterols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porifera</td>
<td>cholesterol, cholestanol, clionasterol, poriferasterol, chalinasterol, neospongasterol, chondrillasterol, haliclonasterol, aptostanol and others.</td>
</tr>
<tr>
<td>Coelenterata</td>
<td>cholesterol, clionasterol, chalinasterol, palysterol and others.</td>
</tr>
<tr>
<td>Annelida</td>
<td>cholesterol</td>
</tr>
<tr>
<td>Arthropoda</td>
<td>cholesterol</td>
</tr>
<tr>
<td>Mollusca</td>
<td>cholesterol</td>
</tr>
<tr>
<td>Pelecypoda</td>
<td>cholesterol</td>
</tr>
<tr>
<td>Gastropoda</td>
<td>cholesterol</td>
</tr>
<tr>
<td>Cephalopoda</td>
<td>cholesterol</td>
</tr>
<tr>
<td>Echinodermata</td>
<td>stellasterols</td>
</tr>
<tr>
<td>Asteroidea</td>
<td>stellasterols</td>
</tr>
<tr>
<td>Holothuroidea</td>
<td>cholesterol</td>
</tr>
<tr>
<td>Echinidea</td>
<td>cholesterol</td>
</tr>
<tr>
<td>Protochordata</td>
<td>cholesterol</td>
</tr>
<tr>
<td>Chordata</td>
<td>cholesterol</td>
</tr>
</tbody>
</table>

Table XII presents a summary of the results of comparative studies on the sterols of animals. It is the summary of a story which is still far from completion. As yet it does not include species of several phyla, while species from other phyla are represented in numbers too inadequate to permit the drawing of far reaching conclusions. It is to be expected, therefore, that the general picture, as it now appears, will
undergo many modifications as soon as more data become available. Nevertheless it can be stated with some certainty already that the greatest diversity of sterols is to be found among the more primitive invertebrates, and that the process of the biochemical evolution of the sterols has led into the direction of the practically exclusive use of cholesterol.

For some time it had been customary among sterol chemists to divide sterols on the basis of their natural occurrence into animal and plant sterols, zoo- and phytosterols. It was then believed that animals were incapable of synthesizing the complex ring system of the sterols, and that they assimilated such compounds from plant material in order to modify them into cholesterol. Schoenheimer (79) and others, however, showed eventually that such is not the case, and that cholesterol is of endogenous origin. In the meantime it had been discovered also that plant sterols are not of the same order \( C_{27} \) as cholesterol, as formerly believed, but of the higher orders \( C_{28} \) and \( C_{29} \). This discovery appeared to re-emphasize the difference between plant and animal sterols.

In the beginning of his studies on the sterols of invertebrates, the present author observed the presence of a phytosterol-like sterol in the oyster, Ostrea virginica, and of cholesterol in the marine gastropod, Fulgar sp. He then interpreted the differences in sterol content of the two species as due to the exogenous origin of sterols in mollusks. The herbivorous oyster was thought to assimilate the plant sterols of its phytoplankton diet, and the carnivorous snail to utilize the cholesterol of its diet. Such assumptions eventually led to the formulation of the tentative hypothesis that invertebrates, and in particular marine invertebrates, are incapable of synthesizing sterols, and that the sterols found in them are of exogenous origin and hence related to the sterol of the diet. Subsequent observations seemed to support this hypothesis for a while, such as the presence of cholesterol in certain carnivorous coelenterates and the presence of phytosterol-like compounds in the herbivorous sponges. However, as more data became available this hypothesis gradually became untenable. The discovery of substantial amounts of cholesterol in the herbivorous silkworm and the earthworm had already made necessary a restriction of this hypothesis to marine invertebrates. It had to be abandoned when sterols of the order \( C_{28} \) were found in the carnivorous starfish (20) and cholesterol in sponges and in such herbivorous gastropods as Haliotis (86).

Therefore, it appears at present that, with the possible exception of parasitic species, all animals are capable of producing their own sterols. As mentioned before, the lowest forms synthesize the greatest
variety of sterols, including cholesterol, the highest only cholesterol and some of its derivative in minor amounts. Such a reduction of a multitude of closely related compounds which seemingly perform closely related functions to a few compounds, if not only one, appears to be a general phenomenon of biochemical evolution. If this were the case one should expect a priori that the distribution of the phosphagens, creatine and arginine phosphoric acid is not as clearly divided among vertebrates and invertebrates as had been believed originally. On the contrary, one should expect the presence of both phosphagens in species of the lower phyla, with the presence of additional phosphagens, such as agmatine phosphoric acid or as yet unknown types, in species of the lowest phyla. Indeed the recent observations by Baldwin and Yudkin (3) on the phosphagens of annelids appear to support this view. In general, therefore, evolutionary development seems to be accompanied, if not caused, by a reduction in the number of chemical products and reactions and by retaining only those which have proved to be the most suitable, the most efficient.

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